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Antifungal Hydroxypropyl Methylcellulose (HPMC)-Lipid Composite Edible Coatings and Modified Atmosphere Packaging (MAP) to Reduce Postharvest Decay and Improve Storability of 'Mollar De Elche' Pomegranates

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Abstract: Pomegranate exhibits important postharvest quality losses that limit its storage potential, caused mainly by weight loss, chilling injury and fungal diseases. In this work, we evaluated the effect of novel hydroxypropyl methylcellulose (HPMC) edible coatings (ECs) formulated with three different lipids (beeswax (BW), carnauba wax, and glycerol monostearate), as hydrophobic components, and two different GRAS salts (potassium bicarbonate (PBC) and sodium benzoate (SB)), as antifungal ingredients, to control weight loss and natural fungal decay of 'Mollar de Elche' pomegranates during storage at 20 °C. Afterwards, selected antifungal ECs and commercial modified atmosphere packaging (MAP) films were assayed alone or in combination to control natural decay and preserve fruit quality of pomegranates stored at 5 °C for 4 months plus 1 week at 20 °C. Results showed that ECs amended with SB reduced pomegranate latent infections caused by *Botrytis cinerea* and wound diseases caused by *Penicillium* spp. Moreover, MAP technologies were confirmed as an efficient mean to preserve freshness, prevent fruit shriveling and rind browning, and reduce fungal decay, thus extending storage life of pomegranates. The combination HPMC-BW-SB + MAP was the most promising treatment as it reduced weight loss and decay, without negatively affecting the fruit physicochemical and sensory quality.

Keywords: GRAS salts; fruit quality; non-polluting postharvest decay control; cold-storage

1. Introduction

The pomegranate (*Punica granatum* L.) belongs to the Lythraceae family and it is widely cultivated worldwide in semi-arid and subtropical regions. Nowadays, in the Mediterranean basin, Spain holds the record for European pomegranate production. More than 90% of Spanish commercial planted area is located in the southeast of the country (Alicante province, Valencia region), where the autochthonous variety 'Mollar de Elche' is the predominant cultivar [1]. This is a late ripening cultivar, harvested from the end of September to the middle of November [2]. Although with lighter external red color, it has soft seeds and outstanding organoleptic properties, such as higher sugar and lower acidity than the well-known worldwide cultivar 'Wonderful'. In recent years, there has been an increasing consumer demand for pomegranate whole fruits, arils, and juice, mainly associated to the high content in phenolic compounds, such as ellagic acid and derivatives, punicalagin, and other hydrolysable tannins, flavanols, and anthocyanins [3,4]. Phenolic compounds of pomegranate account for its high antioxidant activity and several studies

have demonstrated their anti-inflammatory and neuro and hepatoprotective effects, which can contribute to the prevention of cancer and cardiovascular diseases [5].

Pomegranate is a non-climacteric fruit that does not ripen after harvest and, therefore, must be harvested when fully ripe. After harvest, pomegranate fruit exhibits important quality losses that limit its storage potential, caused mainly by weight loss, chilling injury, and decay [6,7]. Several studies with 'Mollar de Elche' and other cultivars recommend storage between 5 and 7 °C and relative humidity (RH) of 90 to 95% to reduce weight loss and avoid chilling injury symptoms such as rind pitting, husk scald, and browning of the arils and internal teguments, among others [8–11]. However, storage under these conditions can favor postharvest decay, especially when the incidence of latent fungal infections at the time of harvest is high or the fruit has small superficial wounds [2]. The most important pathogen causing significant postharvest decay on many pomegranate cultivars worldwide, including 'Mollar de Elche', is *Botrytis cinerea* Pers.: Fr. [2,12–15]. The symptoms of this disease may originate directly from wounds causing gray mold on any part of the fruit, but latent infections in the crown (calyx) are frequently more important. Other relevant fungi attacking the most important pomegranate cultivars are *Alternaria* spp. (the cause of black heart or heart rot), *Aspergillus niger* Tiegh. (the cause of black rot), *Penicillium* spp. (the cause of blue or green mold), *Pilidiella granati* Sacc. (the cause of Pilidiella fruit rot), *Rhizopus* spp. (the cause of soft or watery rot), and *Colletotrichum gloeosporoides* (Penz.) Penz. & Sacc. (the cause of anthracnose) [12,16–18].

Postharvest handling practices to extend the shelf life of pomegranates include cold storage at the optimum temperature, storage under controlled atmosphere (CA) or modified atmosphere packaging (MAP), and the use of synthetic fungicides when allowed by national legislations. In general, MAP is preferred to CA due to its significantly lower implementation and maintenance costs. It has been reported that storage in MAP significantly reduced weight loss, shriveling, and chilling injury of 'Mollar de Elche' [19], 'Primosole' [20], 'Hicrannar' [21], 'Hicaznar' [21,22], and 'Wonderful' [23] pomegranate cultivars. Gas composition recommended for the storage of pomegranate varies depending on fruit cultivar and storage temperature. For instance, for 'Mollar de Elche' pomegranates stored in unperforated polypropylene MAP bags, the best results after 3 months at 2 °C were obtained with 8 kPa O_2 + 10 kPa CO_2 [19], whereas MAP bags creating a steady modified atmosphere of 13.5–17.6 kPa O_2 and 4.4–8.1 kPa CO_2 were suggested to minimize quality losses of 'Hicrannar' and 'Hicaznar' pomegranate cultivars stored at 6 °C for 4 and 7 months, respectively [21,24]. Similarly, Porat et al. [23,25] reported that cold storage of 'Wonderful' pomegranates in MAP using commercial Xtend® bags effectively reduced weight loss and scald incidence and maintained fruit quality for at least 3 months after harvest. However, in many of these studies, storage of pomegranates in MAP bags for longer periods was limited due to enhanced decay development [22,23].

The use of chemical fungicides to control postharvest diseases of pomegranate vary according to national legislations. While they are approved in countries such as USA, Israel, and India, no registered active ingredients are available in the European Union (EU), except for fludioxonil, which received an emergency exception registration for use in 2019 in Spain. This fungicide is a contact fungicide and, therefore, it is recommended to dip the pomegranates in the fungicide solution to kill fungal spores and inactivate latent infections located within the crown of the fruit [15,26]. Although its application is mainly recommended to control gray mold on pomegranate fruit, it can also affect other postharvest diseases caused by *Penicillium* spp., *Alternaria* spp., and *Aspergillus* spp., considerably reducing postharvest pomegranate decay [27]. However, health and environmental issues associated with the use of synthetic fungicides, which generate chemical residues and lead to the proliferation of pathogenic resistant strains, make necessary the search for alternative decay control methods [28]. Among them, the development of novel edible coatings (ECs) with antifungal properties has gained increasing interest in the last years as a sustainable and cost-effective technology to extend shelf life of fresh fruits. These antifungal ECs, in addition to providing antifungal activity, create a semi-permeable barrier against gases and

water vapor, decreasing the respiration rate and moisture loss, thus contributing to reduce fruit weight loss, softening, shriveling, and chilling injury symptoms, and to maintain other quality attributes of coated fruit during storage [29]. These advantages can be achieved using different combinations of hydrophilic (polysaccharides and proteins) and hydrophobic ingredients (lipids) forming composite ECs. The antifungal effect of biopolymer-based coatings is usually achieved by the incorporation of active antimicrobial compounds to the coating [29,30]. Many organic and inorganic salts have antifungal activity and can offer a good alternative to the use of synthetic fungicides. These salts, classified as Generally Recognized as Safe (GRAS) compounds, include carbonates, bicarbonates, sorbates, benzoates, acetates, parabens, and silicates, among others [28]. A considerable number of studies showed that ECs containing GRAS salts significantly controlled major postharvest fungal diseases of different fresh fruits, such as citrus, plums, and tomatoes [31–38]. However, to our knowledge, no studies are available on the application of ECs formulated with GRAS compounds to control pomegranate decay and to preserve fruit quality during cold storage.

The aim of this work was to extend the storage life of 'Mollar de Elche' pomegranates using ECs amended with GRAS salts as antifungal agents and combining them with MAP technology. First, we evaluated the effect of hydroxypropyl methylcellulose (HPMC) ECs formulated with three different lipids (beeswax, carnauba wax, and glycerol monostearate), as hydrophobic components, and two different GRAS salts (potassium bicarbonate and sodium benzoate), as antifungal ingredients, to control weight loss and natural fungal decay during storage at 20 °C. Afterwards, selected antifungal ECs and commercial MAP films were assayed alone or in combination to control natural decay and preserve fruit quality of pomegranates stored at 5 °C and 90% RH for up to 4 months.

2. Materials and Methods

2.1. Fruit

'Mollar de Elche' pomegranates (*Punica granatum* L.) were harvested at commercial maturity in commercial orchards in Elche (Alicante, Spain) and transported the same day to the IVIA CTP facilities. Fruits were selected, randomized, and surface washed with tap water. Pomegranates were allowed to air dry at room temperature and used immediately or stored up to 1 week at 5 °C and 90% RH until the beginning of the experiments.

