

Inhibition of *Penicillium digitatum* and *Penicillium italicum* by Hydroxypropyl Methylcellulose–Lipid Edible Composite Films Containing Food Additives with Antifungal Properties

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New hydroxypropyl methylcellulose (HPMC)–lipid edible composite films containing low-toxicity chemicals with antifungal properties were developed. Tested chemicals were mainly salts of organic acids, salts of parabens, and mineral salts, classified as food additives or generally recognized as safe (GRAS) compounds. Selected films containing food preservatives were used for *in vitro* evaluation (disk diameter test) of their antifungal activity against *Penicillium digitatum* (PD) and *Penicillium italicum* (PI), the most important postharvest pathogens of fresh citrus fruit. Mechanical properties and oxygen (OP) and water vapor permeabilities (WVP) of selected films were also determined. Film disks containing parabens and their mixtures inhibited PD and PI to a higher extent than the other chemicals tested. Among all organic acid salts tested, potassium sorbate (PS) and sodium benzoate (SB) were the most effective salts in controlling both PD and PI. The use of mixtures of parabens or organic acid salts did not provide an additive or synergistic effect for mold inhibition when compared to the use of single chemicals. Barrier and mechanical properties of films were affected by the addition of food preservatives. Results showed that HPMC–lipid films containing an appropriate food additive should promise as potential commercial antifungal edible coatings for fresh citrus fruit.

KEYWORDS: Hydroxypropyl methylcellulose; edible films; food preservatives; *Penicillium digitatum*; *Penicillium italicum*; mechanical and barrier properties

INTRODUCTION

Postharvest diseases of citrus are mainly caused worldwide by the pathogens *Penicillium digitatum* (Pers.:Fr.) Sacc. (PD) and *Penicillium italicum* Wehmer (PI), which cause green and blue molds, respectively (1). Chemical fungicides, such as imazalil, sodium *ortho*-phenyl phenate, or thiabendazole, have been widely applied to control these diseases (1). Consumer concerns about prolonged and extensive use of chemical fungicides to control citrus postharvest decay lead researchers to look for alternative noncontaminant methods that do not deposit harmful residues and contaminate the environment.

Several alternative methods of different nature have been assayed against both PD and PI, including cold storage in conventional controlled hypobaric or ozonated atmospheres, application of heat treatments, use of ionizing radiation, dips

in aqueous solutions of food additives or chemical compounds of low toxicity (2–4), or biological control (5).

The use of edible coatings and films is an alternative method to preserve the postharvest quality of fruits and vegetables (6). In the food industry, they offer opportunities to increase the shelf life of many products. Edible coatings and films form a semipermeable barrier to exchange oxygen, carbon dioxide, and water vapor, leading to weight loss reduction, respiratory rate modification, and senescence delay (7). Furthermore, the visual quality of treated produce is improved because of the gloss provided by edible coatings and films (8).

Polysaccharides, proteins, lipids, and resins are mainly used to form edible coatings. Plasticizers and emulsifiers are usually added to increase flexibility and surface tension between aqueous and lipid phases in those formulations that combine lipids and hydrocolloids (9, 10). In addition, edible coatings and films are a viable mean for incorporating food additives or other substances to enhance flavor, color, and texture, control microbial growth, and improve general coating performance (11, 12). Antimicrobials can be added to edible coatings to retard the

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Table 1. Characteristics and Tested Concentrations of Antifungal Food Preservatives Used To Prepare HPMC–Lipid Emulsion Films

food preservative	molecular formula	E-code	solid concentration tested (%; wb)	food preservative concentration tested (%; wb)
Mineral Salts				
sodium bicarbonate	NaHCO ₃	E-500(I)	6; 8; 10; 12	0.05; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
ammonium bicarbonate	NH ₄ HCO ₃	E-237	6; 8; 10; 12	0.05; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium carbonate	Na ₂ CO ₃	E-500(II)	6; 8; 10; 12	0.05; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium silicate (SiO ₂ ; 27%)	Na ₂ SiO ₃ O ₇		6; 8	1.0; 2.0; 2.5
sodium molybdate	Na ₂ MoO ₄ ·2H ₂ O		6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
ammonium molybdate	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O		6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
Organic Acid Salts				
potassium sorbate	C ₆ H ₇ O ₂ K	E-202	6; 8; 10; 12	0.05; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium benzoate	C ₇ H ₅ O ₂ Na	E-211	6; 8; 10; 12	0.05; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium acetate	CH ₃ COONa	E-262-(i)	6; 8	1.0; 2.0; 2.5
sodium diacetate	(CH ₃ COO) ₂ HNa	E-262-(II)	6; 8	1.0; 2.0; 2.5
sodium propionate	CH ₃ CH ₂ COONa	E-281	6; 8	1.0; 2.0; 2.5
calcium propionate	Ca(CH ₃ CH ₂ COO) ₂	E-282	6; 8	1.0; 2.0; 2.5
sodium formate	HCOONa	E-237	6; 8	1.0; 2.0; 2.5
calcium formate	Ca(HCOO) ₂	E-238	6; 8	1.0; 2.0; 2.5
sodium citrate	C ₆ H ₅ Na ₃ O ₇	E-331(I)	6; 8	1.0; 2.0; 2.5
sodium L-lactate	C ₃ H ₅ NaO ₃	E-325	6; 8	1.0; 2.0; 2.5
sodium L-tartrate	C ₄ H ₄ Na ₂ O ₆ ·2H ₂ O	E-335 (I)	6; 8	1.0; 2.0; 2.5
Organic Acid Salts (Mixtures)				
potassium sorbate + sodium propionate			6	1.5; 2.0
sodium benzoate + potassium sorbate			8	1.5; 2.0; 2.5
sodium benzoate + sodium propionate			8	1.5; 2.0; 2.5
Parabens				
sodium salt of methyl paraben	C ₈ H ₇ NaO ₃	E-219	6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium salt of ethyl paraben	C ₉ H ₉ NaO ₃	E-215	6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
sodium salt of propyl paraben	C ₁₀ H ₁₁ NaO ₃	E-217	6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
Paraben (Mixture)				
sodium salt of methyl paraben + sodium salt of propyl paraben			6; 8; 10; 12	1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5
Other Compounds				
EDTA	C ₁₀ H ₁₂ N ₂ O ₈ CaNa ₂	E-385	6; 8	1.0; 2.0; 2.5
2-deoxy-D-glucose	C ₆ H ₁₂ O ₅		6	0.3; 0.5; 1.0

