Innovative Food Science and Emerging Technologies 34 (2016) 112–121

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

The effects of composite coatings containing chitosan and *Mentha* (*piperita* L. or *x villosa* Huds) essential oil on postharvest mold occurrence and quality of table grape cv. Isabella

Ingrid Conceição Dantas Guerra [a], Priscila Dinah Lima de Oliveira [a], Miriane Moreira Fernandes Santos [c], Ana Sílvia Suassuna Carneiro Lúcio [b], Josean Fechine Tavares [b], José Maria Barbosa-Filho [b], Marta Suely Madruga [c], Evandro Leite de Souza [a,*]

[a] *Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba, João Pessoa, Brazil*
[b] *Department of Pharmaceutical Sciences, Federal University of Paraíba, Paraíba, Brazil*
[c] *Laboratory of Chemical Analysis of Foods, Federal University of Paraíba, Brazil*

ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 30 December 2015
Accepted 7 January 2016
Available online 27 January 2016

Keywords:
Edible coatings
Vitis labrusca L.
Fungi infection
Postharvest treatment

ABSTRACT

The effects of coatings containing shrimp chitosan and the essential oil from *Mentha piperita* L. (MPEO) or *M. villosa* Huds (MVEO) to control common mold infections in table grape cv. Isabella (*Vitis labrusca* L.) that were caused by *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* were evaluated. The effects of the coatings on physicochemical and sensory characteristics of the grapes were also assessed. The coatings containing chitosan (4, 8 mg/mL) and MPEO or MVEO (1.25, 2.5, 5 μL/mL) delayed the mold growth and reduced the incidence of infections caused by all test fungi in grapes during storage at room and low temperatures. The coatings (chitosan 4 mg/mL; MPEO or MVEO 1.25, 2.5 μL/mL) did not negatively affect the physicochemical and sensory characteristics of grapes. From these results, coatings containing chitosan and MPEO or MVEO are potential postharvest treatments to control common mold infections in table grape cv. Isabella.

Industrial relevance: In table grapes, the reduction of losses resulting from fungal rot is a major goal of postharvest technology, which seeks to use effective methods to control the contamination and the growth of phytopathogenic fungi. However, the negative consumer perception of synthetic fungicides used for many years to solve the problem of mold infections in table grapes and the development of fungicide-resistant strains have impelled researchers to study the efficacy of natural compounds against postharvest pathogenic fungi. In this context, edible coatings composed of chitosan and essential oils have been considered as an environmentally friendly and added-value technology to control fungal postharvest decay in table grapes because of their biodegradability and lack of phytotoxicity. In this study, the authors evaluated composite coatings that contained shrimp chitosan and reduced amounts of *Mentha* (*piperita* L. or *x vilosa* Huds) essential oil as postharvest treatment to control the occurrence of mold infections caused by fungi in table grape cv. Isabella (*Vitis labrusca* L.), and their effects on the quality attributes of this fruit during storage. The tested composite coatings are presented as possible alternative technologies to control fungal infections and related post-harvest losses in table grape cv. Isabella.

1. Introduction

The consumption of the table grapes has been associated with several benefits to consumers because grapes are a source of phenolic compounds that possess antioxidant and oxygen radical-scavenging properties (Krikorian et al., 2012), and protective effects against degenerative diseases, particularly cardiovascular diseases (Cadez, Zupan, & Raspor, 2010; Merín, Mendoza, & Morata de Ambrosini, 2014). These characteristics have represented added value for fresh table grapes, which increases both the interest in their daily consumption and the demand for high-quality fruit in the marketplace (Duan, Wu, Strik, & Zhao, 2011). However, the table grape is a highly perishable, non-climacteric fruit that experiences severe postharvest problems, such as loss of firmness, berry drop, stem discoloration, desiccation and fungal rot (Meng, Li, Liu, & Tian, 2008).

