Postharvest Application of Novel Bio-Based Antifungal Composite Edible Coatings to Reduce Sour Rot and Quality Losses of ‘Valencia’ Oranges

Maria Victoria Alvarez 1,2, María Bernardita Pérez-Gago 2, Verónica Taberner 2, Laura Settier-Ramírez 2, Victoria Martínez-Blay 2 and Lluís Palou 2,*

1 Grupo Investigación en Ingeniería en Alimentos, Instituto de Ciencia y Tecnología de Alimentos y Ambiente (INCITAA), Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mar del Plata 8700, Argentina; mvalvarez@fi.mdp.edu.ar
2 Centre de Tecnologia Postcollita (CTP), Institut Valencià d’Investigacions Agràries (IVIA), 46113 Montcada, Valencia, Spain; perez_mbe@gva.es (M.B.P.-G.); taberner_ver@gva.es (V.T.); settier_lau@gva.es (L.S.-R.); vicmarbli@gmail.com (VM.-B.)

* Correspondence: palou_llu@gva.es

Abstract: Sour rot, caused by Geotrichum citri-aurantii, can produce significant postharvest losses of citrus fruits and, currently, cannot be effectively controlled by the postharvest fungicides registered in EU countries. Therefore, novel antifungal edible coatings (ECs) based on citrus pectin and beeswax and enriched with eugenol (EG), geraniol (GR), propolis extract (PR) or essential oils (EOs) from Satureja montana (SA), Cinnamomum zeylanicum (CI), or Commiphora myrrha (CM), were developed as alternatives to reduce sour rot and preserve the postharvest quality of ‘Valencia’ oranges. These natural agents were incorporated into the EC formulation and then applied to inoculated oranges. ECs enriched with EG (2–8 g/kg), GR (4 and 8 g/kg), PR (5–20 g/kg), and CM EO (15 g/kg) reduced disease incidence and severity by 75 to 100% compared to uncoated oranges after 20 days of incubation at 20 °C. ECs containing EG (8 g/kg), GR (4 g/kg), and PR (20 g/kg) reduced weight loss and retained firmness of oranges after 14 days of shelf life at 20 °C. Furthermore, all tested ECs maintained the fruit’s sensory and physicochemical quality. Overall, the EG-enriched pectin EC performed best, showing potential as a safe, bio-based alternative to conventional waxes containing synthetic fungicides for the management of citrus postharvest sour rot.

Keywords: Citrus sinensis; essential oils; natural extracts; edible coatings; postharvest preservation

1. Introduction

Sour rot, caused by the pathogenic fungus Geotrichum citri-aurantii (Ferraris) E.E. Butler, is among the most prevalent postharvest diseases of citrus fruits, second only to green and blue molds, caused by Penicillium digitatum (Pers.) Sacc. and P. italicum Wehmer, respectively, in terms of economic importance worldwide [1]. Due to the lack of effective control means, the incidence of sour rot has significantly increased in recent years causing large economic losses, especially in citrus producing countries with a Mediterranean climate, characterized by heavy rainfalls, hail, and strong winds [1,2].

Commercial synthetic chemicals formulated with fungicidal active ingredients, such as imazalil, thiabendazole, pyrimethanil, and α-phenyl phenol, are commonly used for citrus postharvest disease control [3,4]. While these commercial agrochemicals are generally effective against postharvest decay caused by species of Penicillium, they are unable to inhibit sour rot development [1,5,6]. On the other hand, the application of guazatine and propiconazole, highly effective fungicides for sour rot control, has been prohibited in the European Union (EU) in the last decade due to their high toxicity risks to human health and the environment [2,3]. Therefore, there are currently no approved fungicidal active
ingredients in the EU countries that effectively control citrus sour rot, which is a serious concern for citrus growers and exporters [1,7].

Commercial coatings, generically known as ‘waxes’, formulated with the addition of chemical fungicides, have been used for several decades in citrus packing lines as a conventional strategy to reduce postharvest decay and extend the shelf life of citrus fruits [8–10]. However, the repeated application of synthetic fungicides and conventional waxes formulated with resins, synthetic waxes, such as polyethylene, and ammonia has raised serious concerns due to increased chemical residues on/in fruit, environmental pollution, and induced fungal resistance [8,10,11]. Therefore, the development of eco-friendly and cost-effective alternatives to existing management practices for the control of postharvest infectious diseases caused by fungal pathogens on fresh citrus fruits is currently an urgent challenge for researchers and the industry. In this sense, a great effort has been made in recent years in the search for safe and green alternatives to reduce postharvest losses of citrus fruit by controlling postharvest diseases caused by pathogenic fungi, such as G. citri-aurantii and Penicillium spp. Some of the most investigated alternatives have been physical methods, such as hot water treatments, UV-C irradiation, gamma irradiation, and modified atmospheres; biological methods based on the postharvest application of antagonistic microorganisms as biocontrol agents (e.g., antagonistic yeasts or bacteria) [12,13]; as well as low-risk chemical methods, such as food additives, generally recognized as safe (GRAS) organic and inorganic salts, synthetic elicitors, plant extracts, essential oils (EOs), and antimicrobial polysaccharides, such as chitosan and Aloe vera [4,14].

Among the different low-risk chemical alternatives to control postharvest fungal diseases, natural extracts, EOs, and pure volatile compounds have generated great research interest because of important advantages, such as their low environmental impact, safe consumption at low doses, and high complementarity with other antifungal methods [4,14]. Thus, for example, several works have reported that thyme EO [15,16], thymol [17,18], cassia oil [19], lemongrass EO and citral [15], cinnamaldehyde [11,20–22], menthol [23], cistus extracts [24,25], and isothiocyanates [26] effectively inhibited the growth of G. citri-aurantii in in vitro tests. However, the effectiveness of the direct application of EOs or pure volatile compounds to fruit cannot be anticipated by in vitro results. In fact, the use of natural volatiles in in vivo experiments has typically shown important limitations, such as possible phytotoxic effects, limited efficacy at nonphytotoxic concentrations, and the induction of undesirable off-flavors and aromas [18,21]. Furthermore, some physicochemical properties of EOs, such as low water solubility and high volatility as well as their instability in the presence of oxygen and light, can limit their effectiveness and commercial feasibility as antifungal agents to ensure fruit protection [23]. Hence, the application of some strategies to enhance their properties and overcome these problems could be necessary. Among them, a valid strategy that is lately gaining importance is their incorporation as additional ingredients into edible coating (EC) formulations, which can increase the functionality of the coating by providing antimicrobial properties [10,18].