growth of bacteria, yeasts, and molds during storage and distribution of fresh or minimally processed products. Generally, these compounds include organic acids (acetic, benzoic, lactic, propionic, and sorbic acids) and their salts (13–15), parabens (16), bacteriocins, such as nisin (17), sucrose esters (18), chitosan (19), essential oils (20), or natural antimicrobials, such as natamycin, lactoferrin, lysozyme, or lactoperoxidase (15, 21). Several studies reported that the addition of potassium sorbate to edible coatings controlled microbial proliferation on strawberries (22, 23); the addition of acetic, citric, and sorbic acids effectively controlled the pathogen *Salmonella montevideo* on tomatoes (24); and the addition of parabens reduced coliform bacteria on citrus fruit (25). However, little research has been performed to develop new edible composite coatings with the addition of antifungal compounds as a new technique to control major fungal postharvest diseases of fresh citrus fruit.

The objectives of this study were to develop new hydroxypropyl methylcellulose (HPMC)–lipid edible composite films containing food additives with antifungal properties and evaluate the *in vitro* activity of selected films against PD and PI. Barrier and mechanical properties of selected films were also studied.

MATERIALS AND METHODS

Materials. HPMC (Methocel E15) was from Dow Chemical Co. (Midland, MI). Beeswax (BW) (grade 1) and resin (shellac) were supplied by Fomesa Fruitech, S.L. (Valencia, Spain). Glycerol and stearic acid were from Panreac Química, S.A. (Barcelona, Spain). Ammonia (25%) and silicone antifoam (FG-1510) were from Scharlau (Sentmenat, Spain) and Dow Corning (Belgium), respectively. Food preservatives were purchased to Sigma (Sigma-Aldrich Chemie, Steinheim, Germany) and included mineral salts, salts of organic acids, sodium salts of parabens, and several other compounds. Molecular

formulas and the corresponding E-list code for food additives in the European Union (EU) are shown in **Table 1**. Most of them are likewise classified as food additives or generally recognized as safe (GRAS) compounds by the United States Food and Drug Administration (U.S. FDA).

Film Formation. Films from edible composite emulsions were prepared by combining the hydrophilic phase (HPMC) and the lipid phase (BW and shellac) suspended in water. Glycerol and stearic acid were used as plasticizer and emulsifier, respectively. Ratios of HPMC/glycerol (2:1) dry basis (db) and lipids (BW and shellac)/stearic acid (5:1) (db) were kept constant throughout the study. For emulsion preparation, an aqueous solution of HPMC (5%, w/w) was dispersed in hot water at 90 °C and later hydrated at 20 °C. Shellac was previously dispersed in water at 40 °C, and ammonia [15% (w/w) shellac/ammonia] was added to dissolve the resin. BW, glycerol, stearic acid, and water were added to HPMC solution and heated to 90 °C. Shellac was heated separately at the same temperature and added to the former compounds. Once the lipids were melted, samples were homogenized with a high-shear probe mixer (Ultra-Turrax Model T25, IKA-Werke, Steufen, Germany) for 4 min at 22 000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 °C by placing them in an ice water bath. To ensure completed hydration of HPMC, emulsions were further agitated during 25 min.

A preliminary study was performed to prepare emulsions capable of forming homogeneous composite films containing food preservatives with antifungal properties. Edible composite formulations were optimized on the basis of a solid concentration (SC) range of 4–12% and total lipid concentration (BW–shellac) range of 0–60% (db).

The addition to the coating of food preservatives was from 0.05 to 4.5% (wet basis, wb). In each case, the maximum concentration of food preservative that formed stable emulsions was determined. All food preservatives and concentrations tested to prepare HPMC–lipid emulsions are presented in **Table 1**. Emulsions were degassed, and

films were cast by pipetting emulsions onto rimmed, smooth plates resting on a leveled wooden slab and allowed to dry at room temperature over 24 h.

Determination of *in Vitro* Antifungal Activity. Antifungal activity of selected edible films was evaluated through the disk diameter test. This method was adapted from that described by Min and Krochta (21).

Media. Potato dextrose agar (PDA) supplied by Sigma and dichloran rose-bengal chloramphenicol agar (DRBC) provided by Merck (Darmstadt, Germany) were used to prepare the media for the disk diameter test.