2.2. Formulation and Preparation of Antifungal Edible Coatings

HPMC-lipid composite ECs were prepared by combining the polymer (HPMC, Methocel E19, Dow Chemical Co., Stade, Germany) with three different lipids: beeswax (BW; Guinama S.L., Valencia, Spain), carnauba wax (CW; Decco Ibérica, Paterna, Spain), or glycerol monostearate (GMS; Italmatch Chemicals Spa, Barcelona, Spain), suspended in water. Glycerol (Panreac Química, S.A., Barcelona, Spain) was used as plasticizer in all formulations. Formulations containing BW and CW were prepared using stearic acid (Panreac Química, S.A., Barcelona, Spain) as emulsifier. In the case of the HPMC-GMS formulation, diacetyl tartaric acid esters of mono-diglycerides (DATEM) and sunflower lecithin (LEC; Lasenor, Barcelona, Spain) were used as emulsifiers. Two different GRAS salts, potassium bicarbonate (PBC; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and sodium benzoate (SB; Guinama S.L.U., Valencia, Spain), were tested as antifungal agents. These salts were selected according to their in vitro activity against the fungus *B. cinerea* [31]. Three drops of a silicone antifoam agent (FG-1510, Dow Corning Ibérica, Barcelona, Spain) were added to reduce foam formation during the emulsification process.

Six different emulsions were prepared. Emulsion components and concentrations are shown in Table 1. All formulations were prepared with 5.6% total solid content and the concentrations of the different ingredients were previously optimized to obtain stable emulsions, with low viscosity values (<40 mPa·s) and good wettability of the fruit (data not shown). Emulsions were prepared as described by Valencia-Chamorro et al. [39]. Briefly, an aqueous solution of HPMC (5% *w*/*w*) was prepared by dispersing the HPMC in hot water at 90 °C and later hydrating at 20 °C. Water, the hydrophobic ingredient (BW, CW, or

GMS), glycerol, and emulsifiers were added to the HPMC solution and heated at 92 °C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax IKA® model T25, IKA-Werke, Staufen, Germany) for 1 min at 12,000 rpm and 3 min at 22,000 rpm. After homogenization, emulsions were cooled for 25 min under agitation to a temperature lower than 25 °C by placing them in an ice bath. Viscosity and pH values of the emulsions were determined using a viscometer (Visco Star Plus R, Fungilab, S.A., Barcelona, Spain) and a pH-meter (Consort C830 Multi-Parameter Analyzer, Turnhout, Belgium), respectively. Stability was also determined according to the method described by Valencia-Chamorro et al. [39]. In brief, the emulsions were placed in volumetric tubes and phase separation was assessed after 48 h at 25 °C.

Table 1. Composition of hydroxypropyl methylcellulose (HPMC)-lipid composite edible coatings containing antifungal food preservatives (concentration, % wet basis, wb).

Coating	Biopolymer	Lipid			Plasticizer	Emulsifier		GRAS Salt	
	HPMC	BW	CW	GMS	Gly	SA	DATEM + LEC	PBC	SB
HPMC-BW-PBC	2	0.8	-	-	0.4	0.4	-	2	-
HPMC-BW-SB	2	0.8	-	-	0.4	0.4	-	-	2
HPMC-CW-PBC	2	-	0.8	-	0.4	0.4	-	2	-
HPMC-CW-SB	2	-	0.8	-	0.4	0.4	-	-	2
HPMC-GMS-PBC	2	-	-	0.44	0.3	-	0.43 + 0.43	2	-
HPMC-GMS-SB	2	-	-	0.44	0.3	-	0.43 + 0.43	-	2

BW: beeswax; CW: carnauba wax; GMS: glycerol monostearate; Gly: Glycerol; SA: stearic acid; DATEM: diacetyl tartaric acid esters of mono-diglycerides; LEC: lecithin; PBC: potassium bicarbonate; SB: sodium benzoate.

2.3. Experiment I. Effect of Fruit Coating on Weight Loss and Pomegranate Decay during Storage at 20 °C

A first experiment was conducted to test the effect of the six different antifungal ECs on pomegranate weight loss and natural fungal decay during 2 weeks of storage at 20 °C. Fruits were randomly divided into seven batches, which corresponded to the following treatments: (1) uncoated control (CONTROL), (2) HPMC-BW-PBC, (3) HPMC-BW-SB, (4) HPMC-CW-PBC, (5) HPMC-CW-SB, (6) HPMC-GMS-PBC, and (7) HPMC-GMS-SB (Table 1). Each treatment was applied to 4 replicates of 10 fruits each. Fruits were manually immersed for about 30 s in 1000 mL beakers containing the corresponding coating emulsion at room temperature. Control fruits were dipped for 30 s in tap water at 20 °C. Treated pomegranates were allowed to air-dry at room temperature, arranged on plastic cavity sockets on corrugated carton boxes and stored at 20 °C and 90% RH. Weight loss was evaluated as described below on 20 fruits per treatment after 7 and 14 days of storage at 20 °C. External natural disease incidence and severity caused by latent (crown decay) and wound pathogens (wound decay) were determined on 4 replicates of 10 fruits each per treatment as explained below. These evaluations were conducted weekly for up to 8 weeks.

2.4. Experiment II. Effect of Coatings and MAP on Decay and Quality of Cold-Stored Pomegranates

Selected ECs from Experiment I were applied alone or in combination with MAP to evaluate the effect on pomegranate decay and overall fruit quality during 8 and 15 weeks of cold storage at 5 °C and 90% RH, followed by a shelf life period of 1 week at 20 °C. Seven different treatments were applied: (1) uncoated control fruit (CONTROL), (2) EC1 = HPMC-CW-SB, (3) EC2 = HPMC-BW-SB, (4) EC1 + MAP, (5) EC2 + MAP, (6) Uncoated + MAP, and (7) positive control, fungicide fludioxonil (Scholar® 230 SC, 23% (*w*/*v*) active ingredient (a.i.); Syngenta Agro S.A., Madrid, Spain). Pomegranates were coated or dipped in water (uncoated control) as described above. Fludioxonil was applied at a concentration of 0.6 g a.i./L by completely immersing the pomegranates in the fungicide solution for 1 min. Pomegranates were allowed to air-dry at room temperature and distributed into packing plastic cavity sockets that prevented the contact between fruits. Pomegranates undergoing

MAP were tightly packaged in 5-kg commercial bags (Xtend® 815-PG28/m, Patent No.: 6190710; StePac L.A. Ltd., Tefen, Israel) with an average weight of 5.02 ± 0.14 kg. The 5 kg Xtend® 815-PG28/m (730 mm width, 610 mm length, and 20 µm thickness) is a polyamide based transparent flexible packaging bag specifically designed for pomegranates and characterized by high clarity (>97%), antifog properties (haze < 8%), and good printability. Each treatment was applied to 3 replicates of 16 fruits each. Fruits were stored for 2 and 4 months at 5 °C, followed by 1 more week of shelf life at 20 °C. For MAP treatments, commercial plastic bags were removed after cold storage, just before the beginning of the shelf-life simulation period at 20 °C.

2.5. Assessment of Fruit Quality

Weight loss was determined in both Experiment I and Experiment II. The rest of quality attributes were only determined in Experiment II (after cold storage and shelf life).

2.5.1. Weight Loss

Twenty pomegranates per treatment in Experiment I and 32 fruits per treatment in Experiment II were individually weighted with a calibrated analytical balance at the beginning and at the end of the correspondent storage and shelf-life periods. Results were expressed as the percentage loss of initial weight by using the following formula: % WL = [(Wi − Wf)/Wi] × (100), where % WL = percentage of weight loss, Wi = initial fruit weight (g), and Wf = final fruit weight (g).

2.5.2. Headspace Gas Concentration within MAP Bags

CO_2 an O_2 concentrations inside the package bag were monitored every 4 weeks during cold storage using a O_2/CO_2 analyzer (Systech Instruments Gaspace Advance, GS3M/P, Thame, UK). Sampling of the gases within the package was carried out using a syringe through a septum attached to the film to prevent the entry of air during the measurement. Results were given as percentage of O_2 and CO_2.

2.5.3. Rind Color

External rind color was assessed in 20 fruits per treatment on three equidistant points of the equatorial region using a colorimeter (Chroma Meter CR-400, Minolta, Osaka, Japan). Color was evaluated according to the Commission Internationale de l'Eclairage (CIE) and expressed as lightness (L*), chroma (C*, saturation), hue angle (h°) and the parameters a* and b*. Sixty measurements per treatment were used for color evaluation.

2.5.4. Juice Quality

Arils from 3 replicates of 5 fruits each were homogenized in a commercial blender (Sammic S.L., LI-240, Azkoitia, Spain) to extract the juice. Titratable acidity (TA) and pH were determined with an automatic titrator (Titrator T50, Mettler Toledo, Switzerland) in 5 mL juice samples. TA was calculated to an end point of pH 8.1 and results expressed as percentage of citric acid. The soluble solids concentration (SSC) of the juice was measured using a digital refractometer (model ATC-1, Atago® Co., LTD, Tokyo, Japan) and values were expressed as ° Brix. The maturity index (MI) was calculated as the SSC/TA ratio.