ECs are composed mainly of proteins, polysaccharides, and lipids, alone or in combination. These components form a thin layer of materials surrounding the fruit, providing a partial barrier to the diffusion of water vapor and gasses, reducing respiration and weight loss, and maintaining firmness and overall fruit quality during postharvest [10]. Thus, these natural, biodegradable, composite ECs constitute a promising safe alternative for the replacement of synthetic commercial waxes for the environmentally friendly postharvest management of citrus fruits [4,8]. Most of the studies reporting the effectiveness of natural antifungal compounds to control citrus sour rot focused on the application of aqueous or organic solutions of extracts or EOs [16,17,19,23–25] or the use of shellac or carnauba commercial waxes amended with antifungal EOs or pure volatile compounds [11,15,20,21]. However, the development of bio-based polymeric ECs functionalized through the addition of natural extracts or EOs to reduce citrus sour rot has not been explored. Our research group has recently developed composite pectin–beeswax ECs suitable to be amended with antifungal ingredients. Pectin is a major structural polysaccharide present in many higher
plant cells, allowing for primary cell wall extension and plant growth [27]. This biopolymer can be extracted from different waste biomasses, contributing to waste management and the circular bioeconomy in the food processing industries. Among potential pectin sources at an industrial scale, the generation of citrus fruit peels during juice extraction is one of the most important because of their good properties and high extraction yields [28]. Furthermore, this biopolymer is biodegradable, biocompostable, sustainable, and non-toxic, and it is, therefore, a good alternative to be used as the base for novel EC matrices for the postharvest coating treatment of fresh citrus fruits. Nevertheless, to our knowledge, no information is available on the utilization of composite pectin ECs enriched with natural bioactive compounds with antifungal activity to control sour rot on citrus fruits.

Therefore, this study aimed to (1) assess the in vitro antifungal activity of several natural extracts and EOs against _G. citri-aurantii_, (2) evaluate the ability of novel pectin-based ECs amended with selected antifungal natural agents to reduce the incidence and severity of sour rot on ‘Valencia’ oranges previously wound-inoculated with the pathogen (curative activity), and (3) determine the ability of these ECs to preserve the postharvest quality of coated fruit during simulated commercial shelf-life periods of 7 and 14 days at 20 °C.

2. Materials and Methods

2.1. Antifungal Bioactive Agents and Coating Matrix Ingredients

Geraniol (GR), eugenol (EG), and the EOs from cinnamon (CI, _Cinnamomum zeylanicum_) and lemongrass (LE, _Cymbopogon citratus_) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Myrrh EO (CM, _Commiphora myrrha_) was provided by Essenciales (Barcelona, Catalonia, Spain) and savory EO (SA, _Satureja montana_) by Essential’ Arôms (Lleida, Catalonia, Spain). Dry extracts of green tea (GT) and propolis (PR) were purchased from Guinama (Valencia, Spain). Citrus pectin (CP, Ceampectin RS 4710, DE 70%–75%) was supplied by CEAMSA (Pontevedra, Spain) and beeswax (BW) by Guinama (Valencia, Spain). Glycerol, oleic acid, and palmitic acid were provided by Panreac Química SA (Barcelona, Catalonia, Spain).

2.2. Preparation of Inoculum of _G. citri-aurantii_

This research was performed with the isolate _G. citri-aurantii_ NAV-1 of the IVIA CTP collection of fungal postharvest pathogens, which corresponds to the isolate CECT 13,166 in the Spanish Type Culture Collection (CECT, University of Valencia, Valencia, Spain). Active young colonies of the strain were replated every 7–14 days in Petri dishes containing potato dextrose agar (PDA) medium (Scharlab S.L., Barcelona, Catalonia, Spain) and incubated at 25 °C. For in vitro assays, 5 mm mycelial plugs were employed as inocula. For in vivo assays, arthrospores were taken from the surface of PDA Petri dishes and transferred to 0.5 g/L Tween 80 solution (Panreac-Química S.A., Barcelona, Catalonia, Spain). The suspension was passed through two cheesecloth layers and measured with a hemacytometer. The final inoculum suspension was adjusted to 10^7 arthrospores/mL, and fresh orange juice (10%), thiabendazole (50 mg/L; Textar® 60 T, Decco Ibérica PostCosecha, S.A.U., Paterna, Valencia, Spain), and cycloheximide (5 mg/L; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were included to enhance the virulence of the arthrospore suspension and improve its infectivity in orange rind wounds.

2.3. In Vitro Inhibition of _G. citri-aurantii_

Two different methods were employed to evaluate the antifungal activity of the bioactive compounds depending on their chemical nature. First, pure volatile compounds and EOs were tested according to the volatile exposure method, as described by Plaza et al. [29]. Briefly, 5 mm diameter mycelial plugs were obtained with a sterilized cork-borer from PDA cultures of _G. citri-aurantii_ and placed individually in the center of PDA Petri dishes (90 mm diameter). Subsequently, 55 mm filter paper discs were placed in the lid of each of these inoculated dishes, soaked with 10, 20, or 40 μL of SA, CI, LE, EG, or GR,
sealed with Parafilm, and incubated upside down in the dark at 25 °C. Filter paper in control plates was soaked with sterile distilled water. Second, the antifungal activity of the agents CM, PR, and GT was assessed using the agar dilution method, according to Martinez-Blay et al. [30]. CM was previously dissolved in dimethyl sulfoxide (DMSO), PR in 80% ethanol, and GT in sterile distilled water. Final concentrations of the agents in PDA medium were of 1.25, 2.5, and 5 g/kg for CM; 5 and 10 g/kg for PR; and 5, 10, and 20 g/kg for GT. Plates with PDA, PDA with DMSO (5 g/kg), and PDA with 80% ethanol (50 g/kg) were used as controls. All dishes were inoculated with a 5 mm diameter mycelial plug, as explained previously, and incubated in the dark at 25 °C. For both methods, after 7 days of incubation at 25 °C, two perpendicular diameter measurements of the fungal colony were performed in each plate and the mean value was used as the final value of colony diameter. Five plates were used for each bioactive agent and concentration. For each treatment, results are presented as percentage of fungal radial growth inhibition in relation to the fungal growth in the respective control plates. In Petri dishes with 100% inhibition, the lids containing discs soaked with EOs were removed and replaced with lids containing discs impregnated with sterile distilled water and were observed after incubating for 7 days at 25 °C to evaluate the potential fungicidal effect of the compounds.