Fungal Inoculum. *P. digitatum* isolate NAV-7 and *P. italicum* isolate MAV-1, obtained from decayed oranges from Valencia packinghouses, were isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. These strains were selected for their aggressiveness on the most commercially important mandarin and orange cultivars. Prior to each experiment, the isolates were grown on PDA in Petri dishes at 25 ± 1 °C for 7–10 days. A high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac Química S.A.) in sterile water, passed through two layers of cheesecloth, measured with a hemacytometer, and diluted with sterile water to achieve the desired inoculum density.

Disk Diameter Test. Selected films were cast by pipetting the emulsion onto sterilized HDPE plates (14.1 cm internal diameter). Most of the films were very brittle, which made it difficult to peel them from the plates. Therefore, the plates had to be covered with wax paper (0.2 mm thickness) before using them. The wax paper was disinfected with alcohol (97%). For 6 and 8% SC, 48.3 and 46.6 g of emulsion were weighted respectively to obtain 0.2–0.3 mm thick films. Emulsions on plates were allowed to dry for about 48 h at room temperature under aseptic conditions, inside a previously sterilized laminar flow hood. Dry films were peeled intact from the casting surface and aseptically cut into 16 mm diameter disks using a sterile cork borer. Films that could not be separated from the wax paper were cut together with it. All films were aseptically stored at 5 °C until used. For each emulsion, 3–5 films were prepared. A total of 10 measurements of film thickness on each film were randomly taken, and the mean value of film thickness was reported. Films from emulsions with the selected percentage of SC and BW–shellac ratio but without added food preservatives were used as controls. Three levels of inoculum were prepared for each fungal species: 10^3 , 10^4 , and 10^5 spores/mL. For each inoculum level, 100 μ L of conidial suspension was spread on the surface of DRBC agar plates (9 cm diameter). Film disks were aseptically transferred to the agar surface previously inoculated with PD or PI. The plates were refrigerated at 4 °C for 3 h to allow for the diffusion of film ingredients and then incubated at 25 °C for 5 days. For each pathogen, inoculum density, and film, three agar plates (replicates) were prepared. After incubation, the length of the inhibition zone around the film disk (from the perimeter of the film disk until the edge of the inhibited area) was measured with a digital caliper and reported in millimeters. Four measurements were performed for each Petri dish.

Properties of Selected Emulsions and Films. The pH of the emulsions was measured using a digital pH-meter (Model C830, CONSORT, Turnhout, Belgium). Emulsion viscosity was measured with a viscometer (Model LVF, Brookfield Engineering Laboratories, Inc., Stoughton, MS). Three measurements were performed from replicated emulsion samples. Results were expressed in centipoises (cp). Sample viscosities were measured at 20 °C.

Emulsion stability was measured according to an adapted method from Taherian et al. (26). A total of 60 mL of prepared emulsion was placed in a 100 mL volumetric flask and left there at 25 °C for 48 h. The results were expressed as a percentage of phase separation with respect to the total height of the emulsion in the tube. The determinations were performed in duplicate.

Water Vapor Permeability (WVP). WVP of the films was determined using the gravimetric method of the ASTM E96-92, with modifications by McHugh et al. (27) Films were casted as described before. Two disk samples (5 cm diameter) were cut from each film and mounted on polymethacrylate cups containing 6 mL of distilled water. To study if phase separation occurred during drying, the film surface, which had been exposed to air during drying, was placed either facing the low

relative humidity (RH) environment (“facing up”) or the high RH environment (“facing down”). The film was sealed to the cup base with a ring using four screws located symmetrically around the cup circumference. The cups were placed on desiccator cabinets containing fans and held around 0% RH using anhydrous silica gel (Scharlau Chemie S.A.). Weights were taken periodically after steady state was achieved and used to calculate the RH at the film underside and the resulting WVP.

Oxygen Permeability (OP). An oxygen permeation analyzer (Systech Instruments Model 8001, Oxfordshire, U.K.) was used to measure the oxygen transmission rate through films according to the ASTM method D3985 (28). OP of the films was measured at 23 ± 2 °C and 50 ± 5 % RH. A square film (3 \times 3 cm) was placed on a stainless-steel mask with an open testing area of 5 cm². One side of the film was exposed to flowing nitrogen gas, and the other side was exposed to flowing oxygen gas at the same conditions. OP was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the film thickness (29).

Mechanical Properties. Films were cast onto rectangular 15 \times 24 cm, rimmed, smooth high-density polyethylene (HDPE) plates covered with Teflon by applying 4 g of total solid per plate to minimize thickness variation between treatments. Three replicates of selected emulsions were used to prepare films. Dried films were conditioned at 53% RH in a chamber containing magnesium nitrate hexahydrate (Scharlau Chemie S.A.) saturated solution for more than 2 days. Tensile measurements were tested using an Instron Universal Machine (Model 3343; Instron Corp., Canton, MA) according to the ASTM method D882-97 (30). Each film was clamped between pneumatic grips and stretched at 5 mm/min with a 0.3 kN load cell. Testing conditions were controlled throughout the measurement and held constant at 50 ± 5 % RH and 23 ± 2 °C. The mechanical properties reported were Young’s modulus (Y , in MPa), maximum tensile stress (TS, in MPa), and elongation at break (E , in %). A total of 15 sample strips (50.0 \times 8.0 mm) were measured for each film.

Film Thickness. Film thickness was measured with a digital micrometer (Model Quickmike Series 293-IP-54; Mitutoyo Corp., Kanawava, Japan) at four random positions in the film. Mechanical properties OP and WVP were calculated using the average thickness.