2.5.5. Ethanol and Acetaldehyde Content

These volatile compounds were analyzed from the headspace of pomegranate juice samples using a gas chromatograph (GC) (Thermo Trace, Thermo Finnigan, KAV00106, Milan, Italy) equipped with a flame ionization detector (FID) and a 1.2 × 0.32 cm (i.d.) Poropack QS 80/100 column. The injector, column, and detector temperatures were set at 175, 150, and 200 °C, respectively. Three replicates of 5 fruits each per treatment were analyzed. Samples of 5 mL of juice were placed in 10 mL vials with crimp-top caps and TFE/silicone septum seals. Samples were frozen and stored at −18 °C until the analyses were performed. For the analysis, samples were equilibrated in a water bath at 20 °C for

1 h, followed by 15 min at 60 °C to reach equilibrium in the headspace, and then 1 mL gas was injected into the GC. Results were expressed as mg of acetaldehyde or ethanol per 100 mL of juice.

2.5.6. Physiological Disorders

Pomegranates were visually inspected for external physiological disorders (pitting, husk-scald, and rind sinking) according to the following scale: 0 = none; 1 = slight (<25% of the rind); 2 = moderate (25–50% of the rind); 3 = severe (>50% of the rind) [40]. Following external evaluation, each pomegranate was cut in half along the equator and symptoms of internal physiological disorders (browning of arils, teguments and/or membranes, and paleness of the arils) were assessed in non-decayed fruits using a qualitative scale [40] in which 0 = none, 1 = slight, 2 = moderate symptoms, and 3 = severe symptoms. Evaluations were done at the end of cold storage (external evaluation) and shelf-life periods (external and internal evaluations). Results were given as an average index considering all the fruits in each treatment.

2.5.7. Sensory Evaluation

The flavor of arils and the external aspect of treated fruits were evaluated at the end of each storage period by a panel of 15 trained judges. Six fruits per treatment were peeled and arils were pooled together. Samples were served in cups coded with three-digit random numbers at room temperature and judges were provided with spring water for palate rinsing between samples. Flavor was rated on a 9-point scale where 1 = lowest quality and 9 = highest quality. The presence of off-flavors due to the accumulation of volatiles associated to anaerobic respiration was evaluated according to a 5-point intensity scale where: 1 = absence of off-flavor and 5 very pronounced presence of off-flavor [38]. For external visual aspect, 4 intact fruits per treatment were placed in trays labeled with 3-digit random codes and presented to the panelists to evaluate the appearance according to the following scale: 1 = bad, 2 = acceptable, and 3 = good. All these evaluations were carried out at the sensory laboratory of the IVIA CTP that meets the European Union (UNE87006:1993) standards for this purpose.

2.6. Assessment of Fruit External and Internal Decay

Two different types of natural external decay were considered in Experiment I: crown decay, caused by *B. cinerea* or other latent pathogens established within the fruit calyx (crown) during fruit development in the tree, and wound decay, caused by fungal pathogens developing in rind wounds located at any part of the fruit surface. In contrast, overall external decay and internal decay (assessed after cutting the fruit into two halves) were considered in Experiment II.

In any case, disease incidence, was expressed as the percentage of fruit showing apparent decay symptoms. Crown decay severity was scored in each fruit according to the following qualitative scale: 0 = no lesion or fungal mycelium present in the crown, 1 = mycelium present in the crown, 2 = crown lesion $\leq$ 25% of rind surface, 3 = crown lesion on 26–50% of rind surface, and 4 = crown lesion > 50% of rind surface [41]. Severity of wound decay or overall external decay was assessed using the following similar scale: 0 = no decay, 1 = decay spots < 1 cm^2; 2 = 1 cm^2 < decay lesion < 25% of rind surface, 3 = decay lesion on 26–50% of rind surface, and 4 = decay lesion > 50% of rind surface [41]. Internal decay severity was assessed using this simpler scale: 0 = no decay, 1 = slight decay, 2 = moderate decay, and 3 = severe decay [40]. In all cases, a disease severity index was calculated as: [Σ (number of fruits in each scale category $\times$ score index)]/[total number of fruits] and expressed as an average severity index of the replicates. The number of decayed fruits showing visible fungal spores were also determined.

In Experiment I, external crown and wound decay were determined weekly on 4 replicates of 10 fruits each per treatment for up to 8 weeks of storage at 20 °C. Results are presented after 4 and 8 weeks. In Experiment II, external and internal decay were deter-

mined in 4 replicates of 8 fruits each per treatment after 8 and 15 weeks of cold storage at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C.

2.7. Statistical Analysis

Statistical analyses were performed using the software Statgraphics Centurion XVII (Statgraphics Technologies Inc., The Plains, VA, USA). Differences among means were determined by Fisher's Protected Least Significant Difference test (LSD, $p < 0.05$), applied after an analysis of variance (ANOVA). For disease incidence, data was transformed to the arcsine of the square root of the proportion of decayed fruit to assure the homogeneity of the variance. Non-transformed means are shown.

3. Results

3.1. Experiment I. Effect of Fruit Coating on Weight Loss and Pomegranate Decay during Storage at 20 °C

Figure 1 shows weight loss of uncoated (control) and coated 'Mollar de Elche' pomegranates after 7 and 14 days of storage at 20 °C. Weight loss was in the range of 1.3–1.7% and 2.3–3.3% after 7 and 14 days, respectively. Overall, the most effective coating in reducing weight loss of pomegranates in comparison to uncoated control fruits was HPMC-CW-SB, followed by HPMC-BW-SB ($p < 0.05$). On the other hand, the coatings containing PBC as antifungal agent did not control weight loss of coated pomegranates. Therefore, HPMC-CW-SB and HPMC-BW-SB were selected to be tested under commercial cold storage conditions in Experiment II.

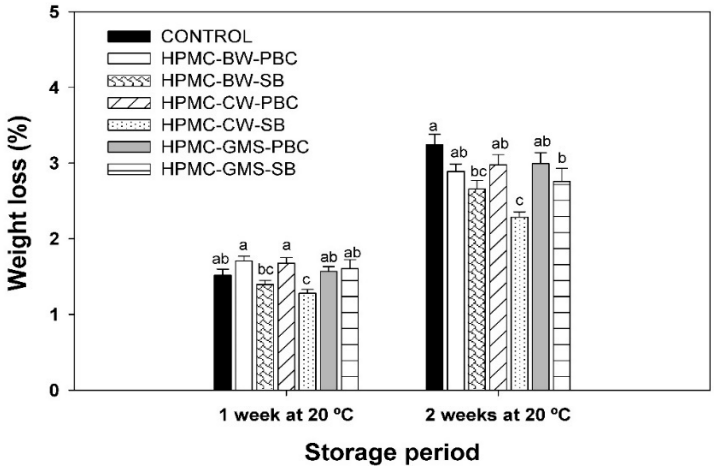

Figure 1. Weight loss of 'Mollar de Elche' pomegranates uncoated (CONTROL) or coated with hydroxypropyl methylcellulose (HPMC)-lipid composite edible coatings amended with GRAS salts and stored at 20 °C and 90% RH for 7 and 14 days. Lipids tested: beeswax (BW), carnauba wax (CW), and glycerol monostearate (GMS). GRAS salts tested: potassium bicarbonate (PBC) and sodium benzoate (SB). For each storage period, columns with different letters indicate significant differences among treatments according to Fisher´s protected LSD test ($p < 0.05$) applied after an ANOVA. Error bars show standard error (n = 20).

Table 2 shows the incidence percentage and severity index of natural disease caused by latent (crown decay) and wound (wound decay) pathogens on pomegranates stored for 4 and 8 weeks at 20 °C. Incidence of latent pathogens in the crown after 4 weeks was low (0–5%), with severity indexes between 0 and 2 (crown lesion < 25% of rind surface), and no significant differences were found among treatments ($p > 0.05$). After 8 weeks of storage at 20 °C, crown decay incidence increased to values that ranged from 7.50 to 37% ($p < 0.05$) and severity index from 2 to 3 ($p > 0.05$). Pomegranates coated with HPMC-GMS-SB showed the lowest percentage of crown decay (7.5%), which was significantly lower than the incidence on uncoated control fruits (35%). Similarly, the incidence of crown

decay on pomegranates coated with HPMC-CW-SB and HPMC-BW-SB was significantly lower than that on uncoated control fruits, whereas coatings formulated with PBC did not reduced crown decay incidence compared to control samples. Nevertheless, no significant differences were found in the severity index of crown decay. On the other hand, wound decay incidence after 4 weeks was in the range of 2.5–20%, with severity indexes between 0 and 2, and no significant differences were found among treatments ($p > 0.05$). After 8 weeks, wound decay incidence increased up to 40% in the uncoated control, with a severity index around 3. Overall, none of the coatings significantly reduced wound decay incidence and severity compared to the control. Main fungi causing latent infections in the crown of 'Mollar de Elche' pomegranates stored at 20 °C were *B. cinerea* (causing gray mold) and *P. granati* (causing Pilidiella fruit rot). The most frequent wound pathogens causing external surface decay on fruit were *A. niger* (causing black rot) and *Penicillium* spp. (causing blue/green mold).