2.4. Bio-Based Coating Preparation

To prepare the composite bio-based ECs used in this research, a citrus pectin (CP, Ceampilpectin RS 4710, DE 70%–75%, Ceamsa, Pontevedra, Spain) solution (20 g/kg, wet basis (wb)) was combined with beeswax (BW, 7 g/kg, wb) as a lipidic ingredient and used as an emulsion matrix. A combination of oleic acid and palmitic acid (1:1) was added as an emulsifier and glycerol as a plasticizer. Ratios of CP:glycerol and BW:emulsifiers were 2:1 and 5:1, respectively. The mixture was heated to 92 °C, homogenized, and allowed to cool to room temperature before adding the antifungal agents. The detailed procedure and the sources of these ingredients were described by Martínez-Blay et al. [31]. EOs, pure volatiles, and natural extracts applied as individual ingredients of the ECs were selected according to the results of the prior in vitro screening assays. The concentrations of these bioactive agents were selected as the highest that did not induce any phytotoxicity symptoms on the surface of oranges in preliminary evaluations of the application of the coatings (data not shown). The CP-based ECs were enriched with SA, CI, EG, and GR at 2, 4, and 8 g/kg; PR at 5, 10, and 20 g/kg; and CM EO at 15 g/kg. The formulated ECs resulted in stable emulsions in all cases, with viscosity values ranging from 50 to 162 cp and pH values from 3.11 to 3.38.

2.5. Orange Fruit

The study was performed with oranges (Citrus sinensis [L.] Osbeck) cv. ‘Valencia’ obtained from commercial orchards in the Valencia area (Spain) at commercial maturity and stored for up to 1 week at 5 °C and 90% relative humidity (RH) before use. No commercial postharvest treatments were applied before the experiments. Oranges were manually selected to use uniform fruit, and diseased or damaged fruit were discarded. Before each assay, selected fruit were disinfected superficially by dipping them for 4 min in a sodium hypochlorite solution (5 g/kg), rinsed with tap water, allowed to air-dry at room temperature, and randomized.

2.6. Effect of Antifungal Edible Coatings on Sour Rot Development

Fruit were wound-inoculated with G. citri-aurantii (10⁷ arthropores/mL) by immersing a stainless-steel rod with a probe tip 1 mm wide and 2 mm in length into the arthropore suspension and wounding each fruit once on the equator. Inoculated oranges were kept for 24 h at 20 °C and 90% RH [3]. Afterwards, ECs were manually applied, simulating the coating application in a roller conveyor in a citrus packinghouse. For this, 400 µL of the corresponding emulsion was applied on the surface of each orange with a micropipette and gently rubbed with gloved hands [31]. Fruit samples used as controls were uncoated. The
CP coating formulated without bioactive compounds was also considered as an additional control treatment. Treated fruit were air-dried at room temperature on plastic grids, then moved to open plastic boxes, and stored in a temperature-controlled cabinet for incubation at 20 °C and 90% RH for up to 20 days. After 7, 14, and 20 days of incubation, sour rot incidence was assessed as the percentage of decayed fruit, and sour rot severity as decay lesion size (diameter in mm). Each treatment was applied to 4 replicates of 5 fruit each. Data obtained after 20 days are presented.

2.7. Impact of Bio-Based Coatings on Orange Quality during Shelf Life at 20 °C

The different antifungal ECs and the PEC-based coating without antifungal agent were applied to intact (non-inoculated) ‘Valencia’ oranges as described above. Immersion for 15 s in tap water at 20 °C was the treatment applied to control fruit. Coated oranges were distributed in plastic boxes and stored at 20 °C and 80% RH for up to 14 days. Orange quality attributes were determined at harvest and for each treatment after 7 and 14 days of storage. A total number of 36 fruit per treatment and shelf-life period were used.

2.7.1. Fruit Weight Loss

Weight loss was determined by individually weighing 15 oranges per treatment at the beginning and at the end of each shelf-life period with a calibrated analytical balance. Results were expressed as weight loss percentage related to initial weight.

2.7.2. Fruit Firmness

Orange firmness was determined in 10 oranges per treatment as the percentage of rind deformation related to initial fruit diameter with an Instron Universal testing machine (Model 3343, Instron Corp., Canton, MA, USA) after the application of a load of 10 N to the fruit’s equatorial area [32].

2.7.3. Juice Physicochemical Quality

Determinations of titratable acidity (TA, g/L of citric acid), pH, and soluble solids content (SSC, °Brix) were performed according to Valencia-Chamorro et al. [33] with 5 mL orange juice samples. For each treatment and shelf-life time, 3 replicates of 3 oranges each were processed. SSC measurements were taken using a digital refractometer (model ATC-1, Atago® Co., LTD, Tokyo, Japan), whereas pH and TA measurements were performed with an automatic titrator (Titrator T50, Mettler Toledo, Switzerland).

2.7.4. Sensory Quality

The sensory quality of coated oranges was evaluated by 10 semi-trained panelists following the methodology described by Valencia-Chamorro et al. [33]. In brief, overall flavor was evaluated in coded fruit pieces from coated and uncoated control oranges by using a 1–9 scale from very poor (score 1) to optimal (score 9). Similarly, the presence of off-flavors was evaluated using a scale from 1 (absence) to 5 (very pronounced). Judges also visually assessed the external appearance of entire oranges by scoring treated and untreated fruit through a scale from bad (score 1) to good (score 3) and ordered the samples from the highest to the lowest gloss. Due to the high number of coating formulations used in this study, sensory evaluation was only performed with oranges subjected to coating treatments containing the highest concentration of each tested antifungal agent. The CP coating formulated without antifungal compounds was also included.

2.8. Statistical Analysis

The experiments were performed with completely randomized designs with treatment as the variation factor. Depending on the experiment, treatments were bioactive agents, concentrations, or antifungal ECs. For each evaluation date, data were subjected to analysis of variance (ANOVA) and Fisher’s Protected Least Significant Difference test (LSD, p < 0.05) was used to separate means (Statgraphics Centurion XVII; Statgraphics
Technologies Inc., The Plains, VA, USA). Sour rot incidence data were arc-sin transformed to ensure the homogeneity of variances in the ANOVA test. Non-transformed means are shown. Friedman test was used for data on sensory gloss. All results are presented as mean value ± standard error.