Statistical Analysis. Statistical analyses of data on film properties or fungal inhibition were performed using the software Statgraphics Plus 2.1 (Manugistics, Inc., Rockville, MD). Specific differences between means were determined by Fisher’s protected least significant difference test (LSD, $p < 0.05$), applied after an analysis of variance (ANOVA). For inhibition data, ANOVA was applied to values transformed to the square root of the value plus 0.5.

RESULTS AND DISCUSSION

Selection of Stable Edible Films. Among the large amount of HPMC–lipid edible composite coatings containing antifungal food preservatives that were prepared (470 emulsions, **Table 1**), only those in **Table 2** were selected to be evaluated for antifungal activity. Their preservative concentration, SC, total lipid content (BW–shellac), pH, and viscosity are presented. To obtain stable emulsions capable of forming homogeneous coatings, HPMC–lipid edible composite coatings were optimized on the basis of the percentage of SC, total lipid content, and food preservative concentration. The emulsions shown in **Table 2** were stable, because they exhibited no phase separation (26). It was found that HPMC–lipid stable emulsions suitable to be used as coatings contained from 6 to 8% SC, 50% (db) total lipid content, and a maximum of 2.5% (wb) food preservative (**Table 2**).

Viscosity is one of the main factors affecting coating thickness (31); therefore, in our HPMC–lipid edible composite coatings, the effects of SC and type and concentration of food preservative on viscosity were evaluated. In this study, emulsions were ranked as follows according to their viscosity: “nonviscous” for emulsion viscosity lower than 40 cp, “viscous” for emulsion

Table 2. Composition, pH, and Viscosity of Selected HPMC–Lipid Edible Composite Emulsions Containing Antifungal Food Preservatives

HPMC–lipid edible coatings with food preservative	food preservative concentration tested (% w/w)	solid concentration tested (%)	BW–shellac (% db)	pH	viscosity (cp)
Mineral Salts					
sodium bicarbonate	2.0	6	45–5	8.88	7.0
ammonium bicarbonate	2.0	6	25–25	9.18	16.5
Organic Acid Salts					
potassium sorbate	2.0	6	25–25	7.42	11.6
sodium benzoate	2.5	8	25–25	7.33	13.3
sodium acetate	1.0	6	50–0	6.89	10.8
sodium diacetate	1.0	6	50–0	4.70	14.7
sodium propionate	2.0	6	25–25	7.39	11.2
calcium propionate	1.0	6	50–0	5.41	17.8
sodium formate	1.0	6	50–0	6.41	14.3
calcium formate	1.0	6	50–0	4.85	18.5
sodium citrate	1.0	6	50–0	3.77	12.7
sodium L-lactate	1.0	6	50–0	7.15	15.2
sodium L-tartrate	1.0	6	50–0	7.04	9.8
Organic Acid Salts (Mixtures)					
potassium sorbate + sodium propionate	1.5 + 0.5	6	25–25	7.46	11.9
sodium benzoate + potassium sorbate	2.0 + 0.5	8	25–25	7.43	15.1
sodium benzoate + sodium propionate	2.0 + 0.5	8	25–25	7.42	16.9
Parabens					
sodium salt of methyl paraben	1.5	6	50–0	9.06	13.2
sodium salt of methyl paraben	1.0	6	50–0	8.73	17.5
sodium salt of ethyl paraben	1.0	6	50–0	9.40	11.1
sodium salt of propyl paraben	1.0	6	50–0	9.43	14.8
Paraben (Mixture)					
sodium salt of methyl paraben + sodium salt of propyl paraben	1.0 + 0.5	6	50–0	9.21	14.7
Other Compounds					
EDTA	1.5	6	45–5	6.52	18.3
2-deoxy-D-glucose	0.5	6	25–25	7.14	22.2
2-deoxy-D-glucose	0.3	6	25–25	6.94	27.5

viscosity between 40–70 cp, and “very viscous” for emulsion viscosity higher than 70 cp. Viscosity of selected emulsions was on the range of 7–22 cp, corresponding to nonviscous emulsions. Differences in emulsion SC of selected formulations did not translate in important differences on emulsion viscosity (Table 2). Cisneros-Zevallos and Krochta (31) found that viscosity of HPMC solutions increased as SC increased. In our emulsions, the formulations not only differed on SC but also on lipid ratio and food preservative concentration, which also contribute to the final viscosity value.

Food preservatives added to the emulsions in a concentration lower than 2.5%, resulting in nonviscous emulsions. However, food preservatives added at a concentration up to 4.5% resulted on viscous emulsions or gels (data not shown). When a mixture of two food preservatives, such as two salts of organic acids or two sodium salts of parabens, was added to the emulsions, their viscosity did not increase and the resulting emulsion was nonviscous.

Emulsions with food preservatives that contained calcium propionate or calcium formate produced gels when shellac was included in the formulation. Stable and nonviscous emulsions were obtained when shellac was not present in the emulsion.

Because sodium carbonate, sodium molybdate, and ammonium molybdate showed promise for the control of postharvest decay of citrus fruits (3, 32–34), these compounds were also evaluated as ingredients of HPMC–lipid emulsions. Unfortunately, the addition of all of them resulted in unstable emulsions or gels. Emulsions containing sodium carbonate formed gels, even when shellac was not included, and sodium carbonate content was as low as 1% (data not shown). Aleuritic acid is one of the most important derivatives of shellac. It is obtained from shellac by saponification (35). It seems that saponification of shellac leading to gel formation occurred in

our HPMC–lipid emulsions containing sodium carbonate, probably because of the alkaline properties of sodium carbonate.

