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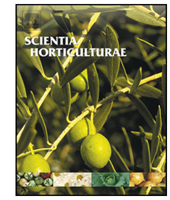

Hydroxypropyl methylcellulose-beeswax edible coatings formulated with antifungal food additives to reduce alternaria black spot and maintain postharvest quality of cold-stored cherry tomatoes

Cristiane Fagundes[a], Lluís Palou[b,*], Alcilene R. Monteiro[a], María B. Pérez-Gago[b,c]

[a] Universidade Federal de Santa Catarina, Departamento de Engenharia Química e Engenharia de Alimentos, Campus Universitário-Trindade, 88040-900 Florianópolis, SC, Brazil

[b] Centre de Tecnologia Postcollita (CTP), Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain

[c] Fundació AGROALIMED, Apartat Oficial, 46113 Montcada, València, Spain

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ABSTRACT

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and food preservatives with antifungal properties were formulated and evaluated on cherry tomatoes during cold storage. Selected food preservatives included: sodium methyl paraben (SMP), sodium ethyl paraben (SEP) and sodium benzoate (SB). Cherry tomatoes artificially inoculated with *Alternaria alternata* were coated and stored up to 21 d at 5 °C followed by 4 d of shelf-life at 20 °C. All antifungal coatings reduced the incidence and severity of alternaria black spot on inoculated cherry tomatoes, being the SB-based coating the most effective. Analytical and sensory fruit quality was evaluated on intact and cold-stored tomatoes. In contrast to coatings containing SMP or SEP, the SB-based coating was effective to reduce weight loss and respiration rate and maintain the firmness of coated cherry tomatoes. Peel color, ethanol and acetaldehyde content of the juice, sensory flavor, off-flavors, and fruit appearance were not adversely affected by the application of the antifungal coatings. In conclusion, HPMC-BW coatings containing the food additive SB at 2% showed potential for industrial application, including the production and commercialization of organic cherry tomatoes.

1. Introduction

Tomato fruits have a relatively short postharvest life and during fruit ripening many processes reducing fruit quality may take place, leading to important economic losses. Therefore, the development of new technologies to effectively control ripening and decay would be of great economic importance (Hoeberichts et al., 2002). *Alternaria alternata* (Fr.) Keissl., causing black spot, is among the most common fungal pathogens responsible for postharvest decay of cherry tomato fruit (Wang et al., 2008). The use of synthetic chemical fungicides as antimicrobial agents to control fungal spoilage of fresh horticultural products has been practiced for many years. However, concerns about environmental contamination and human health risks associated with fungicide residues on/in produce, as well as the proliferation of fungicide-resistant strains of the pathogens, have led to serious restrictions or even bans of many synthetic fungicides (Palou et al., 2008). At present, there is a lack of authorized postharvest treatments and/or registered fungicides available for the control of postharvest diseases of high value commercial fruits, such as tomato. Alternative methods that have been proposed for the control of postharvest diseases include biological control with antagonistic microorganisms, physical methods such as heat or radiations, and the use of low-toxicity chemicals with antimicrobial activity (Montesinos-Herrero et al., 2009; Palou et al., 2002, 2008; Valencia-Chamorro et al., 2009a). The latest include natural or synthetic compounds of known and low toxicity, usually classified as safe food-grade additives or Generally Regarded as Safe (GRAS) substances by international authorities (Larrigaudière et al., 2002; Palou et al., 2002).

In recent years, the release of antimicrobial agents incorporated into biodegradable edible films and coatings has emerged as a new, effective, and environmentally-friendly alternative mean to extend the shelf-life of many products including fresh fruits and vegetables. Edible coatings provide a semi-permeable barrier to water vapor, oxygen (O_2), and carbon dioxide (CO_2) that reduce weight loss and respiration. Additional advantages of edi-

* Corresponding author.
E-mail address: palou_llu@gva.es (L. Palou).