3. Results

3.1. In Vitro Inhibitory Activity of Natural Agents against G. citri-aurantii

The effect of the different EOs, pure volatiles, and natural extracts on the mycelial growth of G. citri-aurantii after 7 days of incubation at 25 °C is shown in Table 1. The results with both volatile and agar-diluted compounds suggested that, in general, the presence of bioactive antifungal compounds affected the growth of G. citri-aurantii in a dose-dependent manner. The volatiles SA, CI, LE, and GR, at a dose of 20 µL, were highly effective and inhibited the fungal radial growth by 90%–100%. Furthermore, SA and EG at doses of 20 and 40 µL, respectively, showed significant in vitro fungicidal activity since the fungus did not grow during further incubation for 7 days at 25 °C after removing the lids containing the soaked discs. Among the natural agents assayed by direct contact with the pathogen, CM and PR showed a moderate effect, with growth inhibition close to 50% when applied at 1.25 and 10 g/kg, respectively, while GT showed a similar result at the highest dose tested (20 g/kg) (Table 1). Based on these results, SA, CI, EG, GR, PR, and CM were the bioactive compounds selected to be used as EC ingredients in the in vivo experiments.

Table 1. Radial growth inhibition of Geotrichum citri-aurantii exerted by natural bioactive agents after 7 days of incubation at 25 °C.

<table>
<thead>
<tr>
<th>Bioactive Agent</th>
<th>Dose (µL)</th>
<th>Radial Growth Inhibition (%) 1</th>
<th>Bioactive Agent</th>
<th>Dose (g/kg)</th>
<th>Radial Growth Inhibition (%) 1</th>
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<tbody>
<tr>
<td>SA</td>
<td>10</td>
<td>77.5 ± 6.0 bcd</td>
<td>GT</td>
<td>5.00</td>
<td>0.0 ± 0.0 f</td>
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<td></td>
<td>20</td>
<td>100.0 ± 0.0 * a</td>
<td></td>
<td>10.00</td>
<td>26.7 ± 3.8 d</td>
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<td></td>
<td>40</td>
<td>100.0 ± 0.0 * a</td>
<td></td>
<td>20.00</td>
<td>53.9 ± 2.7 b</td>
</tr>
<tr>
<td>CI</td>
<td>10</td>
<td>62.1 ± 2.4 d</td>
<td>CM</td>
<td>1.25</td>
<td>58.2 ± 5.6 ab</td>
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<tr>
<td></td>
<td>20</td>
<td>100.0 ± 0.0 a</td>
<td></td>
<td>2.50</td>
<td>62.1 ± 1.4 a</td>
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<td></td>
<td>40</td>
<td>100.0 ± 0.0 a</td>
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<td>5.00</td>
<td>63.6 ± 3.1 a</td>
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<tr>
<td>LE</td>
<td>10</td>
<td>82.1 ± 4.8 abc</td>
<td>PR</td>
<td>5.00</td>
<td>16.6 ± 8.8 e</td>
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<td></td>
<td>20</td>
<td>91.1 ± 0.8 ab</td>
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<td>10.00</td>
<td>47.0 ± 6.1 c</td>
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<td></td>
<td>40</td>
<td>93.4 ± 1.9 ab</td>
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<tr>
<td>EG</td>
<td>10</td>
<td>67.1 ± 5.6 cd</td>
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<td></td>
<td>20</td>
<td>80.11 ± 6.6 bcd</td>
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<td></td>
<td>40</td>
<td>100.0 ± 0.0 * a</td>
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<td>GR</td>
<td>10</td>
<td>88.0 ± 5.5 ab</td>
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<td></td>
<td>20</td>
<td>90.8 ± 0.9 ab</td>
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<tr>
<td></td>
<td>40</td>
<td>90.0 ± 1.7 ab</td>
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</table>

1 Data are percentage inhibition with respect to control plates without natural agents (means ± standard error; n = 5). Means in columns with different lowercase letters are significantly different (LSD test, p < 0.05). SA: savory essential oil; CI: cinnamon essential oil; LE: lemongrass essential oil; EG: eugenol; GR: geraniol; GT: green tea extract; CM: myrrh essential oil; PR: propolis extract. * Fungicidal effect: no fungal growth during incubation at 25 °C for 7 days after removing the plate lids with soaked discs.

3.2. Effect of Antifungal Edible Coatings on Sour Rot Development and Quality of Oranges

3.2.1. In Vivo Sour Rot Control

The development of sour rot on ‘Valencia’ oranges artificially inoculated with G. citri-aurantii, treated with antifungal CP-based ECs, and incubated for 20 days at 20 °C is presented in Figure 1. Sour rot incidence on uncoated oranges (control) reached 60%, while it decreased to 21% on samples coated with CP (EC matrix without bioactive compounds) (Figure 1A). ECs containing EG (2, 4, and 8 g/kg), GR (4 and 8 g/kg), PR (5, 10 and
20 g/kg), or CM (15 g/kg) showed the highest disease incidence reductions (75 to 100% with respect to the uncoated control (p < 0.05)). Moreover, ECs formulated with CI (4 and 8 g/kg) showed a moderate but significant effect, reducing disease incidence by 67%–75%. By contrast, the addition of SA did not exert significant changes in sour rot incidence compared to the control at any of the concentrations tested (p > 0.05). In general, no significant differences in sour rot incidence were observed between treatments containing the same antifungal agent at different concentrations.

Regarding sour rot severity, the average lesion diameter on uncoated oranges after 20 days at 20 °C was 21 mm. Although the differences between uncoated control fruit and oranges coated with CP (matrix without bioactive compounds) were non-significant (p > 0.05), the latter reduced disease severity with respect to the former by more than 50% (Figure 1B). Except for SA, the addition of antifungal agents to the CP-based coating resulted in lesion diameters significantly lower than those on control oranges after 20 days. In particular, CI (4 and 8 g/kg), EG (2, 4 and 8 g/kg), GR (4 and 8 g/kg), PR (10 and 20 g/kg), and CM (15 g/kg) reduced sour rot severity by 70 to 100% with respect to the uncoated control (p < 0.05).
3.2.2. Fruit Weight Loss and Firmness

Figure 2 shows the weight loss of control and coated ‘Valencia’ oranges after 7 and 14 days of shelf life at 20 °C. Orange weight loss in oranges ranged from 0.7% to 1.1% after 7 days and from 1.8% to 2.9% after 14 days. No differences in weight loss were observed between oranges coated with CP (without antifungal agents) and uncoated oranges during shelf life (p > 0.05). ECs containing EG (8 g/kg), GR (4 g/kg), or PR (20 g/kg) were the most effective treatments to reduce the weight loss of oranges, with significant reductions of up to 27% compared to uncoated fruit after 14 days of shelf life.