Among all mineral salts tested, only sodium bicarbonate (SBC) and ammonium bicarbonate (ABC) were selected as ingredients of HPMC–lipid emulsions.

Most of the HPMC–lipid emulsions containing shellac and salts of organic acids or sodium salts of parabens precipitated or formed gels. When shellac was removed from most of these emulsions, nonviscous and stable emulsions were obtained (Table 2). Potassium sorbate (PS), sodium benzoate (SB), and sodium propionate (SP) were three of the organic acid salts tested that permitted the inclusion of shellac in their formulations.

Acid or alkaline properties of the emulsions depended upon the kind of food preservatives that were added to them. Dependent upon the food preservative that was added into the emulsions, a wide range of pH was found, from acid emulsions containing sodium citrate (pH 3.77), through neutral emulsions, such as those containing sodium tartrate or sodium lactate, to basic emulsions containing parabens (pH 9.40).

In Vitro Antifungal Activity. The determination of film antifungal activity in this study was based on the disk diameter test. In this assay, the inhibition area surrounding the film disks was measured and compared to control films, in which food preservatives were not added, and hence, there was no inhibition area. Results on the antifungal activity against different inoculum concentrations of both PD and PI are shown in Table 3.

Mineral Salts. Among HPMC–lipid films with mineral salts, those with 2% SBC significantly inhibited ($p < 0.05$) the growth of both PD and PI on DRBC agar, irrespective of the inoculum density tested (Table 3). Films with SBC better controlled PD than PI. On the contrary, films with ABC produced no significant inhibition of both PD and PI. At all inoculum densities, pH values of all of these films were above 8.5 (Table 2), the pH

Table 3. Antifungal Activity of HPMC–Lipid Edible Composite Films with Food Preservatives against *P. digitatum* and *P. italicum* at Different Inoculum Concentrations

HPMC–lipid films with food preservative ^b	length of inhibition zone (mm) ^a					
	PD inoculum concentration (spores/mL)			PI inoculum concentration (spores/mL)		
	10 ³	10 ⁴	10 ⁵	10 ³	10 ⁴	10 ⁵
Mineral Salts						
sodium bicarbonate	8.9 defg	6.2 b	9.0 cd	4.3 cd	5.0 c	3.8 b
ammonium bicarbonate	1.0 ab	1.1 a	0.0 a	0.0 a	0.0 a	0.0 a
Organic Acid Salts						
potassium sorbate	16.8 ghi	15.8 ef	17.3 ef	9.2 cde	6.6 e	5.9 de
sodium benzoate	12.8 fgh	11.3 d	7.2 c	9.8 de	3.9 cd	2.9 bc
sodium acetate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium diacetate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium propionate	6.0 cd	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
calcium propionate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium formate	6.6 cde	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
calcium formate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium citrate	1.2 ab	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium L-lactate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
sodium L-tartrate	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Organic Acid Salts (Mixtures)						
potassium sorbate + sodium propionate	16.6 ghi	12.0 d	4.3 b	0.0 a	0.0 a	0.0 a
sodium benzoate + potassium sorbate	13.6 fgh	10.6 cd	9.1 cd	11.0 e	4.5 de	4.3 cd
sodium benzoate + sodium propionate	10.2 efgh	7.8 bc	0.7 ab	2.8 b	1.1 b	0.0 a
Parabens						
sodium salt of methyl paraben (1.5%)	22.1 i	24.2 g	21.1 f	18.3 f	18.5 g	19.9 g
sodium salt of methyl paraben (1.0%)	22.3 i	21.9 fg	17.4 ef	15.8 f	14.8 f	15.8 f
sodium salt of ethyl paraben	15.3 fghi	15.2 de	12.2 de	15.3 f	14.3 f	13.3 f
sodium salt of propyl paraben	2.8 bc	4.9 b	5.7 c	7.8 cde	6.1 e	7.0 e
Paraben (Mixture)						
sodium salt of methyl paraben + sodium salt of propyl paraben	21.3 hi	18.9 efg	19.9 f	18.8 f	17.4 fg	19.8 g
Other Compounds						
EDTA	7.5 de	4.7 b	0.0 a	6.3 c	0.0 a	0.0 a
2-deoxy-D-glucose (0.5%)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Controls^c						
control (8% SC; 25% BW–25% shellac)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
control (6% SC; 45% BW–5% shellac)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
control (6% SC; 25% BW–25% shellac)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
control (6% SC; 50% BW–0% shellac)	0.6 ab	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

^a Values are measurements of length (mm) of inhibitory zones around film disk (from the perimeter of the film disk until the edge of the inhibited area). Values within columns followed by unlike letters are different by the Fisher protected LSD test ($p < 0.05$), applied after an analysis of variance of the square root of the inhibition zone plus 0.5. Nontransformed data are shown. ^b Characteristics of these films are presented in **Table 2**. ^c HPMC–lipid films without food preservatives at 6 and 8% SC and a different percentage of BW–shellac were used as control films.

value at which the germination and growth of PD are inhibited (36). However, results from this study suggest that factors other than emulsion pH influence the antifungal activity against PD or PI of these edible films. Smilanick et al. (36) observed in an *in vitro* study that sodium salts were superior to ammonium or potassium carbonates and bicarbonates for the control of green mold, suggesting that the sodium cation played some important role in the control of PD. Furthermore, brief immersions in water solutions of SBC, alone or in a combination with other nonpolluting control methods, have been reported to be effective for the control of PD and PI on citrus fruit (4, 5, 37).