Table 2. External decay caused by latent (crown) and wound pathogens on 'Mollar de Elche' pomegranates treated with antifungal edible coatings and stored for 4 and 8 weeks at 20 °C and 90% RH.

External Decay	Treatment [3]	Storage Period			
		4 Weeks		8 Weeks	
		% Incidence	Severity Index (0–4)	% Incidence	Severity Index (0–4)
Crown Decay [1]	CONTROL	5.00 ± 2.89 a	2.00 ± 1.15 a	35.00 ± 6.45 a	3.13 ± 0.13 a
	HPMC-BW-PBC	5.00 ± 2.89 a	1.75 ± 1.03 a	37.50 ± 7.50 a	3.38 ± 0.36 a
	HPMC-BW-SB	2.50 ± 2.50 a	0.75 ± 0.75 a	15.00 ± 6.45 bc	1.75 ± 0.63 a
	HPMC-CW-PBC	0.00 ± 0.00 a	0.00 ± 0.00 a	15.00 ± 5.00 abc	2.83 ± 0.44 a
	HPMC-CW-SB	5.00 ± 2.89 a	1.50 ± 0.87 a	12.50 ± 4.79 bc	2.63 ± 0.94 a
	HPMC-GMS-PBC	2.50 ± 2.50 a	0.50 ± 0.50 a	25.00 ± 6.45 ab	2.48 ± 0.22 a
	HPMC-GMS-SB	2.50 ± 2.50 a	1.00 ± 1.00 a	7.50 ± 4.79 c	2.00 ± 1.15 a
Wound Decay [2]	CONTROL	15.00 ± 6.45 a	2.38 ± 0.80 a	40.00 ± 12.25 a	2.70 ± 0.57 a
	HPMC-BW-PBC	5.00 ± 5.00 a	0.63 ± 0.63 a	27.5.0 ± 6.29 a	2.81 ± 0.64 a
	HPMC-BW-SB	20.00 ± 8.16 a	1.31 ± 0.45 a	35.00 ± 6.45 a	2.55 ± 0.12 a
	HPMC-CW-PBC	2.50 ± 2.50 a	0.75 ± 0.75 a	12.50 ± 4.79 a	2.38 ± 0.85 a
	HPMC-CW-SB	20.00 ± 4.08 a	1.71 ± 0.29 a	35.00 ± 6.45 a	3.04 ± 0.38 a
	HPMC-GMS-PBC	10.00 ± 4.08 a	1.38 ± 0.47 a	22.50 ± 4.79 a	2.67 ± 0.58 a
	HPMC-GMS-SB	12.50 ± 7.50 a	0.96 ± 0.60 a	37.50 ± 7.50 a	2.37 ± 0.32 a

Mean ± standard error (SE) (n = 4). [1] Crown decay severity index: 0 = no lesion or fungal mycelium present in the crown; 1 = mycelium present in the crown; 2 = crown lesion ≤ 25% of rind surface; 3 = crown lesion on 26–50% of rind surface; and 4 = crown lesion > 50% of rind surface. [2] Wound decay severity index: 0 = no decay; 1 = decay lesion < 1 cm^2; 2 = 1 cm^2 < decay lesion < 25% of rind surface; 3 = decay lesion on 26–50% of rind surface; and 4 = decay lesion > 50% of rind surface. [3] CONTROL: Uncoated; HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; GMS: glycerol monostearate; PBC: potassium bicarbonate; SB: sodium benzoate. For each type of decay and storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

3.2. Experiment II. Effect of Coating and MAP on Decay and Quality of Cold-Stored Pomegranates

3.2.1. Weight Loss

Figure 2 shows weight loss of uncoated (control) and coated pomegranates for air and MAP storage after 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C. Pomegranates stored in MAP showed the lowest weight loss and at the end of the storage period (15 weeks at 5 °C + 1 week at 20 °C) values ranged between 2.6 and 4.2%, and the combination of HPMC-CW-SB + MAP was the most effective treatment to reduce weight loss, while unwrapped samples reached weight loss values of 11–13%. On the other hand, after 8 weeks at 5 °C plus the shelf life period, the application of the HPMC-CW-SB and HPMC-BW-SB coatings reduced slightly but significantly weight loss of pomegranates with respect to control fruits. However, these treatments did not control weight loss of pomegranates after 15 weeks at 5 °C plus 1 week at 20 °C. Fludioxonil treatment significantly reduced weight loss compared to control fruits at the end of both

shelf-life periods, but weight loss values were quite high in comparison to those observed with MAP treatments.

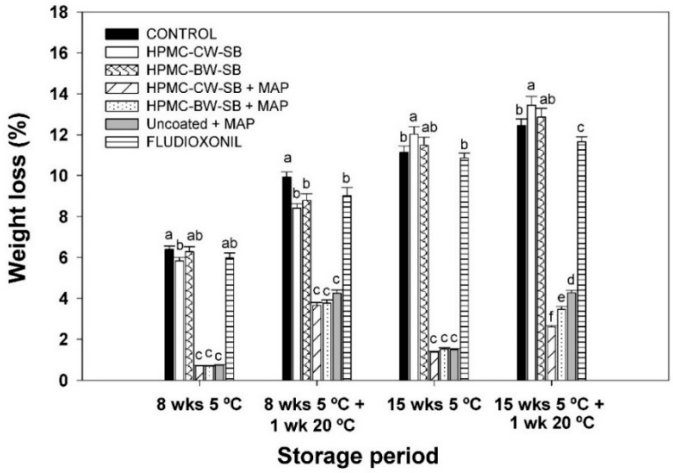

Figure 2. Weight loss of 'Mollar de Elche' pomegranates coated with hydroxypropyl methylcellulose (HPMC)-lipid antifungal edible coatings alone or in combination with modified atmosphere packaging (MAP) for 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C. CONTROL: uncoated fruits dipped in tap water for 60 s. Lipids tested: beeswax (BW) and carnauba wax (CW). Antifungal: sodium benzoate (SB). For each storage period, columns with different letters indicate significant differences among treatments according to Fisher´s protected LSD test ($p < 0.05$) applied after an ANOVA. Error bars show standard error (n = 32) gas composition within MAP bags.

Variations of O_2 and CO_2 concentrations inside the MAP bags are presented in Table 3. The levels of O_2 and CO_2 were maintained fairly constant throughout storage time at 5 °C ($p > 0.05$), with O_2 and CO_2 concentrations in the range of 18.2–19.53 kPa and 2.63–4.67 kPa, respectively. Similarly, no significant differences were observed among treatments for each storage period.

Table 3. O_2 and CO_2 concentrations (kPa) in the headspace of modified atmosphere packaging (MAP) bags of coated and uncoated 'Mollar de Elche' pomegranates during storage at 5 °C and 90% RH.

Treatment	Storage Time					
	30 Days		60 Days		90 Days	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
HPMC-CW-SB + MAP	18.43 a	3.63 a	18.44 a	4.50 a	18.93 a	3.37 a
HPMC-BW-SB + MAP	19.53 a	2.63 a	19.50 a	3.02 a	19.40 a	2.87 a
Uncoated + MAP	18.20 a	4.00 a	18.07 a	4.67 a	18.97 a	3.33 a

HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; SB: sodium benzoate. For each gas and storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

3.2.2. Rind Color

In general, the a* and h° values of pomegranate rind decreased and increased, respectively, with storage time, while C* remained almost unalterable (Table 4). After 8 weeks at 5 °C plus 1 week at 20 °C, fruits from all the treatments, except HPMC-CW-SB, maintained higher lightness values (L*) than control samples (uncoated and unpacked) ($p < 0.05$), whereas no significant differences were found among treatments for a* and h° values. After 15 weeks at 5 °C plus 1 week at 20 °C, only fruits from the treatments HPMC-BW-SB + MAP, uncoated + MAP, and fludioxonil showed significant differences with control samples for all the color parameters, with higher L* and h° values, and lower a*, b* and C* values ($p < 0.05$).

Table 4. Rind color (CIELab parameters) of 'Mollar de Elche' pomegranates treated with antifungal edible coatings and stored in modified atmosphere package (MAP) for 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C.