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ble coatings are the possibility to maintain the firmness of the fruit and provide gloss to coated products (Greener-Donhowe and Fennema, 1994). Edible coatings are based on polysaccharides, proteins and lipids or a mixture of these. Other food-grade ingredients such as antimicrobial agents, antioxidants, flavors, color pigments, and vitamins can also be incorporated into the basic formulation of these coatings with the aim to improve their functional properties (Valencia-Chamorro et al., 2011a,b). Among the active ingredients used in antimicrobial edible coatings, compounds such as plant essential oils, food aromas, organic acids, parabens, their salts and other permitted food additives or GRAS compounds, have been preferred for fruit and vegetables (Das et al., 2013; Fagundes et al., 2013; Valencia-Chamorro et al., 2009a; Xu et al., 2007). Our research group optimized stand-alone hydroxypropyl methylcellulose (HPMC)-lipid edible composite films containing a wide variety of food additives and GRAS compounds such as mineral salts, organic acid salts and their mixtures, and sodium salts of parabens and their mixtures to provide antifungal activity against the citrus pathogens *Penicillium digitatum* and *Penicillium italicum* (Valencia-Chamorro et al., 2008). Then, selected coatings were tested *in vivo* against green and blue molds on different citrus cultivars. The inhibitory activity of the coatings was strongly dependent on the susceptibility of each citrus cultivar to penicillium decay and the storage temperature (Valencia-Chamorro et al., 2009a,b, 2010, 2011b). Similar studies also proved the antifungal activity of several mineral salts, organic acid salts, and paraben salts incorporated to HPMC-BW coatings against the pathogens *Monilinia fructicola* in artificially inoculated plums (Karaca et al., 2014) and *Botrytis cinerea* and *A. alternata* in inoculated cherry tomatoes during shelf-life at 20 °C (Fagundes et al., 2013). In a recent work, the best coatings against *Botrytis cinerea* were evaluated on cherry tomatoes cold-stored at 5 °C and it was observed that the effect of the coatings on disease development and fruit quality during storage was dependent on the storage temperature, remarking the need to evaluate the coatings under commercial storage conditions (Fagundes et al., 2014). In our previous study to select appropriate antifungal coatings for the control of alternaria black spot of cherry tomato, the best results after incubation of coated fruit at 20 °C were obtained with HPMC-BW coatings containing 2.0% sodium benzoate (SB), sodium ethyl paraben (SEP), or sodium methyl paraben (SMP) (Fagundes et al., 2013). The objective of the present research was to determine the effect of selected HPMC-BW edible coatings formulated with antifungal food additives on the development of alternaria black spot and the physico-chemical and sensory quality of cherry tomatoes during cold storage. This information is needed for potential commercial development of suitable antifungal edible coatings.

2. Materials and methods

2.1. Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). BW (grade 1) was supplied by Fomesa Fruitech, S.L. (Beniparrell, València, Spain). Oleic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Fluka Chemie AG (Buchs, Switzerland) and Merck KGaA (Darmstadt, Germany), and included SMP ($C_8H_7NaO_3$; E-218), SEP ($C_9H_9NaO_3$; E-214), and SB ($C_7H_5O_2Na$; E-211). All these chemicals are classified as food additives (with their correspondent E-number) or GRAS compounds by the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (US FDA).

2.2. Emulsions preparation

HPMC–lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. All the formulations contained 30% BW (dry basis, db) and the ratios of HPMC–glycerol (3:1) (db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w) to improve wetting of the coating and adherence to the tomato fruit. All formulations contained 2.0% (w/w) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 °C and later hydration at 20 °C. The corresponding food preservative, BW, glycerol, oleic acid, and water were added to the HPMC solution and heated at 98 °C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12.000 and 3 min at 22.000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 °C by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The emulsions were prepared with a final solid concentration of 10% and had a viscosity in the range of 140–147 cp. Table 1 shows the viscosity and pH of the emulsions containing selected food preservatives. Emulsions were kept 1 d at 5 °C before use. These formulations were stable and no phase separation was observed.

2.3. Effect of coatings on disease development

2.3.1. Fungal inoculum

The strain TAV-6 of *A. alternata*, obtained from decayed tomato fruit in Valencia packinghouses, was isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolate was grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in petri dishes at 25 °C for 7–14 d. From this culture, a high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) and sterile water. This suspension was passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1×10^6 spores/mL of *A. alternata.*

2.3.2. Fruit inoculation and coating application

Cherry tomatoes (*Solanum lycopersicum* L. var. *cerasiforme* cv. Josefina; syn.: *Lycopersicon esculentum* Mill.) 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. Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent at 6% (v/v) (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the pathogen by placing 10 μl of a spore suspension containing 1×10^6 spores/ml of *A. alternata*. After incubation at 20 °C for 24 h, inoculated fruit were coated by immersion for 30 s in the selected HPMC-BW edible composite emulsions, drained, and allowed to air-dry at 20 °C. Inoculated but uncoated fruit were used as controls. Coated fruit were placed on plastic trays on corrugated cartons and stored up to 21 d at 5 °C and 90–95% RH, followed by 4 d of shelf-life at 20 °C. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.