Organic Acid Salts and Mixtures. Among all organic acid salts added into HPMC–lipid films, only films containing PS (pH 7.42) or SB (pH 7.33) clearly inhibited ($p < 0.05$) the growth of both PD and PI at 10³–10⁵ spores/mL on DRBC agar (**Figure 1**). Films with PS or SB better controlled PD than PI. Films containing 2.0% PS produced larger inhibition zones for PD (16.8–17.3 mm) and PI (5.9–9.2 mm) than films with 2.5% SB (7.2–12.8 mm for PD and 2.9–9.8 mm for PI) (**Table 3**). In general, films with other organic acid salts did not inhibit both PD and PI. The only exceptions were SP, sodium formate, and sodium citrate that inhibited PD at 10³ spores/mL.

PS, the most soluble form of sorbate, is well-known for its potent antifungal activity. In food systems, PS is one of the most widely used compounds to prevent the growth of molds and thus extend produce shelf life (38). Sorbates inhibit mold species, including *Penicillium*. The antimicrobial activity of PS against PD and PI has been observed in both *in vitro* and *in vivo* studies. For instance, both pathogens were inhibited in PDA agar when PS were added at a concentration of 0.15–0.20 g/L (39). Moreover, aqueous solutions of PS and other common food preservatives, such as SB, applied to citrus fruit controlled to some extent both postharvest green and blue molds (2, 4, 40). The antimicrobial action of sorbate is pH-dependent. In general, PS activity is greater at low pH values, although sorbates may be effective at pH values as high as 7 (38). In our work, pH of HPMC–lipid films containing PS was around 7 (**Table 2**). In *in vivo* trials with Valencia oranges artificially inoculated with conidia of PD, brief dips in warm water solutions of 2–3% PS at neutral pH significantly inhibited mold development (40). In contrast, other common organic-acid-based food preservatives, such as propionates or benzoates, only showed considerable antimicrobial activity at low pH values, such as 5–5.5 and 4–4.5, respectively (38).

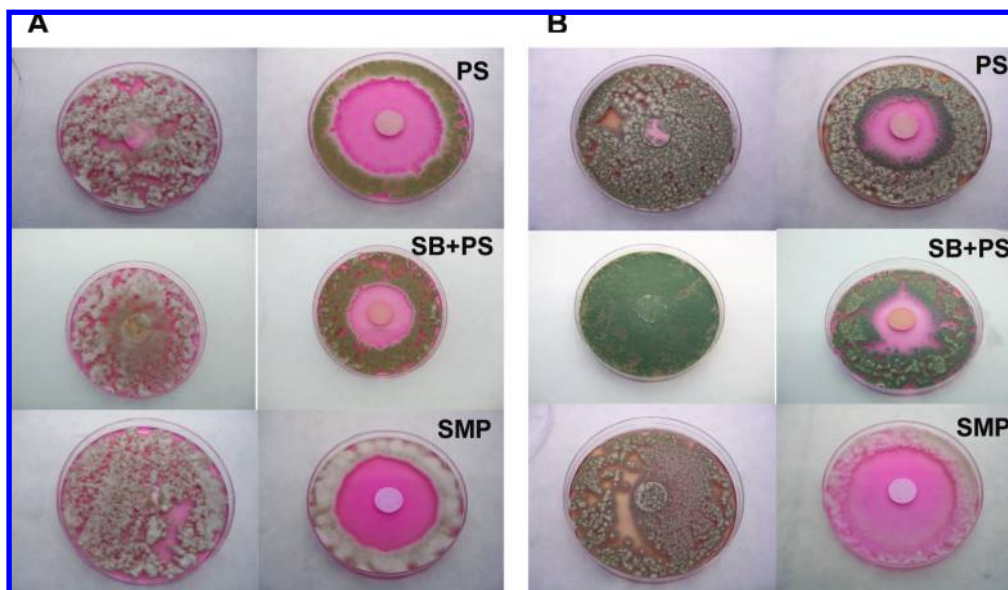


Figure 1. Disk diameter tests on DRBC agar plates for evaluation of the *in vitro* inhibition of (A) *P. digitatum* and (B) *P. italicum* by control HPMC–lipid films (left-side images) and HPMC–lipid films containing potassium sorbate (PS), a mixture of sodium benzoate and potassium sorbate (SB + PS) and sodium salt of methyl paraben (SMP) (right-side images). Inoculum density = 10^5 spores/mL.

In this study, mixtures of the most effective organic acid salts were added to HPMC–lipid films with the goal of achieving an additive or synergistic effect for the inhibition of PD or PI. Films with a mixture of PS + SP significantly inhibited ($p < 0.05$) the growth of PD on DRBC agar at all inoculum concentrations tested. PD at 10^3 spores/mL was more intensely inhibited (16.6 mm) than at 10^4 (12.0 mm) or 10^5 spores/mL (4.3 mm). The inhibition ability was therefore greatly dependent upon the pathogenic inoculum density (Table 3). In contrast, PI was not inhibited at all by films formulated with this mixture. Films containing a mixture of SB + PS significantly inhibited ($p < 0.05$) the growth of PD and PI on DRBC agar (Table 3). Jarret et al. (38) observed that the combination of sorbates with either benzoates or propionates may be used to inhibit microorganisms using lower concentrations of each preservative. However, in this work, the use of mixtures of PS + SP, SB + PS, and SB + SP incorporated to HPMC–lipid films resulted in smaller inhibition zones ($p < 0.05$) than the use of films containing PS or SB alone. Thus, there was not an additive effect for the inhibition of PD or PI (Table 3).