Storage Time	Treatment	Rind Color				
		L*	a*	b*	C*	h°
8 weeks at 5 °C + 1 week at 20 °C	CONTROL	65.83 ± 1.34 c	15.40 ± 1.12 a	37.95 ± 0.53 cd	41.65 ± 0.36 bc	66.92 ± 1.84 a
	HPMC-CW-SB	68.07 ± 1.33 bc	14.69 ± 1.24 a	40.58 ± 0.31 a	43.41 ± 1.05 a	70.26 ± 1.63 a
	HPMC-BW-SB	69.78 ± 1.25 ab	12.64 ± 1.08 a	40.42 ± 0.32 ab	42.76 ± 0.25 ab	72.86 ± 1.47 a
	HPMC-CW-SB + MAP	69.85 ± 0.97 ab	12.99 ± 0.88 a	39.12 ± 0.28 bc	41.47 ± 0.38 c	71.83 ± 1.17 a
	HPMC-BW-SB + MAP	71.47 ± 1.25 ab	10.41 ± 0.96 a	37.49 ± 0.53 d	39.65 ± 0.41 d	74.54 ± 1.41 a
	Uncoated + MAP	71.78 ± 1.35 a	11.77 ± 1.21 a	39.06 ± 0.33 bc	41.32 ± 0.29 c	73.42 ± 1.70 a
	FLUDIOXONIL	69.53 ± 1.29 ab	14.10 ± 1.27 a	38.82 ± 0.38 cd	41.55 ± 0.32 c	70.04 ± 1.83 a
15 weeks at 5 °C + 1 week at 20 °C	CONTROL	69.65 ± 1.11 c	10.87 ± 0.65 a	41.33 ± 0.42 ab	43.45 ± 0.26 a	75.33 ± 0.90 c
	HPMC-CW-SB	71.22 ± 0.82 bc	11.04 ± 0.98 a	41.87 ± 0.29 a	43.62 ± 0.23 a	75.31 ± 1.30 c
	HPMC-BW-SB	71.49 ± 0.72 bc	9.98 ± 0.57 ab	41.95 ± 0.42 a	43.43 ± 0.37 a	76.71 ± 0.76 bc
	HPMC-CW-SB + MAP	70.91 ± 0.70 c	10.02 ± 0.48 ab	41.09 ± 0.34 ab	43.05 ± 0.27 a	75.86 ± 0.77 bc
	HPMC-BW-SB + MAP	73.31 ± 0.55 a	7.92 ± 0.37 bc	40.34 ± 0.33 bc	41.23 ± 0.36 b	78.54 ± 0.57 ab
	Uncoated + MAP	73.15 ± 0.64 a	7.22 ± 0.40 c	39.31 ±0.34 c	40.51 ± 0.28 b	79.62 ± 0.58 a
	FLUDIOXONIL	73.25 ± 0.43 a	7.97 ± 0.45 bc	39.18 ± 0.57 c	40.54 ± 0.39 b	79.98 ± 0.57 a

Mean ± standard error (SE) (n = 20). CONTROL: uncoated; HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; SB: sodium benzoate. For each storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

3.2.3. Juice Quality and Ethanol and Acetaldehyde Content

Juice SSC and TA decreased and pH and MI increased after 8 weeks of cold storage at 5 °C and 1 week of shelf life compared to values at harvest (Table 5). However, in general, these values remained almost constant until the end of the cold storage period. Thus, TA values decreased from 0.23 at harvest to 0.16–0.18 after 15 weeks at 5 °C plus 1 week at 20 °C, and MI increased from 69.13 at harvest to 84–92 at the end of the storage period. Significant differences among treatments in SSC, pH, and MI were only observed after 8 weeks at 5 °C plus 1 week at 20 °C, with pomegranates treated with HPMC-BW-SB and HPMC-CW-SB + MAP having significantly higher SSC and MI than control fruits. However, after 15 weeks, no significant differences were found among treatments in any of the juice quality parameters.

Table 5. Juice quality of 'Mollar de Elche' pomegranates at harvest and after treatment with antifungal edible coatings and/or modified atmosphere package (MAP), followed by storage at 5 °C and 90% RH plus a shelf-life period of 1 week at 20 °C.

Storage Time	Treatments	Juice Quality					
		SSC (° Brix)	TA (% Citric Acid)	pH	MI	Acetaldehyde (mg/100 mL)	Ethanol (mg/100 mL)
At harvest	-	15.90 ± 0.03	0.23 ± 0.01	4.16 ± 0.05	69.13 ± 0.58	0.18 ± 0.03	0.52 ± 0.10
8 weeks at 5 °C + 1 week at 20 °C	CONTROL	15.15 ± 0.10 b	0.18 ± 0.00 b	5.10 ± 0.02 a	82.88 ± 0.95 b	0.23 ± 0.02 a	1.69 ± 0.25 c
	HPMC-CW-SB	15.28 ± 0.12 b	0.18 ± 0.00 b	5.10 ± 0.04 a	83.03 ± 0.43 b	0.27 ± 0.02 a	1.63 ± 0.31 c
	HPMC-BW-SB	15.92 ± 0.22 ab	0.18 ± 0.00 b	5.08 ± 0.03 a	90.58 ± 2.63 a	0.21 ± 0.02 a	0.85 ± 0.34 c
	HPMC-CW-SB + MAP	16.42 ± 0.41 a	0.18 ± 0.00 b	5.01 ± 0.04 ab	90.82 ± 1.95 a	0.09 ± 0.04 b	4.33 ± 0.79 a
	HPMC-BW-SB + MAP	15.15 ± 0.13 b	0.18 ± 0.00 b	4.93 ± 0.01 bc	84.36 ± 2.59 ab	0.23 ± 0.02 a	3.22 ± 1.05 ab
	Uncoated + MAP	15.77 ± 0.47 ab	0.20 ± 0.01 a	4.87 ± 0.04 cd	80.40 ± 3.92 b	0.21 ± 0.02 a	1.83 ± 0.10 bc
	FLUDIOXONIL	15.52 ± 0.13 b	0.20 ± 0.00 a	4.79 ± 0.01 d	78.60 ± 0.73 b	0.23 ± 0.02 a	1.44 ± 0.11 c
15 weeks at 5 °C + 1 week at 20 °C	CONTROL	15.07 ± 0.25 a	0.17 ± 0.01 a	4.71 ± 0.02 a	86.95 ± 4.17 a	3.06 ± 0.12 cd	27.37 ± 0.85 bc
	HPMC-CW-SB	15.10 ± 0.05 a	0.17 ± 0.01 a	4.80 ± 0.05 a	90.99 ± 2.43 a	3.88 ± 0.06 ab	26.22 ± 0.28 cd
	HPMC-BW-SB	15.17 ± 0.07 a	0.18 ± 0.01 a	4.71 ± 0.09 a	84.48 ± 2.92 a	4.28 ± 0.35 a	30.55 ± 1.25 b
	HPMC-CW-SB + MAP	15.33 ± 0.12 a	0.16 ± 0.01 a	4.87 ± 0.03 a	93.58 ± 3.54 a	2.77 ± 0.23 cd	23.24 ± 1.77 de
	HPMC-BW-SB + MAP	14.67 ± 0.32 a	0.16 ± 0.01 a	4.91 ± 0.06 a	92.85 ± 3.36 a	3.45 ± 0.23 bc	21.31 ± 0.83 e
	Uncoated + MAP	15.23 ± 0.27 a	0.16 ± 0.00 a	4.75 ± 0.08 a	92.76 ± 2.12 a	2.53 ± 0.07 d	29.74 ± 3.04 bc
	FLUDIOXONIL	15.07 ± 0.13 a	0.17 ± 0.00 a	4.86 ± 0.05 a	89.37 ± 2.76 a	3.81 ± 0.38 ab	35.61 ± 0.92 a

Mean ± standard error (SE) (n = 3). SSC: Soluble solid content; TA: Titratable acidity; MI: Maturity index. CONTROL: uncoated; HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; SB: sodium benzoate. For each storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

The concentrations of acetaldehyde and ethanol in the juice increased with storage time from 0.18 and 0.52 mg/100 mL at harvest to 2.77–4.49 and 21.31–35.61 mg/100 mL at the end of the storage period, respectively (Table 5). At the end of the experiment (15 weeks of cold storage plus shelf life), only fludioxonil-treated pomegranates had significantly higher ethanol and acetaldehyde content than the uncoated control fruits.

3.2.4. External and Internal Physiological Disorders

External and internal physiological disorders of 'Mollar de Elche' pomegranates after cold storage and shelf life are presented in Table 6. Fruit rind sinking of pomegranates packed under MAP showed significantly lower values than control and unpacked samples at the end of both shelf-life periods ($p < 0.05$). Thus, at the end of the storage period (15 weeks at 5 °C + 1 week at 20 °C), the fruits stored under MAP conditions were evaluated with a slight rind sinking index (values around 1), whereas fruits from the rest of the treatments were assigned a moderate index (values around 2). Physiological disorders due to chilling injury were nil or very low for all treatments after 8 weeks of storage, without significant differences. However, after 15 weeks plus shelf-life period, severity indexes for these disorders increased, with values of 0.7–1.6 for external pitting, 1.6–2.0 for pitting of teguments, and 0.4–1.1 for browning of arils. Overall, pomegranates from the treatments HPMC-CW-SB + MAP and uncoated + MAP showed significantly lower external pitting and browning of arils than control fruits, whereas no significant differences were found among treatments for pitting of teguments.

Table 6. External and internal physiological disorders of 'Mollar de Elche' pomegranates treated with antifungal edible coatings and/or stored in modified atmosphere package (MAP) for 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C.