Figure 2. Weight loss of ‘Valencia’ oranges untreated (control) or treated with citrus pectin–beeswax (CP) composite edible coatings enriched with savory (SA), cinnamon (CI), or myrrh (CM) essential oils, and eugenol (EG), geraniol (GR), or propolis extract (PR) at different doses, and stored for 7 days (A) or 14 days (B) at 20 °C as simulated shelf-life periods. For each evaluation period, different letters in columns indicate significant differences between means (LSD test, p < 0.05).

Figure 3 shows the changes in firmness for coated and uncoated oranges stored for 7 and 14 days at 20 °C. The mean firmness of oranges at harvest was 2.36% rind deformation. After 7 days, rind deformation increased (lower firmness) and ranged from 2.36% to 3.63%, with significant differences between coated and control fruit. While CP-based ECs containing EG (8 g/kg), GR (2, 4, and 8 g/kg), PR (5 and 20 g/kg), or CM (15 g/kg) helped to maintain firmness with significantly lower rind deformation values than the uncoated control (p < 0.05), CP coating (without antifungal agent) showed no significant differences with the control. However, after 14 days, rind deformation was in the range of 2.98%–3.94% without significant differences between coated and uncoated fruit.
control (p < 0.05), CP coating (without antifungal agent) showed no significant differences between treatments.

Regarding the visual quality of the entire fruit, scores changed from 3.0 (good) at harvest to 2.5 in average (fair-good) at the end of shelf life, without significant differences (p > 0.05) between coated and uncoated samples throughout the shelf-life period. Overall flavor scores decreased throughout shelf life, from values ranging from 10.8 to 12.2 °Brix after 14 days.

Figure 3. Firmness of ‘Valencia’ oranges untreated (control) or treated with citrus pectin–beeswax (CP) composite edible coatings enriched with savory (SA), cinnamon (CI), or myrrh (CM) essential oils, and eugenol (EG), geraniol (GR), or propolis extract (PR) at different doses, and stored for 7 days (A) or 14 days (B) at 20 °C as simulated shelf-life periods. For each evaluation period, different letters in columns or ‘n.s.’ indicate significant and non-significant differences, respectively, between means (LSD test, p < 0.05).

3.2.3. Internal Physicochemical Quality and Sensory Evaluation

Values of internal and sensorial quality attributes of untreated and treated ‘Valencia’ oranges after 7 and 14 days of simulated shelf-life period at 20 °C are shown in Table 2. Coated oranges showed slightly higher values of juice pH (3.60–3.68) (p < 0.05) than the uncoated control (3.44) after 7 days of shelf life, while non-significant differences were found after 14 days (p > 0.05). TA values decreased as shelf-life time increased; however, no differences in TA were observed between coated and uncoated samples throughout the shelf-life period. Similarly, SSC was not significantly affected by coating application, with values ranging from 10.8 to 12.2 °Brix after 14 days.

The application of bio-based antifungal ECs did not exert significant changes on orange sensory properties, such as global flavor, off-flavors, and visual quality along the entire shelf-life period. Overall flavor scores decreased throughout shelf life, from values of 7.1 (good quality) at harvest to values of 4.8–6.0 (acceptable) at the end of the shelf-life period, without significant differences (p > 0.05) between coated and uncoated fruit. Similarly, values in the off-flavor scale increased from absence at harvest to 1.5–2.1 (very slight presence) after 14 days. Regarding the visual quality of the entire fruit, scores changed from 3.0 (good) at harvest to 2.5 in average (fair-good) at the end of shelf life, without significant differences between treatments.

Figure 3. Firmness of ‘Valencia’ oranges untreated (control) or treated with citrus pectin–beeswax (CP) composite edible coatings enriched with savory (SA), cinnamon (CI), or myrrh (CM) essential oils, and eugenol (EG), geraniol (GR), or propolis extract (PR) at different doses, and stored for 7 days (A) or 14 days (B) at 20 °C as simulated shelf-life periods. For each evaluation period, different letters in columns or ‘n.s.’ indicate significant and non-significant differences, respectively, between means (LSD test, p < 0.05).

3.2.3. Internal Physicochemical Quality and Sensory Evaluation

Values of internal and sensorial quality attributes of untreated and treated ‘Valencia’ oranges after 7 and 14 days of simulated shelf-life period at 20 °C are shown in Table 2. Coated oranges showed slightly higher values of juice pH (3.60–3.68) (p < 0.05) than the uncoated control (3.44) after 7 days of shelf life, while non-significant differences were found after 14 days (p > 0.05). TA values decreased as shelf-life time increased; however, no differences in TA were observed between coated and uncoated samples throughout the shelf-life period. Similarly, SSC was not significantly affected by coating application, with values ranging from 10.8 to 12.2 °Brix after 14 days.

The application of bio-based antifungal ECs did not exert significant changes on orange sensory properties, such as global flavor, off-flavors, and visual quality along the entire shelf-life period. Overall flavor scores decreased throughout shelf life, from values of 7.1 (good quality) at harvest to values of 4.8–6.0 (acceptable) at the end of the shelf-life period, without significant differences (p > 0.05) between coated and uncoated fruit. Similarly, values in the off-flavor scale increased from absence at harvest to 1.5–2.1 (very slight presence) after 14 days. Regarding the visual quality of the entire fruit, scores changed from 3.0 (good) at harvest to 2.5 in average (fair-good) at the end of shelf life, without significant differences between treatments.
Table 2. Physicochemical and sensory quality attributes of ‘Valencia’ oranges uncoated (control) or coated with citrus pectin–beeswax (CP) composite edible coatings after 7 or 14 days of storage at 20 °C as simulated shelf-life periods.