The postharvest fungal decay of table grapes is a major problem that affects fruit quality during storage and frequently results in grapes becoming unmarketable. These harmful effects on table grapes are typically associated with mold infections that are induced by *Botrytis cinerea*, *Penicillium expansum*, *Aspergillus niger* and *Rhizopus stolonifer*

* Corresponding author at: Universidade Federal da Paraíba, Centro de Ciências da Saúde, Departamento de Nutrição, Campus I, 58051-900, Cidade Universitária, João Pessoa, Paraíba, Brazil. Tel.: +55 83 3216 7807; fax: +55 83 3216 7094.
E-mail address: evandroleitesouza@ccs.ufpb.br (E.L. de Souza).

http://dx.doi.org/10.1016/j.ifset.2016.01.008
1466-8564/

(Abdolahi, Hassani, Ghosta, Bernousi, & Meshkatalsadat, 2010; Santos et al., 2012; Sousa et al., 2013; Tian et al., 2014). The colonization of table grapes by *A. niger*, *B. cinerea*, *P. expansum* or *R. stolonifer* can result in the development of a characteristic set of symptoms in infected fruit, which are termed black, gray, blue and soft-rot mold, respectively (Lichter et al., 2002; Romanazzi & Feliziani, 2014).

The control of mold infections in this fruit has conventionally been achieved through the use of chemical fungicides, primarily SO_2, during both pre and postharvest periods. However, SO_2 application is becoming restrictive in many countries because SO_2 residues are potentially hazardous to human health (Pastor et al., 2011; Sánchez-González et al., 2011). Additionally, SO_2 frequently has injurious effects on fresh table grapes and can induce bleaching of the berries (Sánchez-González et al., 2011). As an alternative to the use of synthetic fungicides in table grapes, researchers have perceived added value in the use of edible coatings that contain chitosan and essential oils (EOs), which could provide environmentally friendly treatments to control mold infections and maintain fruit quality during storage (Liu, Tian, Meng, & Xu, 2007; Oliveira et al., 2014; Santos et al., 2012). These coatings create a semi-permeable barrier on fruit that can control microbial growth and reduce moisture loss and respiration rate, thereby preventing infection and physical damage and enhancing product appearance (Fagundes, Palou, Monteiro, & Pérez-Gago, 2014; Gao, Zhu, & Zhang, 2013). Because chitosan and EOs are generally recognized as safe (GRAS) by the FDA, this facilitates their use in the formulation and application of coatings as preservation techniques for fruit (Fagundes et al., 2014).

Chitosan is a high molecular weight cationic polysaccharide that is obtained by the deacetylation, in alkaline media, of chitin (β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units), which is extracted from the exoskeleton of crustaceans, fungi and insects (Kanatt, Chander, & Sharma, 2008). Studies have shown that the incorporation of EOs into chitosan dispersions can markedly improve their antimicrobial activities and some of their physicochemical properties, such as biodegradability and the ability to form films and water vapor barriers, thereby extending the shelf life of fruit (Kanatt et al., 2008; Sánchez-González et al., 2011).

In the food industry, the EOs of *Mentha* spp., primarily *Mentha piperita* L. essential oil (MPEO), are used as flavoring agents in foods and beverages and are commonly exploited because of their antioxidant, antimicrobial and sensory properties (Riahi et al., 2013; Tyagi & Malik, 2011). *M. x villosa* Hudson is an aromatic plant hybrid of *M. spicata* L. and *M. suaveolens* Ehrh. that is commonly used in Brazilian folk medicine (Lahlou, Carneiro-Leão, & Leal-Cardoso, 2002). MPEO and *M. villosa* Huds essential oil (MVEO) have shown promising results in the inhibition of phytopathogenic fungi (Lima et al., 2014; Riahi et al., 2013). Although the application of chitosan and some EOs has proven to be effective in controlling mold infections in table grapes (Oliveira et al., 2014; Santos et al., 2012; Sousa et al., 2013), studies verifying the efficacy of composite coatings containing chitosan and MPEO or MVEO to control the occurrence of different mold infections in fruit are still scarce (Guerra et al., 2015).

This study evaluated composite coatings that contained shrimp chitosan and reduced amounts of MPEO or MVEO as postharvest treatments to prevent the occurrence of mold infections caused by *A. niger*, *B. cinerea*, *P. expansum* or *R. stolonifer* in cv. Isabella table grapes that had been artificially contaminated with each tested fungi. Additionally, the effects of the tested composite coatings on some physicochemical and sensory characteristics of table grapes during storage were verified.