Storage Time	Treatments [1]	Rind Sinking [2]	External Pitting/Browning [2]	Pitting of Teguments [3]	Browning of Arils [3]
		Severity (0–3)	Severity (0–3)	Severity (0–3)	Severity (0–3)
8 weeks at 5 °C + 1 week at 20 °C	CONTROL	1.23 ± 0.08 a	0.83 ± 0.08 a	0.00 ± 0.00 a	0.08 ± 0.02 a
	HPMC-CW-SB	1.08 ± 0.33 a	0.85 ± 0.31 a	0.04 ± 0.04 a	0.02 ± 0.02 a
	HPMC-BW-SB	1.10 ± 0.13 a	0.48 ± 0.06 a	0.00 ± 0.00 a	0.08 ± 0.02 a
	HPMC-CW-SB + MAP	0.29 ± 0.10 b	0.35 ± 0.09 a	0.04 ± 0.04 a	0.13 ± 0.10 a
	HPMC-BW-SB + MAP	0.47 ± 0.08 b	0.90 ± 0.08 a	0.19 ± 0.04 a	0.08 ± 0.02 a
	Uncoated + MAP	0.42 ± 0.13 b	0.31 ± 0.16 a	0.10 ± 0.02 a	0.08 ± 0.04 a
	FLUDIOXONIL	1.09 ± 0.16 a	0.88 ± 0.18 a	0.10 ± 0.08 a	0.13 ± 0.00 a
15 weeks at 5 °C + 1 week at 20 °C	CONTROL	2.00 ± 0.23 a	1.52 ± 0.06 a	2.02 ± 0.13 a	1.08 ± 0.18 a
	HPMC-CW-SB	1.96 ± 0.11 a	1.54 ± 0.08 a	1.81 ± 0.07 a	1.15 ± 0.25 a
	HPMC-BW-SB	1.96 ± 0.09 a	1.54 ± 0.12 a	1.90 ± 0.15 a	0.77 ± 0.11 abc
	HPMC-CW-SB + MAP	0.98 ± 0.02 b	0.94 ± 0.13 b	1.67 ± 0.11 a	0.48 ± 0.08 bc
	HPMC-BW-SB + MAP	1.19 ± 0.10 b	1.65 ± 0.13 a	1.88 ± 0.10 a	0.81 ± 0.06 ab
	Uncoated + MAP	1.17 ± 0.15 b	0.73 ± 0.17 b	1.56 ± 0.04 a	0.38 ± 0.11 c
	FLUDIOXONIL	1.96 ± 0.06 a	1.54 ± 0.06 a	1.94 ± 0.00 a	0.94 ± 0.04 a

Mean ± standard error (SE) (n = 3). [1] CONTROL: uncoated. HPMC: hydroxypropyl methylcellulose. BW: beeswax, CW: carnauba wax. SB: sodium benzoate. [2] Rind sinking and pitting/browning severity index: 0 = none; 1 = slight (< 25% of the rind); 2 = moderate (25–50% of the rind); 3 = severe (> 50% of the rind). [3] Pitting of teguments and browning of arils severity index: 0 = none, 1 = slight, 2 = moderate, and 3 = severe. For each storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

3.2.5. Sensory Evaluation

Flavor of 'Mollar de Elche' pomegranates was not affected by the different treatments after the first storage period of 8 weeks at 5 °C plus shelf life at 20 °C (Table 7), and was considered as good with an average score value around 7. After 15 weeks of cold storage, flavor score significantly decreased to values below 6, and the pomegranates coated with HPMC-CW-SB received the lowest score, although it was not significantly different than that of control fruits. Off-flavor index increased from values around 1

(absence) to values around 2 (very slight) as cold storage increased from 8 to 15 weeks. However, not significant differences were observed among treatments. Fruit external aspect was evaluated according to dehydration, external physiological disorders, the presence or absence of cracks, blemishes, strains, and homogeneity of the coating. Overall, the appearance of coated pomegranates was not optimal after both storage periods. The worst results were obtained with the treatment HPMC-CW-SB, after both 8 and 15 weeks; whereas the best aspect was that of uncoated pomegranates packed in MAP (Uncoated + MAP), which were scored as good (3) and fair-good (2.57) after 8 and 15 weeks of cold storage plus shelf life, respectively.

Table 7. Flavor, off flavors, and visual quality of 'Mollar de Elche' pomegranates coated with antifungal edible coatings and/or stored in modified atmosphere packaging (MAP) for 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C.

Storage Time	Treatments [1]	Sensory Analysis		
		Flavor [2] (1–9 Scale)	Off Flavor [3] (1–5 Scale)	Visual Quality [4] (1–3 Scale)
8 weeks at 5 °C + 1 week at 20 °C	CONTROL	6.38 ± 0.27 a	1.27 ± 0.12 a	1.27 ± 0.15 d
	HPMC-CW-SB	7.15 ± 0.32 a	1.15 ± 0.10 a	1.27 ± 0.12 d
	HPMC-BW-SB	7.62 ± 0.24 a	1.13 ± 0.09 a	1.75 ± 0.23 c
	HPMC-CW-SB + MAP	7.29 ± 0.38 a	1.20 ± 0.11 a	2.40 ± 0.25 b
	HPMC-BW-SB + MAP	7.64 ± 0.47 a	1.27 ± 0.12 a	1.67 ± 0.41 c
	Uncoated + MAP	7.75 ± 0.30 a	1.20 ± 0.11 a	3.00 ± 0.00 a
	FLUDIOXONIL	6.61 ± 0.40 a	1.31 ± 0.13 a	2.57 ± 0.32 b
15 weeks at 5 °C + 1 week at 20 °C	CONTROL	5.00 ± 0.28 ab	1.79 ± 0.21 a	1.60 ± 0.16 c
	HPMC-CW-SB	4.42 ± 0.38 b	2.77 ± 0.34 a	1.00 ± 0.07 d
	HPMC-BW-SB	5.42 ± 0.32 a	1.77 ± 0.30 a	2.36 ± 0.19 ab
	HPMC-CW-SB + MAP	5.92 ± 0.36 a	1.86 ± 0.42 a	1.93 ± 0.16 bc
	HPMC-BW-SB + MAP	5.69 ± 0.36 a	1.57 ± 0.17 a	1.71 ± 0.19 c
	Uncoated + MAP	5.71 ± 0.22 a	1.71 ± 0.29 a	2.57 ± 0.17 a
	FLUDIOXONIL	5.83 ± 0.27 a	1.79 ± 0.24 a	1.50 ± 0.14 cd

[1] CONTROL: uncoated; HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; SB: sodium benzoate. [2] Flavor scale: 1–3 = poor quality; 4–6 = acetable quality; 7–9 = excellent quality. [3] Off flavors scale: 1 = absence of off-flavor and 5 = very pronounced presence. [4] Visual quality scale: 1 = bad; 2 = acceptable; 3 = good. For each storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

3.2.6. External and Internal Fungal Decay

External fungal decay during cold storage was mainly caused by wound pathogens as latent infection in the crown was nearly negligible. Causal fungi of external and internal decay were not always directly identified because of the lack of sporulation after cold storage and shelf life, but some symptomatic samples were taken after the experiment and incubated at 25 °C for several additional days in order to favor disease development for identification purposes. Similarly to Experiment I, the main postharvest pathogens causing external wound decay were *Penicillium* spp. Overall, external and internal fungal decay were nil or very low for all the treatments after 8 weeks of storage, without significant differences among treatments (Table 8). At the end of the storage period, after 15 weeks at 5 °C plus 1 week at 20 °C, external disease incidence on control samples reached 35%, and pomegranates treated with fludioxonil, HPMC-CW-SB + MAP, and uncoated + MAP showed significantly lower incidence than control fruits. Values of internal disease incidence were between 2 and 22%, without significant differences among treatments. In general, disease severity indexes for both external and internal fungal decay were low, and no significant differences among treatments were found after both storage periods.

Table 8. External and internal decay on 'Mollar de Elche' pomegranates treated with antifungal edible coatings and/or stored in modified atmosphere packaging (MAP) for 8 and 15 weeks at 5 °C and 90% RH followed by a shelf-life period of 1 week at 20 °C.