Paraben Salts and Mixtures. Films with sodium salts of parabens and mixtures were the most effective among all food preservatives tested for the inhibition of both PD and PI (Table 3). Large inhibition zones ($p < 0.05$) were observed on DRBC agar at all inoculum densities (Figure 1). Films containing sodium salt of methyl paraben (SMP) inhibited the growth of PD (22.1–24.2 mm) and PI (18.3–19.9 mm) in DRBC agar more than films containing other parabens and mixtures. However, an increase of SMP concentration in the film from 1.0 to 1.5% (wb) did not significantly increase the inhibition zone ($p > 0.05$) for both PD and PI (Table 3). When sodium salt of propyl paraben (SPP) was mixed with SMP (SMP + SPP), the resulting film was not superior to films with SMP alone in reducing the development of PD and PI ($p > 0.05$). Hence, the mixture of parabens did not provide a synergistic effect to control PD or PI.

Effective diffusion of antimicrobials from a film disk depends upon the size, shape (linear, branched, or cyclic) and polarity of the diffusing molecule, chemical structure of the film, and the degree of molecular cross-linking (41). Cagri et al. (42) reported that the application of whey protein isolated (WPI)

films containing sorbic acid (linear structure) resulted in higher inhibition of *Listeria monocytogenes* than the application of films containing *p*-aminobenzoic acid (cyclic structure), which was related to differences in diffusion rates. It is unknown how these factors affect the antifungal properties of HPMC–lipid films.

Other Compounds. Films with ethylenediaminetetraacetic acid (EDTA) showed clear zones of inhibition on DRBC agar for PD at 10^3 and 10^4 spores/mL and for PI at 10^3 spores/mL (Table 3). Shelef and Seiter (43) reported that EDTA may act as a direct inhibitor of several species of microorganisms and may act synergistically with other antimicrobial agents to promote bacterial destruction. Films containing 0.5% 2-deoxy-D-glucose did not inhibit the growth of PD and PI ($p > 0.05$) (Table 3). *In vivo* studies showed that the combination of low doses of 2-deoxy-D-glucose (0.2%, w/w) with *Candida saitoana* applied to fruit wounds before artificial inoculation with PD was effective for decay control on oranges and lemons, but this effect was reduced when either 2-deoxy-D-glucose or *C. saitoana* were applied separately (44).

Properties of Selected Films. WVP. HPMC–lipid films containing PS, SB, and parabens and their mixtures exhibited higher antifungal activity than other food preservatives tested (Table 3). Therefore, permeability and mechanical properties of these films were determined.

WVP is a measure of the ease with which a material can be penetrated by water vapor (45). In general, cellulose-based films present a poor moisture barrier because of their hydrophilic character. Incorporation of hydrophobic material improves film resistance to water vapor (46). In this study, HPMC–lipid films with 50% (db) total lipid content were used to increase the moisture barrier of the films. Films containing organic acid salts and their mixtures were made with a BW/shellac ratio of 1:1. Films with sodium salt of parabens only contained BW (Table 2).

The effect of food preservatives on WVP of selected HPMC–lipid films is shown in Table 4. Films containing PS alone or the mixture PS + SP exhibited higher WVP than the rest ($p < 0.05$). These values of WVP were around 2 times higher than those of films containing SB alone or the mixtures SB + PS and SB + SP ($p < 0.05$). These results indicate that

Table 4. Water Vapor and Oxygen Permeabilities of Selected HPMC–Lipid Edible Composite Films Containing Food Preservatives

HPMC–lipid films with food preservative ^b	permeabilities ^a		
	water vapor (g mm/kPa h m ²)		oxygen (cm ³ μm m ⁻² day ⁻¹ kPa ⁻¹)
	up ^c	down ^c	
potassium sorbate	6.62 c B	5.21 b A	153.11 b
sodium benzoate	2.85 b B	1.77 a A	82.01 a
potassium sorbate + sodium propionate	7.38 c A	6.21 b A	293.86 c
sodium benzoate + potassium sorbate	2.45 b A	2.56 a A	164.58 b
sodium benzoate + sodium propionate	3.24 b A	2.27 a A	170.44 b
sodium salt of ethyl paraben	1.11 a A	1.02 a A	713.73 d
sodium salt of propyl paraben	0.86 a A	1.00 a B	866.27 e

^a Water vapor and oxygen permeability means ($n = 3$) in columns with different lowercase letters are significantly different according to the Fisher protected LSD test ($p < 0.05$). Water vapor permeability means ($n = 3$) in rows with different capital letters are significantly different according to the Fisher protected LSD test ($p < 0.05$).

^b Characteristics of the films are shown in **Table 2**. ^c Film orientation during the water vapor permeability test according to the drying direction on casting plates.