<table>
<thead>
<tr>
<th>Internal Quality Attributes</th>
<th>Shelf-Life Period</th>
<th>Coating Treatments ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Uncoated)</td>
<td>CP</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At harvest</td>
<td>3.53 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>3.44 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>3.60 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Titratable Acidity (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At harvest</td>
<td>10.10 ± 0.55</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>9.42 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Citric Acid (°Brix)</td>
<td>8.04 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>Soluble Solids Content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At harvest</td>
<td>11.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>11.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>10.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Sensory Quality Attributes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Flavor</td>
<td>7.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>6.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>5.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Off-Flavors</td>
<td>1.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>1.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>1.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Visual Quality</td>
<td>2.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>2.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>2.7 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

1 Data are means ± standard error. In each row, different lowercase letters and ‘ns’ indicate significant and non-significant differences, respectively, among coating treatments (LSD test, p < 0.05). ² CP: citrus-pectin-based edible coating; SA: savory essential oil; CI: cinnamon essential oil; EG: eugenol; GR: geraniol; PR: propolis extract; CM: myrrh essential oil. ³ Overall flavor scored from 1 = very poor to 9 = optimum, off-flavors from 1 = absence to 5 = high presence, and visual quality from 1 = bad to 3 = good.

Table 3 shows the effect of the application of bio-based coatings on the rind gloss of ‘Valencia’ oranges after 7 and 14 days of shelf life at 20 °C. After 7 days, fruit coated with CP-EG (8 g/kg) were scored with the highest gloss, and they were the only oranges showing significantly higher gloss than untreated fruit (p < 0.05), whereas oranges treated with CP-PR (20 g/kg) presented the lowest gloss. At the end of the experiment, CP-EG (8 g/kg) coating maintained the highest rind gloss, followed by CP-GR (8 g/kg), CP-CI (8 g/kg), and CP-CM (15 g/kg), while no significant differences were observed between oranges coated with CP (without natural bioactive agents) and control oranges (p > 0.05).

Table 3. Gloss rating of ‘Valencia’ oranges untreated (control) or treated with citrus pectin–beeswax (CP) composite edible coatings after 7 or 14 days of storage at 20 °C as simulated shelf-life periods.

<table>
<thead>
<tr>
<th>Coating Treatments ¹</th>
<th>Shelf-Life Period (20 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 Days</td>
</tr>
<tr>
<td>Gloss Rank ²</td>
<td></td>
</tr>
<tr>
<td>Glossier</td>
<td></td>
</tr>
<tr>
<td>CP-EG (8 g/kg)</td>
<td>a</td>
</tr>
<tr>
<td>CP-CM (15 g/kg)</td>
<td>ab</td>
</tr>
<tr>
<td>CP-GR (8 g/kg)</td>
<td>ab</td>
</tr>
<tr>
<td>CP-EG (8 g/kg)</td>
<td>b</td>
</tr>
<tr>
<td>CONTROL</td>
<td>b</td>
</tr>
<tr>
<td>CP</td>
<td>bc</td>
</tr>
<tr>
<td>CP-SA (8 g/kg)</td>
<td>bc</td>
</tr>
</tbody>
</table>

¹ CP: citrus-pectin-based edible coating; SA: savory essential oil; CI: cinnamon essential oil; EG: eugenol; GR: geraniol; PR: propolis extract; CM: myrrh essential oil. ² For each shelf-life period, different letters indicate significant differences among treatments (Friedman test, p < 0.05, n = 10).

4. Discussion

This research study focuses on the antifungal capacity of different natural bioactive extracts, EOs, and pure volatiles against G. citriaurantii and their suitability as antifungal...
ingredients of composite edible emulsions formulated with pectin for sour rot control and quality maintenance of ‘Valencia’ oranges. Such plant-derived natural compounds are increasingly attracting the attention of scientists and the fresh produce industry due to their wide biological activity, including antimicrobial and antioxidant actions, and contribution to bio-circular postharvest practices [34]. To the best of our knowledge, this is the first report of composite ECs enriched with natural EOs or plant extracts used to control orange sour rot caused by *G. citri-aurantii*. The present research complements a previous work by our research group in which a similar study with antifungal CP-based ECs was conducted to control citrus green mold, caused by the fungus *P. digitatum*, and preserve the postharvest quality of cold-stored ‘Valencia’ oranges [35].

In the present work, the in vitro antifungal activity of EOs and pure volatile compounds was evaluated using the ‘volatile exposure’ method due to the high volatility of terpenes and phenols (major components of these compounds), which are expected to have a stronger and longer activity applied as volatiles [29]. Among the tested volatiles, SA showed a very high effectiveness, with 100% growth inhibition of *G. citri-aurantii*. The activity was not only fungistatic but also fungicidal at a dose of 20 µL. Inhibitory activity similar to that of SA was found against *P. digitatum* using the volatile exposure assay in our previous research [35]. Also, the same EO totally inhibited the mycelial growth of *Alternaria alternata* Fr. Keissl. and other postharvest fungi when tested when amended to agar medium at 0.3 g/L [36]. In these works, the antifungal activity of SA oil was explained by that of its major components (24% carvacrol, 15.9% γ-terpinene, and 14.2% p-cymene).

In a study performed by Regnier et al. [15], a large set of commercial EOs and their major components were tested in vitro against *G. citri-aurantii* by incorporating these bioactive compounds into the culture medium (‘disc diffusion-toxic medium’). Oregano, clove, and thyme EOs at 0.5 mL/L were the most effective oils, with mycelial growth inhibitions from 72% to 100% after 6 days of incubation at 24 °C, while LE had a moderate effect and inhibited the fungal growth by 52%. In addition, EG, GR, carvacrol, and thymol totally suppressed the mycelial growth of *G. citri-aurantii* at 0.5 mL/L. In the same study, the most effective EOs were also tested as vapors against *G. citri-aurantii*. As a result, LE EO inhibited fungal growth by 100% at a dose of 10 µL, exceeding the effect of thyme and oregano EOs by more than 50% [15]. In our study, we confirmed the high effectiveness of LE, EG, and GR applied in the vapor phase against this pathogen. Furthermore, a fungicidal effect by EG at a dose of 40 µL was observed after the incubation period, while GR at the same dose only provided a fungistatic effect. In accordance with our results, CI EO has also been reported as an effective antifungal agent able to reduce the growth of *P. digitatum* when tested in vitro in the vapor phase [29,35]. Moreover, Combrink et al. [37] showed total growth inhibition of *P. digitatum* and *Alternaria citri* Ellis and N. Pierce isolated from oranges when CI EO was applied by the agar dilution method at 2–3 g/L. The activity of CI against fungi was attributed mainly to the high levels of EG and cinnamaldehyde in its composition [37]. On the other hand, CM EO showed a moderate inhibitory activity against *G. citri-aurantii* at 1.25-5 g/kg by the agar dilution method, with similar results to those observed in a previous in vitro study against *P. digitatum* [35], whereas Prakash et al. [38] reported a complete growth inhibition of nine molds, including *P. italicum*, by CM EO at concentrations of 2.5–3.5 mL/L. Differences observed between research works can be attributed to the different composition of the EOs used. As reported by the supplier, the most important components of the commercial CM EO used in the present work were curzerene (40.7%), lindestrene (25.6%), and furanoeudesma-1,3-diene (81%).