Storage Time	Treatment [1]	Storage Period			
		External Decay		Internal Decay	
		% Incidence	Severity Index (0–4) [2]	% Incidence	Severity Index (0–4) [3]
8 weeks at 5 °C + 1 week at 20 °C	CONTROL	4.17 ± 2.08 a	0.67 ± 0.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	HPMC-CW-SB	2.08 ± 2.08 a	0.67 ± 0.67 a	2.08 ± 2.08 a	0.67 ± 0.67 a
	HPMC-BW-SB	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	HPMC-CW-SB + MAP	4.17 ± 4.17 a	1.00 ± 0.50 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	HPMC-BW-SB + MAP	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Uncoated + MAP	6.25 ± 3.60 a	0.83 ± 0.44 a	4.17 ± 2.08 a	0.00 ± 0.00 a
	FLUDIOXONIL	4.17 ± 2.08 a	1.33 ± 0.88 a	4.17 ± 2.08 a	1.00 ± 0.58 a
15 weeks at 5 °C + 1 week at 20 °C	CONTROL	35.42 ± 5.51 a	1.41 ± 0.21 a	22.92 ± 7.51 a	1.78 ± 0.20 a
	HPMC-CW-SB	20.83 ± 9.08 ab	1.17 ± 0.17 a	10.42 ± 4.17 a	1.17 ± 0.17 a
	HPMC-BW-SB	16.67 ± 2.08 abc	1.39 ± 0.06 a	8.33 ± 4.17 a	1.00 ± 0.58 a
	HPMC-CW-SB + MAP	2.08 ± 2.08 d	1.00 ± 0.58 a	2.08 ± 2.08 a	0.33 ± 0.33 a
	HPMC-BW-SB + MAP	18.75 ± 6.25 abc	1.17 ± 0.08 a	14.58 ± 7.51 a	1.06 ± 0.53 a
	Uncoated + MAP	8.33 ± 2.08 bcd	1.33 ± 0.33 a	2.08 ± 2.08 a	0.67 ± 0.67 a
	FLUDIOXONIL	6.25 ± 3.61 cd	1.17 ± 0.60 a	6.25 ± 0.00 a	2.00 ± 0.58 a

Mean ± standard error (SE) (n = 4). [1] CONTROL: uncoated; HPMC: hydroxypropyl methylcellulose; BW: beeswax; CW: carnauba wax; SB: sodium benzoate. [2] External decay severity index: 0 = no decay; 1 = decay lesion < 1 cm^2; 2 = 1 cm^2 < decay lesion < 25% of rind surface; 3 = decay lesion on 26–50% of rind surface; and 4 = decay lesion > 50% of rind surface. [3] Internal decay severity index: 0 = none; 1 = slight; 2 = moderate; and 3 = severe. For each type of decay and storage time, means in columns with different letters are significantly different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

4. Discussion

The present work highlights the antifungal effect of HPMC-lipid composite ECs formulated with GRAS salts and MAP technology to reduce postharvest decay and improve storability of 'Mollar de Elche' pomegranates. Weight loss is one of the major causes of fresh pomegranate consumer rejection, as excessive weight loss may result in shriveling, hardening of the husk, and browning of the rind, reducing the visual quality and marketability [14,41]. Thus, Fawole and Opara [42] reported that storage potential of pomegranate fruit significantly decreased as storage temperature increased from 5 to 22 °C, mainly due to augmented weight loss which reached values of 20–25% after 4 weeks of storage at the highest temperature. Coatings containing hydrophobic compounds, deposited as an additional layer over the rind, generally should improve the moisture resistance of the fruit. Herein, in the first experiment, the results showed that, among the coatings tested, HPMC-CW-SB was the most effective to reduce weight loss, followed by HPMC-BW-SB, whereas the rest of the coatings did not reduce weight loss of pomegranates (Figure 1). Similar results on the effect of SB incorporated into HPMC-BW coatings on fruit weight loss reduction were reported for example on cherry tomatoes [43]. However, several works have confirmed that the addition of food additives or GRAS salts to HPMC-lipid based ECs greatly affects the moisture barrier properties of stand-alone films and coatings when applied to different fruits such as cherry tomatoes, citrus, and table grapes [32,35,44–47]. This may explain why the HPMC-BW and HPMC-CW coatings containing the salt PBC were less effective in reducing weight loss than those containing SB, in spite of having the same content of wax (Table 1). In the case of coatings formulated with GMS, the lower lipid content and the less polar degree of GMS may have contributed to their lower effectiveness to reduce weight loss of coated pomegranates.

Regarding natural decay caused by latent (crown) and wound pathogens on coated and uncoated 'Mollar de Elche' pomegranates during 8 weeks at 20 °C (Experiment I), results showed that the three coatings containing SB significantly reduced crown decay incidence at the end of the storage period (Table 2). In general, 'Mollar de Elche' pomegranates

stored at 20 °C showed a low incidence of crown natural infections, with most decay caused by *B. cinerea*, as previously reported in other works [13,41]. Typically, pomegranate gray mold starts as small, superficial, rounded, soft and decolorized spots that under high RH conditions promptly expand from the crown (calyx containing the stamens and pistils) to the peduncular area, inducing a dark brown lesion [12]. Incidence of latent infections caused by *B. cinerea* depends on different factors such as cultivar, grove location, growing season, and environmental conditions. In fact, Palou et al. [41], working with naturally infected 'Wonderful' pomegranates in California, reported a very high incidence of gray mold compared to the low incidence levels observed in this study. On the other hand, our results also showed that among wound pathogens, species of the genera *Penicillium* and *Aspergillus*, were the most abundant, and these fungi were similarly controlled by coatings containing GRAS salts than crown decay. Both salts, SB and PBC, are commonly used as additives in the food industry and their potential to effectively control postharvest fungal decay on a variety of fresh fruits and vegetables during storage has been shown in several research works [28,44,48]. The number of studies on the use of GRAS salts to control pomegranate postharvest diseases is scarce [49]. The use of aqueous solutions of some salts such as sodium bicarbonate, sodium carbonate, and potassium sorbate was tested alone or in mixtures by Palou et al. [41]. The effectiveness of these treatments was inferior to that of the fungicide fludioxonil and, in some case, rind phytotoxicity caused by dips in salt solutions was observed. The addition of GRAS salts to ECs, in an attempt to provide an antifungal functionality to the coating emulsion, may provide a solution for such a phytotoxicity problem and could be a suitable option for disease control alternative to synthetic fungicides [50]. To our knowledge, this is the first report on the potential of HPMC-based coatings to reduce pomegranate postharvest diseases. The antifungal activity of HPMC-lipid coatings amended with SB or PBC has been proved in previous works by our group to control different postharvest diseases on a variety of fresh fruits such as citrus, tomatoes, and plums [31,32,34,35,37,43]. For instance, recent research showed that coatings formulated with SB effectively reduced severity of stem-end rot on mandarins artificially inoculated with *Lasiodiplodia theobromae* [32] and anthracnose on oranges and mandarins artificially inoculated with *Colletotrichum gloeosporioides* [35]. Similar results were reported with mandarins artificially inoculated with *Penicillium digitatum* or *Penicillium italicum* [47]. Moreover, Fagundes et al. [31] showed that among a wide range of food preservatives tested as ingredients of HPMC-based ECs, PBC and SB were among the most promising GRAS salts to control *B. cinerea* in vitro and on inoculated cherry tomato fruits. Considering the major importance of gray mold on the etiology of postharvest diseases of 'Mollar de Elche' pomegranates in our local conditions in Spain [2], these two particular salts were selected as antifungal ingredients of the ECs to be tested in the Experiment I.

Considering the overall effects of the tested ECs on weight loss and decay reduction seen in Experiment I, HPMC-CW-SB and HPMC-BW-SB were selected as antifungal ECs to be tested, alone and in combination with MAP, under commercial prolonged cold storage conditions in the Experiment II. As expected, weight loss of pomegranates significantly increased with storage time (Figure 2). Overall, the selected coatings only reduced weight loss of air-stored pomegranates compared to control fruits during 8 weeks at 5 °C plus the shelf-life period, while the extension of cold storage reduced their effectiveness. This may be probably due to changes originated in the permeability of the pomegranate cuticle and/or coating integrity during extended storage. In this respect, some works with caseinate-based stand-alone films revealed a re-arrangement of polymer chains during prolonged storage that translated into a loss of mechanical resistance, stretchability, and optical properties that affected the film barrier properties [51,52]. Similarly, Kamper and Fennema [53] observed approximately a six-fold increase in water vapor permeability through a HPMC-stearic/palmitic acid bi-layer film upon decrease of the temperature from 25 to 5 °C, probably due to shrinking and breaking of the film as the components became more rigid. In this sense, the greater weight loss observed at the end of the storage period on fruits treated with the HPMC coating formulated with CW compared to the coating

containing BW could also be attributed to the more viscoelastic character of BW, that may have yielded more easily to the internal forces related to shrinkage of the polymer matrix during storage, better maintaining the integrity of the coating [54]. On the other hand, MAP of pomegranates greatly reduced weight loss after cold storage and shelf-life conditions. Furthermore, the combination of coating application and MAP showed a synergic effect in reducing weight loss and while MAP reduced weight loss of uncoated pomegranates by 65%, a reduction of 79% was obtained when HPMC-CW-SB and MAP were combined. Similar results on the efficacy of MAP to reduce weight loss of pomegranates have also been reported in previous works [19,24,25,55]. The effect of MAP on weight loss reduction can be directly attributed to a limitation of water vapor diffusion through the film that generates higher RH within the package [49]. High RH could also prevent the coating from shrinking and breaking after prolonged storage, which would explain the synergic effect observed with the combination of both technologies at the end of the storage period.

