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Antifungal activity of food additives in vitro and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria alternata* on cherry tomato fruit

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ABSTRACT

The antifungal activity of food additives or 'generally recognized as safe' (GRAS) compounds was tested in vitro against *Botrytis cinerea* and *Alternaria alternata*. Radial mycelial growth of each pathogen was measured in PDA Petri dishes amended with food preservatives at 0.2, 1.0, or 2.0% (v/v) after 3, 5, and 7 days of incubation at 25 °C. Selected additives and concentrations were tested as antifungal ingredients of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings. The curative activity of stable coatings was tested in in vivo experiments. Cherry tomatoes were artificially inoculated with the pathogens, coated by immersion about 24 h later, and incubated at 20 °C and 90% RH. Disease incidence and severity (lesion diameter) were determined after 6, 10, and 15 days of incubation and the 'area under the disease progress stairs' (AUDPS) was calculated. In general, HPMC-lipid antifungal coatings controlled black spot caused by *A. alternata* more effectively than gray mold caused by *B. cinerea*. Overall, the best results for reduction of gray mold on cherry tomato fruit were obtained with coatings containing 2.0% of potassium carbonate, ammonium phosphate, potassium bicarbonate, or ammonium carbonate, while 2.0% sodium methylparaben, sodium ethylparaben, and sodium propylparaben were the best ingredients for coatings against black rot.

1. Introduction

Cherry tomato (*Lycopersicon esculentum* L.) is a widely produced and consumed horticultural crop worldwide, for both fresh produce markets and processed food industries (Feng et al., 2011). This fruit is susceptible to postharvest disease caused by various pathogenic fungi. *Botrytis cinerea* Pers.: Fr. and *Alternaria alternata* (Fr.) Keissl., causing gray mold and black spot, respectively, are among the most common fungal pathogens responsible for postharvest decay on cherry tomato fruit (Wang et al., 2010).

Synthetic chemical fungicides have been used to reduce postharvest fungal spoilage, but because of problems regarding toxicity, fungicide resistance, and negative impact on both the environment and human health, alternative measures for disease control are increasingly demanded. In general, decay control methods that are alternatives to conventional synthetic fungicides can be classified as physical, chemical, or biological (Palou et al., 2008). Alternative methods that have been assayed against both *B. cinerea* and *A. alternata*, include cold storage in conventional controlled atmospheres, application of heat treatments (Zhong et al., 2010), use of ionizing radiation (Charles et al., 2009), biological control (Wang et al., 2008, 2010), or dips in aqueous solutions of food additives or other chemical compounds. Alternative chemical control methods comprise the use of natural or synthetic compounds with known and low toxicity, usually classified as food additives or 'generally recognized as safe' (GRAS) substances by most of food authorities worldwide (Palou et al., 2002).

The use of edible films and coatings is an alternative chemical method to preserve the postharvest quality of fruits and vegetables (Dhall, 2013). Consumer pressure for natural healthy products has led researchers to develop new edible films and coatings as an environmentally-friendly technology that may enhance food quality, safety, stability, and the mechanical handling properties by providing a semi-permeable barrier to water vapor, oxygen, and carbon dioxide between the food and the surrounding atmosphere (Dhall, 2013). In the last decade, several studies have focused on the development of coatings based on proteins or polysaccharides with natural food preservatives to control microbial growth on fruits and vegetables. Antimicrobials can be added to edible coatings to retard the growth of bacteria, yeasts, and molds during storage and distribution of fresh or minimally processed products (Lucera et al., 2012; Valencia-Chamorro et al., 2011). Coatings containing antimicrobials, such as some organic acids and their salts (Franssen et al., 2004), parabens and other food additives (Valencia-Chamorro et al., 2009b; Yildirim and Yapici, 2007), chitosan, essential oils, or natural plant extracts (Falguera et al., 2011; Sánchez-González et al., 2011) have been effective in delaying the growth of contaminating

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microorganisms and maintaining the quality during storage and distribution of fresh and fresh-cut horticultural products.

Different treatments have been evaluated for the control of postharvest decay of tomatoes. Essential oils including those from thyme, sage, cassia, or dill showed significant inhibitory activity against fungal pathogens such as *A. alternata* or *Aspergillus* spp. (Feng et al., 2011; Tian et al., 2011). Treatments with chitosan provided an effective control of tomato diseases caused by *B. cinerea* and *Penicillium expansum* (Liu et al., 2007). According to Pane et al. (2012), compost teas showed high biological control ability, both in vitro and in vivo on tomato, against *A. alternata*, *B. cinerea* and *Pyrenochaeta lycopersici*. Wang et al. (2010) studied the control of postharvest decay on cherry tomatoes by the marine yeast *Rhodosporidium paludigenum* and calcium chloride and verified that the combined treatments showed high activities to reduce black rot caused by *A. alternata*. A combination of heat treatment at 38 °C and the biocontrol agent *Pichia guilliermondii* prevented cherry tomato spoilage caused by the pathogens *B. cinerea*, *A. alternata*, and *Rhizopus stolonifer* (Zhao et al., 2010). However, available information on the development of new edible composite coatings with the addition of antifungal compounds as a new technique to control major fungal postharvest diseases of cherry tomatoes is very limited.

The objectives of this study were to: evaluate the in vitro activity of food additives with antifungal properties against *B. cinerea* and *A. alternata*; formulate stable hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing selected antifungal food preservatives; and determine the curative activity of these coatings for the control of gray mold and black rot on artificially inoculated cherry tomatoes.