Studies analyzing the effects of natural plant extracts and EOs against *G. citri-aurantii* proposed that chemical compounds can cause disruptions in the cell membrane, disorganize the cytoplasm, and block fungal reproduction [5,16,22]. Functional properties of PR extracts have been widely studied for food and pharmacological applications [39,40]. It is known that their composition is usually variable depending on their botanical and geographical origin. PR extracts have been reported as effective antibacterial, antifungal, antioxidant, and anti-inflammatory agents due to their high content of bioactive compounds, such as
polyphenols, mainly flavonoids, coumarins, and sesquiterpene quinine, among others [40]. For instance, Moreno et al. [39] found that the growth of *P. digitatum* and *P. italicum* was suppressed by an ethanolic PR extract at 0.14 and 0.41 g/L, respectively, in a broth microdilution assay. However, as far as we know, no reports showing the effect of PR against *G. citri-aurantii* are currently available in the literature.

In this research, the EOs SA, CI, and CM, the pure compounds EG and GR, and the PR extract were selected to enrich the CP-based emulsions used for assessment of curative activity in the in vivo trials. ‘Valencia’ oranges previously inoculated with *G. citri-aurantii* were coated with these bio-based antifungal ECs and incubated at 20 °C. Although not the optimal temperature for the development of *G. citri-aurantii*, which is around 28 °C, this temperature is typically chosen for simulation of fruit shelf life and allows for a very good growth of most postharvest fungal pathogens infecting fresh fruits and vegetables. The use of natural extracts and EOs within coating-forming polymer materials provides advantages over the direct application of liquid solutions onto the fruit surface, such as a slower diffusion rate of the antifungal compounds and the reduction in any possible phytotoxic effect. This can allow for the continuous presence of the active agent at optimal concentrations on the surface of the fruit where fungal contamination occurs and infection takes place, typically occurring when wounds or microwounds are inflicted to the fruit peel [41].

In our study, the CP coating without the additional bioactive ingredients reduced sour rot incidence after incubation at 20 °C compared to the uncoated control. This effect could be attributed to the gas barrier created by the film layer formed on the surface of the fruit, affecting the environmental conditions needed for the development of the infection, and/or to a potential antifungal effect of some of the coating matrix ingredients, such as the fatty acids used as emulsifiers in the coating formulation. Thus, for example, several published works confirmed the antifungal activity of fatty acids, such as palmitic acid [42,43] and oleic acid [44], as well as that of botanical extracts rich in these compounds. Similarly, Duan et al. [11] also observed a reduction in the incidence of sour rot on citrus fruit coated with a commercial wax composed of food-grade shellac, resin, and fatty acid salts, without any antifungal compounds as additional ingredients, and incubated at 28 °C. In any case, despite the slight effect of the base coating matrix (CP-BW) on sour rot reduction, the addition of antifungal ingredients to this coating matrix was needed to improve the antifungal properties of the coating and achieve a significant protection of coated oranges against sour rot.

Among the different antifungal agents added to the ECs, SA was the least effective in reducing the incidence and severity of sour rot on coated oranges, while it was highly effective in the previous in vitro assay. On the contrary, PR and CM, which showed a moderate inhibition in vitro against *G. citri-aurantii* at the tested concentrations, were highly effective in reducing the development of sour rot in the in vivo tests at concentrations of 10 g/kg or higher, with incidence and severity reductions after 20 days of incubation at 20 °C above 90% and 80%, respectively. Differences between the effects of antifungal compounds when tested in vitro and in vivo have been repeatedly observed in previous work, and have been generally imputed to differences in the release rate and diffusion of the bioactive compounds from the coating to the fruit peel compared to direct inhibition in the culture medium [29,35]. Moreover, interactions between bioactive compounds and the food matrix and volatility could reduce the antimicrobial effects and higher levels of EOs or pure compounds could be necessary to achieve the desired inhibition in in vivo assays [16].

In this study, the best results regarding the reduction in sour rot incidence and severity on oranges were obtained with ECs containing EG at the lowest concentration (2 g/kg), GR (from 4 g/kg), PR (from 10 g/kg), and CM (15 g/kg). In addition, a greater effectiveness was not always found by increasing the concentrations of the antifungal agent. In general, the capability of natural antifungals to control disease development is affected by complex interactions between the host, fungal pathogen, and environment occurring during the infection process. Several authors reported that particular interactions between
the bioactive compounds and the fruit host involving biochemical reactions may strongly induce plant defense mechanisms, such as the production of defense-related enzymes that enhance resistance to phytopathogens and contribute to disease control [11,20,23]. Furthermore, several factors should be considered when the effectiveness of ECs amended with antifungal agents is evaluated. Thus, composition and coating properties, possible interactions between the active ingredients and the coating materials, and volatility may affect the availability and release of the antifungal agents with a significant influence on the ability of the coating to control fungal infections [31,45,46].

In a previous study, we reported for the first time the feasibility of CP-based ECs as carriers of natural bioactive agents to control the postharvest diseases of citrus fruit [35]. We demonstrated that CP-BW ECs formulated with GR (2 g/kg), EG (4 and 8 g/kg), and CM EO (15 g/kg) showed curative activity and reduced green mold incidence by up to 58% on ‘Valencia’ oranges artificially inoculated with *P. digitatum* and incubated for 8 days at 20 °C, while CI (8 g/kg) reduced green mold severity effectively. On the contrary, SA and PR showed no notable inhibitory effects against green mold during incubation at 20 °C. Similarly, an increment of the concentration of antifungals not always resulted in a higher inhibition of disease development. Furthermore, ECs enriched with EG (8 g/kg) or GR (2 g/kg) were the most effective treatments to control green mold on oranges during long-term cold storage, with respective reductions of disease incidence of 56 and 48% after 4 weeks at 5 °C [35]. These results illustrate that the effectiveness of natural antifungals can be different according to the pathogen under study, the environmental conditions, as well as the application technique and the fruit host.