ANOVA analysis showed no effect of storage time and treatment in the concentrations of O_2 and CO_2 inside the MAP, indicating that the equilibrium-modified atmosphere was achieved within the tested MAP before 30 days of storage. Overall, the final O_2 and CO_2 concentrations in MAP bags of coated and uncoated pomegranates ranged from 18.2 kPa to 19.53 kPa and from 2.63 to 4.67 kPa, respectively, showing that at the tested conditions the Xtend® packaging sheets provided a greater barrier to CO_2 than O_2. These values differ from those obtained for 'Mollar de Elche' in unperforated polypropylene MAP bags, with 8 kPa of O_2 and 10 kPa of CO_2 after 12 weeks at 2 °C [19]. However, in general, CO_2 levels inside the tested packaging material were in the range of those reported for other pomegranate cultivars packed within Xtend® patented film, while O_2 levels were slightly higher. For instance, O_2 and CO_2 levels of 13.5–17.60 kPa and 3.90–4.40 kPa, respectively, were reported for the Turkish pomegranates cultivars 'Hicrannar', 'Hicaznar', and' Beinari' after 120 days at 6 °C [21,22,24,56], whereas values of 15.0 kPa O_2 and 5.0 kPa CO_2 were recorded for 'Wonderful' pomegranates packed in 80 kg Xtend® bags after 16 weeks at 7 °C [25]. The differences in the atmosphere inside Xtend® MAP bags can be attributed to several factors that affect the respiration rate, such as cultivar, fruit maturity stage, and storage temperature, as well as to possible differences in film surface area, product weight, and free space within the package [10].

Rind color is one of the most important quality attributes of pomegranates. It is characteristic of each cultivar and, in general, uniform and intense red tonalities are desirable for many markets. In the case of the Spanish 'Mollar de Elche' cultivar, the rind reaches a yellow-pink or light red rind color at harvest and, as a non-climacteric fruit, little variations are further observed after harvest. The changes in rind color associated with prolonged storage of pomegranates usually translate into a decrease in L*, due to peel dehydration, and an increase in h° values, indicating a change to a less red color. In this sense, our results confirmed this tendency, and higher L* values were observed in fruits from those treatments that most reduced weight loss (i.e., coated and uncoated pomegranates packed in MAP and treated with Fludioxonil) than in control samples, except for those fruits treated with HPMC-CW-SB + MAP (Table 4). This could be directly attributed to the effect of the HPMC-CW-SB coating, since the incorporation of SB to the HPMC-CW formulation negatively affected the appearance of coated pomegranates, reducing fruit gloss (Table 7). Similar results have also been reported for citrus, cherry tomatoes, and plums treated with HPMC-lipid coatings amended with different GRAS salts [33,39,43]. On the other hand, in control samples, a decrease in skin redness (lower a*) and an increase in yellowness (higher b*) were observed during storage. The application of the coatings and MAP helped to maintain b*, but not a* values. In other works, conversely, the use of MAP helped maintaining the peel color of other pomegranate cultivars [20,24,56].

Pomegranate juice quality was analyzed monitoring SSC, TA, pH, and MI (Table 5). TA and SSC are important quality attributes that determine consumer eating acceptance. In the case of 'Mollar de Elche' pomegranates, this cultivar is highly appreciated due to its sweet taste (15.5–17.6% SSC) and very low acid level (0.17–0.27% citric acid) [57]. Herein,

in agreement with previous studies, SSC and TA decreased significantly during storage and after the shelf-life period in comparison to the initial values at harvest [21,22,42,55,56]. This resulted in an increase of MI from 69.13 to 86.95%. The pH increase in comparison to values at harvest is probably associated to a reduction of citric acid as it is being consumed in the respiratory process of the fruit [21]. Similarly, SSC showed a slight but significant reduction during storage, which could also be related with the hydrolysis of sucrose and the utilization of the corresponding reducing sugars during the respiration process [21]. However, some previous studies by Ghafir et al. [58] and Selcuk and Erkan [24] reported an increase in SSC content of 'Shlefy' and 'Hicrannar' pomegranates during cold storage, which was attributed to moisture loss. On the hand, our results show no effect of coating and MAP application on SSC and TA of 'Mollar de Elche' pomegranates, in agreement with other works reported for other pomegranate cultivars packed with Xtend® film [21,23,24,56] or treated with chitosan + MAP [22].

Pomegranate fruits are susceptible to different physiological disorders during postharvest storage, such as chilling injury and husk scald, which, together with fruit dehydration, negatively affect consumer acceptance. Dehydration is manifested as rind sinking and, in the present work, it was significantly reduced by the use of MAP, in correlation with the lower weight loss observed on MAP-exposed fruits (Table 6). Chilling injury symptoms are usually manifested after transferring long-term cold-stored fruits to ambient temperatures as surface pitting and rind browning, as well as internal rind browning. Husk scald is also manifested as rind browning and is mainly due to the oxidation of phenolic compounds in the peel. In this work, at the end of the storage period, main symptoms were manifested as rind pitting and browning, so both symptoms are reported together. As expected, these physiological disorders were mainly observed in control fruits after 15 weeks of cold storage plus shelf life. Overall, the MAP treatments, except for the combination HPMC-BW-SB + MAP, reduced surface pitting and skin browning compared to control samples, whereas no differences were found for internal pitting. As previously mentioned, the beneficial effect of MAP to reduce physiological disorders has been already reported for different pomegranate cultivars, including 'Mollar de Elche' [19–23]. Furthermore, Candir et al. [22] also reported a reduction of husk scald on 'Hicaznar' pomegranates stored in MAP or coated with chitosan in combination with MAP, which was associated to a reduction or delay in the oxidation of phenolic compounds on the husk at lower O_2 and higher CO_2 levels. In our case, the application of MAP reduced the external pitting/browning of pomegranates, except for the combination HPMC-BW-SB + MAP. This could be related to different changes in the atmosphere surrounding the fruit inside the MAP bag when this coating was applied (Table 3).

Sensory evaluation was carried out by a trained panel based on overall flavor, off-flavors, and external visual aspect of the pomegranates. Coating and MAP application did not modify fruit flavor compared to uncoated pomegranates and, at the end of the storage period, flavor was scored in the acceptable range (4.07–5.71) (Table 7). The decrease in overall flavor after 15 weeks at 5 °C plus the shelf-life period at 20 °C was correlated with a slight increase in off-flavor, with HPMC-CW-SB coated pomegranates receiving the highest off-flavor score (2.77), but without significant differences among treatments. This slight increase in off-flavor can be attributed to an increase of ethanol and acetaldehyde contents in the juice after long-term cold storage (Table 5), as these volatile components are associated with anaerobic fermentation [59,60]. In general, the HPMC-CW-SB coating was the worst treatment to preserve fruit visual appearance after storage, whereas the treatment uncoated + MAP gave the best results. Although there is no information available on the effect of HPMC-based coatings on visual quality of pomegranates, our results are in accordance with previous works on citrus, tomato, and plums. In fact, it was reported in these studies that HPMC-based coatings amended with the salt SB did not provide intense brightness to coated fruits, probably due to the macro emulsion character of the coating formulation [32,35,38,43]. Moreover, some studies have also reported the presence of white spots on the surface of coated mandarins or oranges that reduced the

general good appearance of the fruit when using HPMC-based coatings amended with SB [32,39]. This white residue was also observed on pomegranate surface coated with HPMC-CW-SB, resulting in a reduction of the global appearance of the fruits. Thus, in general, uncoated + MAP pomegranates had the best aspect at the end of both storage periods and also the best global quality, showing that MAP treatment is able to maintain external fruit quality.

In general, the application of coatings containing SB gave equal results to prevent external surface decay during cold storage than the application of the fungicide fludioxonil, confirming the potential of this salt and this type of coatings to prevent postharvest pomegranate diseases (Table 8). This similar effectiveness of the coatings containing SB compared to fludioxonil was not expected as the effect of GRAS salts is typically rather fungistatic than fungicidal and not very persistent in comparison to synthetic chemical fungicides [41]. On the other hand, the positive results observed with MAP for decay reduction during prolonged cold storage are in agreement with previous findings on the pomegranate varieties 'Beynari', 'Hicrannar', or 'Primosole' [20,24,56]. In general, in this work, the severity indexes of internal and external decay were low, probably due to a generally reduced amount of natural fungal inoculum present on the fruits, and *Penicillium* spp. causing green/blue mold were the most frequent pathogens. This genus has been already reported among the most important causal agents of postharvest decay on long-term cold-stored pomegranates [2,20]. Species cited as pathogenic on pomegranate include *P. expansum*, *P. sclerotiorum*, *P. implicatum*, *P. glabrum*, and *P. chrysogenum* [12].

5. Conclusions

This study has focused on the use of antifungal HPMC-lipid composite ECs containing SB and MAP to reduce postharvest decay and improve storability of 'Mollar de Elche' pomegranates stored under simulated commercial conditions. Results have shown that HPMC-based coatings amended with SB have the potential to reduce pomegranate postharvest diseases, mainly latent infections caused by *B. cinerea* and wound diseases caused by *Penicillium* spp., and may provide a non-polluting alternative to the use of synthetic fungicides such as fludioxonil. On the other hand, MAP technologies have been confirmed as an efficient mean to preserve pomegranates freshness, prevent fruit shriveling and rind browning and reduce fungal decay, thus improving fruit postharvest quality and extending storage life. Overall, the combination HPMC-BW-SB + MAP was the most promising treatment as it reduced weight loss and decay, without negatively affecting the fruit physicochemical and sensory quality. Further studies should focus on improving the physical characteristics of the coatings to achieve similar results to MAP technologies in order to minimize plastic use and benefit from this sustainable and environmentally friendly alternative to conventional postharvest practices.

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