Table 5. Mechanical Properties of Selected HPMC–Lipid Edible Composite Films Containing Food Preservatives

HPMC–lipid films with food preservative ^b	mechanical properties ^a		
	Young's modulus (MPa)	tensile strength (MPa)	elongation at break (%)
potassium sorbate	64.18 ab	0.23 a	5.05 c
sodium benzoate			
potassium sorbate + sodium propionate	32.11 a	0.14 a	7.78 d
sodium benzoate + potassium sorbate	331.04 d	1.98 c	0.94 a
sodium benzoate + sodium propionate	329.52 d	1.61 cd	0.92 a
sodium salt of ethyl paraben	135.53 bc	0.60 ab	2.93 b
sodium salt of propyl paraben	171.86 c	1.13 bc	4.76 c

^a Mechanical properties means ($n = 3$) in columns with different letters are significantly different according to the Fisher protected LSD test ($p < 0.05$). ^b Characteristics of the films are shown in **Table 2**.

PS alone or in combination with SP modified the HPMC–lipid film structure in a greater extent than films containing SB alone or in combination. Park et al. (23) reported that the addition of PS or chitosan to HPMC films increased WVP. This was attributed to a disruption of the crystalline structure of the homogeneous polymer network, leading to an increase of WVP.

HPMC–lipid films containing parabens had the lowest WVP. This could be due to interactions of parabens with the polymer matrix and/or differences in lipid composition of these films. These films had BW as the only hydrophobic component compared to films with organic acid salts that also had shellac on their formulation. Waxes have been shown to be more effective moisture barriers than resins, such as shellac (47).

To determine the lipid-phase separation in HPMC–lipid composite films, WVP was measured as a function of film orientation during drying. Phase separation was only observed on films containing PS or SB, with higher WVP when the films were in the “up” position than when the films were in the “down” position, indicating creaming of the lipid phase during drying. This finding contrasted with emulsion stability measurements that showed no phase separation after 48 h at 25 °C. Surprisingly, when SPP was incorporated into the HPMC–lipid film, the “down” position showed higher WVP than the “up” position ($p < 0.05$), indicating some enrichment on the lipid phase of the film surface in contact with the casting plate during drying. These results show that food additives have an important role in film morphology and final barrier properties.

Oxygen Permeability. The OP of HPMC–lipid films containing food preservatives is shown in **Table 4**. Films containing SB acted as good oxygen barriers and exhibited the lowest OP values. When HPMC–lipid film containing SB was combined with PS or SP, OP values were about 2-fold higher than that of the film with SB alone ($p < 0.05$). On the other hand, OP values of films containing PS were lower than that of films with the mixture PS + SP ($p < 0.05$). The mixture of two food preservatives added to the coating formulation might modify

the film matrix structure by increasing polymer mobility that permits oxygen migration through the film, thus increasing OP values. The films containing sodium salts of parabens exhibited the highest values of OP among all films tested ($p < 0.05$). These results, as with WVP values, could be due to the presence of the paraben salts and/or the difference in lipid composition of the films. The total amount of lipid in all of the films was 50% (db). However, films containing organic acid salts presented a combination of BW and shellac, while films with parabens contained only BW. Hagenmaier and Shaw (48) reported that OP was generally lower for coatings with shellac and rosin than for coatings with natural or synthetic waxes.

Mechanical Properties. **Table 5** shows mechanical properties of HPMC–lipid films containing selected food preservatives. Films containing SB were very brittle, and film samples were not possible to obtain for this analysis. The type of food preservative had a significant effect on Y , TS , and E . In general, HPMC–lipid films containing food preservatives presented low TS . HPMC–lipid films containing PS and the mixture PS + SP showed lower Y and TS and higher E than the rest of the assayed films ($p < 0.05$). Low Y and high E values generally indicate high flexibility of films. The addition of parabens to HPMC–lipid films resulted in an important increase of Y and TS values and a reduction of E values (**Table 5**), reducing the flexibility of the films and conferring stiffness and less extensibility. The different behavior of HPMC–lipid films with PS and parabens could be related to the different chemical structure of these food preservatives. Structurally, PS is a straight chain, which can penetrate more easily into the film matrix than parabens, which have a benzene ring. Therefore, PS may confer more mobility between HPMC chains resulting in lower Y and TS and greater flexibility of films. Accordingly, similar behavior related to TS and E was reported by Cagri et al. (42) in their study of WPI films containing stearic acid (straight-chain structure) and p -aminobenzoic acid (benzene ring). HPMC–lipid films containing mixtures of SB + PS and SB + SP showed

the highest *Y* and *TS* and the smallest *E* of all of the films tested ($p < 0.05$) (Table 5). These samples contained a total concentration of food preservatives higher than the rest (Table 2). In addition, these films contained a benzene ring (SB) that may influence the mobility in the film matrix and thus reduce film flexibility.

In conclusion, stable emulsions were obtained from HPMC–lipid containing food preservatives. HPMC–lipid stable emulsion suitable to use as coatings contained from 6 to 8% SC, 50% (db) total lipid content, and a maximum of 2.5% food preservative. Films containing sodium salts of parabens, PS, and SB and their mixtures exhibited clear inhibition activity against PD and PI. Barrier and mechanical properties of selected HPMC–lipid composite films depended upon lipid composition and the properties of the food preservative. Films containing parabens formulated with BW had lower WVP and higher OP than films with organic acid salts formulated with BW and shellac. Further studies should follow to determine the ability of these selected antifungal edible coatings to control PD and PI in *in vivo* tests with fresh citrus fruits, as well as the effect of their application on fruit postharvest quality. On the other hand, these films with antimicrobial properties could also be evaluated as low-toxicity means to reduce the risks associated with the presence of microbes of food safety concern.

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