Some authors have evaluated the ability of natural antifungal compounds to protect citrus fruit against postharvest sour rot. Regnier et al. [15] studied the curative activity of several EOs and volatile compounds applied as aqueous dip treatments or mixed with a carnauba-based commercial wax as coatings to reduce sour rot on ‘Valencia’ oranges. In general, antifungal coatings were more effective than aqueous dip treatments. In addition, LE EO and citral, as its major constituent, added to the commercial wax were able to reduce the incidence of sour rot after 7 days at 28 °C by 90%, although only 10 fruit per treatment were evaluated. In a study by Moussa et al. [17], thymol (1 g/L) was applied as a preventive treatment to oranges in a dip solution, and the results showed almost a complete inhibition of sour rot after 7 days of incubation at 22 °C. Similarly, thymol (50 mM) encapsulated in β-cyclodextrin reduced the incidence and severity of sour rot after 15 days of incubation at 20 °C by 60% in a curative assay performed with lemons [18]. In a recent work, Cai et al. [23] demonstrated the effectiveness of menthol (128 mM) added to corn oil and applied by immersion to navel oranges previously inoculated with *G. citri-aurantii*. After 6 days of incubation at 25 °C, decay incidence was reduced by 43%, and the lesion diameter was minimized compared to control fruit [23]. On the other hand, the use of composite ECs based on polymeric matrices amended with bioactive antifungal agents to control citrus sour rot has scarcely been addressed and GRAS salts, such as sodium benzoate, have been the active ingredients more often incorporated into EC formulations for this purpose [1,3]. An exception, in which a volatile compound was used, is the study by Faten et al. [47], which reported that chitosan ECs (6–8 g/L) amended with citral (4–5 mL/L) reduced sour rot incidence and severity by more than 89% and 93%, respectively, in a curative assay with lime fruit stored at 20 °C for 15 days.

To assess the viability of the studied antifungal ECs as an effective postharvest tool, their impact on the physicochemical and sensory quality of ‘Valencia’ oranges stored at 20 °C was also evaluated. In general, fruit coatings are able to reduce physiological weight loss and respiration during storage and transportation mainly due to the water vapor and oxygen barrier provided by the formulations containing combinations of biopolymers and hydrophobic ingredients [41]. In this work, the CP coating (formulated without antifungals) did not exert a barrier effect to water loss in coated oranges during storage, while the addition of EG (8 g/kg), GR (4 g/kg), or PR (20 g/kg) to the coating formulations enhanced the moisture barrier and helped reduce weight loss after 7 and 14 days of shelf life at 20 °C.
Changes in the composition of ECs lead to changes in their physical properties. Water vapor permeability is affected by the relative concentrations of major and minor ingredients (polymer, lipids, plasticizers, and emulsifiers) and the interactions between added bioactive compounds and the coating components. Thus, an increase in the hydrophobic character of the coating due to the addition of lipophilic compounds, such as EOs, might translate into a stronger barrier to moisture [45]. The ability of ECs to reduce weight loss was also reflected by a higher firmness of coated oranges during the first 7 days compared to control samples, while changes became non-significant after 14 days at simulated room temperature. The impact of coatings on fruit firmness depends on the coating composition, the storage conditions, and also the citrus species and cultivar. In general, composite ECs better maintain the firmness of mandarins than that of oranges [31,33].

On the other hand, the internal quality attributes (pH, TA, and SSC) of oranges during 14 days of shelf life at 20 °C was not affected by the application of the different antifungal ECs. Similar results were reported with 'Tomango' oranges coated with a commercial carnauba wax amended with *Mentha spicata* and *Lippia scavenrims* EOs [48] and 'Navel Powell' oranges coated with chitosan enriched with bergamot EO [49]. Similarly, the antifungal ECs developed in this study can be considered as a feasible postharvest treatment also in terms of sensory quality since they did not negatively affect the overall flavor, nor did they induce the development of off-flavors compared to uncoated fruit. Furthermore, some antifungal ECs significantly improved rind gloss, being that EG, the antifungal compound that added to the CP coating, provided the highest gloss, which could be attributable to differences in the optical properties of the coated surface depending on the coating composition.

In a previous study [35], the impact of selected CP-BW-based antifungal ECs on quality attributes of 'Valencia' oranges was evaluated during a long-term cold-storage period of 2 months at 5 °C plus a shelf-life period of 7 days at 20 °C. In accordance with our current results, the EC containing EG effectively reduced weight loss after the storage period. However, the coating containing CM EO also protected fruit against weight loss during cold storage, whereas this effect was not observed in the present study with coated oranges kept at 20 °C. The barrier properties of ECs are affected by environmental conditions [35,50], and therefore, the observed differences could be attributed to different storage temperatures. In addition, in the previous research, CP-BW-based ECs enriched with CI, EG, GR, and CM also maintained the fruit’s sensory and physicochemical quality after long-term cold storage, and the coating containing EG also improved fruit gloss compared to uncoated oranges after cold storage, improving fruit appearance [35].

5. Conclusions

The results of the present study revealed that composite ECs based on CP and BW functionalized by the addition of selected natural antifungal substances, such as EOs, pure volatiles, and plant extracts, could be effective alternatives for the replacement of conventional commercial waxes containing synthetic fungicides for the postharvest preservation of 'Valencia' oranges. Among the different active agents studied, EG, GR, CM EO, and PR extract improved the antifungal properties of CP-BW-based ECs and were able to reduce sour rot incidence and severity on artificially inoculated oranges during shelf life at 20 °C. Considering previous results that also showed the potential of similar antifungal ECs containing EG and GR to reduce green mold caused by *P. digitatum* on cold-stored 'Valencia' oranges, these ECs could be considered as an effective multi-target protection strategy against some of the most important postharvest pathogens of citrus fruit, i.e., *G. citri-aurentii* and *P. digitatum*. Furthermore, CP ECs containing EG, GR, or PR also reduced weight loss and retained firmness during shelf life at 20 °C, while EG also improved fruit gloss. Therefore, the CP-BW-based EC enriched with EG showed the best potential for overall quality preservation of 'Valencia' oranges. Further research should focus on the evaluation of these bio-based antifungal ECs in other relevant citrus species and cultivars at different storage conditions in order to increase their potential for commercial application.

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