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Effect of antifungal hydroxypropyl methylcellulose-beeswax edible coatings on gray mold development and quality attributes of cold-stored cherry tomato fruit

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ABSTRACT

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and food preservatives with antifungal properties, were evaluated on cherry tomatoes during cold storage. Food preservatives selected from previous research work included sodium propionate (SP), potassium carbonate (PC), ammonium phosphate (APh) and ammonium carbonate (AC). Cherry tomatoes artificially inoculated with *Botrytis cinerea* were coated and stored up to 15 d at 5 °C followed by 7 d of shelf-life at 20 °C. All antifungal HPMC-BW coatings significantly reduced gray mold development on inoculated and cold-stored cherry tomatoes, the SP-based coating being the most effective. Analytical and sensory fruit quality was also evaluated after cold storage and shelf-life. The AC-based coating was the most effective to control weight loss and maintain the firmness of coated cherry tomatoes. Respiration rate, firmness, color, sensory flavor, off-flavor, and fruit appearance were not adversely affected by the application of the antifungal coatings. Overall, the application of HPMC-BW edible composite coatings containing AC could be a promising treatment to extend the postharvest life of cherry tomatoes. Further studies should focus on the modification of some physical characteristics of the coatings in order to enhance the general performance and provide higher peel gloss.

1. Introduction

During the last decades, there has been an increased interest by consumers in natural healthy fresh fruit and vegetables. Tomato (*Solanum lycopersicum* L.), being a climacteric fruit, has a relatively short postharvest life, generally limited by transpiration, postharvest diseases, increased ripening and senescence (Zapata et al., 2008). Although storage under optimum cold storage conditions has been effective in extending shelf-life as it reduces the rate of respiration of the fruit, the benefits from refrigeration are not important enough to preserve produce quality. Tomato fruit is susceptible to postharvest diseases caused by various pathogenic fungi that cause important economic losses. *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).

Several technologies have been developed to extend the shelf-life of fruit and vegetables, which include the control of diseases caused by fungi. One of these techniques is the release of antimicrobial agents incorporated into biodegradable edible films and coatings (Valencia-Chamorro et al., 2011). Edible coatings are considered an environmentally friendly technology able to extend the shelf-life of fruit and vegetables by reducing moisture loss and respiration rate, preventing physical damage, and enhancing product appearance. These coatings are commonly based on polysaccharides, proteins, and lipids, alone or in combination. In fruit and vegetables, composite coatings based on polysaccharides or proteins and lipids are usually used to achieve good moisture and gas barriers provided by the lipid and polymer components, respectively. Among the hydrophobic materials, waxes such as BW have been the most widely used for protective moisture barriers in fresh commodities. Furthermore, the addition of food preservatives can improve the functional properties of the coatings by retarding the growth of bacteria, yeasts, and molds during storage and distribution of fresh fruit and vegetables (Valencia-Chamorro et al., 2011; Lucera et al., 2012).

In tomato, the development of antifungal edible coatings has been mainly focused on chitosan-based formulations. These

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coatings have been effective controlling black spot caused by *A. alternata* (Reddy et al., 2000), gray and blue molds caused by *B. cinerea* and *Penicillium expansum*, respectively (Liu et al., 2007; Badawy and Rabea, 2009), anthracnose caused by *Colletotrichum* spp. (Muñoz et al., 2009), and rhizopus rot caused by *Rhizopus stolonifer*, when combined with essential oils (Ramos-García et al., 2012). The addition of food additives, or 'generally recognized as safe' (GRAS) compounds with antifungal properties, to other hydrocolloids has been less studied in tomato. Pea starch coatings amended with potassium sorbate showed some antifungal activity against *P. expansum* and *Cladosporium fluvum*, although decay was only significantly controlled for 5 days at 5 °C (Mehyar et al., 2011). In recent work, we studied the *in vitro* activity of a wide variety of food additives (mineral salts, organic acid salts, paraben salts, and other GRAS compounds) with antifungal properties against *B. cinerea*, formulated stable hydroxypropyl methylcellulose (HPMC)-beeswax (BW) edible composite coatings containing selected antifungal food preservatives, and determined the beneficial activity of these coatings against gray mold on cherry tomatoes artificially inoculated with *B. cinerea* (Fagundes et al., 2013). Overall, the best results for reduction of gray mold on cherry tomato fruit incubated at 20 °C were obtained with coatings containing 2.0% sodium propionate (SP), potassium carbonate (PC), ammonium phosphate (APh), or ammonium carbonate (AC). The next research step for potential commercial development of these antifungal coatings is the evaluation of their performance on cold-stored cherry tomatoes. Therefore, the objective of this work was to determine the effect of selected HPMC-lipid edible composite coatings containing food additives with antifungal properties on the development of gray mold and the physico-chemical and sensory quality of cherry tomatoes during cold storage.

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 Sigma–Aldrich Chemie (Steinheim, Germany) and included SP (CH_3CH_2COONa; E-number E-281), PC (K_2CO_3; E-501 (i)), Aph ($NH_4H_2PO_4$; E-342 (i)), and AC [$(NH_4)_2CO_3$; E-503 (i)]. 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. Emulsion preparations

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 emulsions contained 30% BW (w/w, db). Ratios of HPMC-glycerol (3:1) (dry basis) and BW-oleic acid (5:1) (dry basis) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w, wet basis; Panreac-Química S.A., Barcelona, Spain) to improve wetting of the coating and adherence to tomato surface. All formulations contained 2% (w/w, wet basis) 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, Tween 80, and water were added to the HPMC solution and heated at 98 °C to melt the lipid. 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 final solid concentration of the emulsions were optimized to obtain formulations with a viscosity range of 100–150 cp. Table 1 shows the solid concentration, viscosity and pH of the emulsions containing selected food preservatives. Emulsions were kept 1 day 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 TAA-1 of *B. cinerea*, obtained from decayed tomatoes in Valencia packinghouses, was isolated, identified, tested for pathogenicity, 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 days. A high-density conidial suspension was prepared in Tween 80 (0.05%, w/v) in sterile water, 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 *B. cinerea*.

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). Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with a fruit biodegradable detergent at 6% (v/v) (Essasol V., Dydsa, 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 *B. cinerea*. After incubation at 20 °C for 24 h to resemble common fungal infections, inoculated fruit were coated by immersion for 30 s in the selected HPMC-lipid edible composite emulsions, drained, and allowed to air-dry at 20 °C. Inoculated but uncoated fruit were used as control. Coated fruit were placed on plastic trays on corrugated cartons that avoided fruit contact and stored for 14 days at 5 °C, followed by 7 d at 20 °C and 85–90% RH. These conditions simulated typical commercial cold storage and shelf-life for Spanish cherry tomatoes. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.

2.3.3. Determination of disease incidence and severity

Gray mold incidence was calculated as the percentage of decayed fruit. Disease severity was determined as the diameter of the lesion (mm). Both incidence and severity were assessed after 7 and 14 d of storage at 5 °C, and also after a shelf-life period of 7 d at 20 °C following cold storage.

2.4. Effect of coatings on fruit quality

2.4.1. Fruit coating and storage

For the quality study, fruit were selected, randomized, washed with biodegradable detergent, rinsed with tap water, and allowed to air-dry at room temperature. Fruit were then divided into 5 groups of 120 fruit each, which corresponded to the four coating