2. Materials and methods

2.1. Pathogens and fungal inoculum

The strains TAA-1 of *B. cinerea* and TAV-6 of *A. alternata*, obtained from decayed tomatoes in Valencia packinghouses, were isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolates were grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in Petri dishes at 25 °C for 7–14 days. Depending on the experiment, mycelial plugs from these cultures were used, or high-density conidial suspensions were prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) in sterile water, passed through two layers of cheesecloth, measured with a hemacytometer, and diluted with sterile water to achieve an inoculum density of 1×10^6 spores/ml of *B. cinerea* or *A. alternata*.

2.2. Food preservatives

Food preservatives used in this work, molecular formulas, and the corresponding E-code list for food additives in the European Union (EU) are shown in Table 1. Most of them are likewise classified as food additives or GRAS compounds by the United States Food and Drug Administration (US FDA). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Sigma-Aldrich Chemie, Fluka Chemie AG (Buchs, Switzerland), Panreac Química S.L.U., or Merck KGaA (Darmstadt, Germany). Potassium silicate (PSi) was purchased from PQ Corporation, (Valley Forge, PA, USA) as the commercial product Sil-Matrix® (29% PSi).

2.3. Fruit

Cherry tomatoes (*L. esculentum* L.) used in the experiments were commercially grown and collected in the Valencia area (Spain) and stored up to 24 h at 5 °C until use. Fruits were free from previous postharvest treatments or coatings. Before each experiment, fruits were selected, randomized, washed with biodegradable fruit detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature.

Table 1
Characteristics of antifungal food preservatives tested in vitro or in vivo for inhibition of *Botrytis cinerea* and *Alternaria alternata*.

Food preservative	Acronym	Molecular formula	E-code[a]	MW[b]
Potassium sorbate	PS	$C_6H_7O_2K$	E-202	150.22
Sodium acetate	SA	CH_3COONa	E-262 (i)	82.03
Sodium methylparaben	SMP	$C_8H_7NaO_3$	E-219	174.13
Sodium propylparaben	SPP	$C_{10}H_{11}NaO_3$	–[c]	202.19
Sodium ethylparaben	SEP	$C_9H_9NaO_3$	E-215	188.16
Sodium propionate	SP	CH_3CH_2COONa	E-281	96.06
Sodium benzoate	SB	$C_7H_5O_2Na$	E-211	144.11
Potassium carbonate	PC	K_2CO_3	E-501 (i)	138.21
Ammonium phosphate	APh	$NH_4H_2PO_4$	E-342 (i)	132.07
Ammonium carbonate	AC	$(NH_4)_2CO_3$	E-503 (i)	114.1
Potassium silicate	PSi	K_2SiO_3	E-560	154.26
Sodium formate	SF	$HCOONa$	E-237	68.01
Sodium bicarbonate	SBC	$NaHCO_3$	E-500 (ii)	84.01
Potassium bicarbonate	PBC	$KHCO_3$	E-501 (ii)	100.12
Ammonium bicarbonate	ABC	NH_4HCO_3	E-503 (ii)	79.06

[a] E-code = code number for food additives approved by the European Union.
[b] Molecular weight.
[c] Excluded from the EU list of food additives according to CR EU (2011).

2.4. Determination of in vitro antifungal activity of food preservatives

The effect of potassium sorbate (PS), sodium benzoate (SB), sodium acetate (SA), sodium propionate (SP), sodium formate (SF), sodium methylparaben (SMP), sodium ethylparaben (SEP), sodium propylparaben (SPP), and PSi (Table 1) on mycelial growth of *B. cinerea* and *A. alternata* was evaluated on 90 mm plastic Petri dishes with PDA medium amended at 45–55 °C with sterile aqueous solutions of food additives. Stock solutions of 20% of each salt were used to achieve final salt concentrations of 0.2, 1.0 and 2.0% (v/v). Plates with PDA without salts served as control. The center of each test plate was inoculated with a 5-mm diameter plug of 7–15 day-old cultures of *B. cinerea* or *A. alternata* and incubated for 7 days at 25 °C in the dark in a growth cabinet. Radial mycelial growth was determined in each plate after 3, 5 and 7 days of incubation by calculating the mean of two perpendicular diameters of the fungal colony. For each pathogen, salt, and salt concentration, four replicate plates were used. The results were expressed as percentage of mycelial growth inhibition according to the formula: $(dc - dt) / dc \times 100$, where dc = average diameter of the fungal colony on control plates and dt = average diameter of the fungal colony on salt-amended plates.

Other food preservatives (Table 1) that were used in this research in in vivo trials, but that were not tested in vitro because their in vitro antifungal activity against *B. cinerea* and *A. alternata* has already been reported in the literature include: potassium carbonate (PC; Nigro et al., 2006), ammonium phosphate (APh; Nigro et al., 2006), ammonium carbonate (AC; Palmer et al., 1997), sodium bicarbonate (SBC; Mills et al., 2004; Nigro et al., 2006; Palmer et al., 1997), potassium bicarbonate (PBC; Palmer et al., 1997), and ammonium bicarbonate (ABC; Nigro et al., 2006; Palmer et al., 1997).

2.5. Formulation and preparation of antifungal coatings

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA) and beeswax (BW) (grade 1) was supplied by Fomesa Fruitech S.L. (Valencia, Spain). Oleic acid and glycerol were from Panreac Química S.L.U. HPMC-lipid edible composite emulsions were prepared by combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. Ratios of HPMC–