2. Materials and methods

2.1. Materials

Commercially mature cv. Isabella table grapes (*Vitis labrusca* L.), *i.e.*, grapes with purple color, were obtained from EMPASA (Supplies and Services Company of Paraíba, João Pessoa, Brazil). Grapes with no visible sign of mechanical damage or fungal infection were selected and standardized according to size, appearance, color and shape. Prior to the assays with artificially contaminated fruit and for evaluation of physicochemical quality parameters, the grapes were dissected from the bunch (but maintaining the stem-cap) using scissors, surface-disinfected via immersion in a sodium hypochlorite solution (150 ppm, pH 7.2 adjusted using 1 M NaOH) for 5 min, washed with sterile distilled water and dried for 2 h in a safety cabinet.

A. niger URM 5162, *B. cinerea* URM 2802, *P. expansum* URM 3396 and *R. stolonifer* URM 3482 were obtained from the University of Recife Mycology Culture Collection (Center for Biological Sciences, Federal University of Pernambuco, Recife, Brazil). The stock cultures were subcultured in Sabouraud agar (Himedia, India) at 25 °C for 7 days to facilitate sufficient sporulation. The fungal spores were collected in a sterile saline solution (0.85 g/100 mL NaCl) in Sabouraud broth (Himedia, India), and the resulting suspension was filtered through a triple layer of sterile gauze to retain the hyphal fragments. The number of spores present in the suspension was quantified using a hemocytometer. The spore concentration was adjusted with sterile saline solution to yield an inoculum of approximately 10^6 spores/mL (Rasooli & Owlia, 2005).

The shrimp shells used for chitosan extraction came from a single species of shrimp, *Litopenaeus vannamei* (Boone, 1931), and were provided by Aquamaris Aquaculture S/A (João Pessoa, Brazil). The chitosan was extracted from the shrimp shells using a previously described procedure (Guerra et al., 2015). The CHI in powder form was vacuum-packaged and stored under refrigeration (7 °C) for a maximum period of 30 days until use in subsequent assays. The obtained shrimp chitosan presented a deacetylation degree of 83% and a degree of cristalinity of 71% (Guerra et al., 2015), and its identity based on the diffraction peaks at angles of 10° and 20° 2Θ, relative to the crystallographic letter (ICDD 00-035-1974), was confirmed (Guerra et al., 2015). The average viscosimetric molar weight of the obtained chitosan was 11.62×10^4 g/mol, as determined using a procedure described elsewhere (dos Santos, Soares, Dockal, Campana Filho, & Cavalheiro, 2003; de Oliveira et al., 2014).

M. piperita and *M. villosa* leaves were collected at the Medicinal Plants Garden, Institute for Research in Drugs and Medicines, Federal University of Paraíba (João Pessoa, Brazil) in July 2011 (7°08′29″S, 34°50′48″W). Voucher specimens for *M. piperita* and *M. villosa* were deposited at the Herbarium Prisco Bezerra, Federal University of Ceará (Fortaleza, Brazil) under numbers 14423 and 14996, respectively. The EOs were extracted by hydrodistillation using a Clevenger apparatus as described elsewhere (Guerra et al., 2015; Mkadden et al., 2009). A previous study identified the individual constituents forming the MPEO and MVEO tested in this study, and found L(−)-menthol (30.31%) as the majority constituent in MPEO, followed by isomenthone (26.70%), menthol acetate (8.52%) and eucalyptol (7.03%), while rotundifolone (70.2%) was found as the majority compound in MVEO, followed by limonene (6.42%), β-pinene (4.30%) and eucalyptol (4.29%) (Guerra et al., 2015).

2.2. Production of coatings containing chitosan and EOs

Chitosan concentrations of 4 and 8 mg/mL were used to form the edible coatings because 4 mg/mL was the lowest concentration that was capable of forming a viscous solution with coating features that permitted its application as a coating for grapes. CHI concentrations greater than 8 mg/mL formed highly viscous dispersions that were not capable of forming homogeneous and continuous films when they were applied to fruit. For the tested EOs, the concentrations were set at 1.25, 2.5 and 5 μL/mL because researchers have stated that EOs could be incorporated at low concentrations (<10 μL/mL) in coating dispersions to minimize their impact on the olfactory perception of consumers (Guerra et al., 2015; Perdones, Sanchez-Gonzalez, Chiralt, & Vargas, 2012). Studies assessing the *in vitro* antifungal properties of EOs against common mold-causing fungi have found inhibitory effects when these substances