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Potential application of natural phenolic antimicrobials and edible film technology against bacterial plant pathogens

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ABSTRACT

The aim of the present study is to use antimicrobial edible film technology and natural phenolic antimicrobials for inhibition of major bacterial plant pathogens such as *Erwinia amylovora, Erwinia carotovora*, *Xanthomonas vesicatoria* and *Pseudomonas syringae.* For this purpose phenolic acids (PAs) (gallic (GA), vanillic (VA), cinnamic acids (CA)), essential oils (EOs) (carvacrol (CAR), thymol (THY), eugenol (EUG) citral (CIT)), phenolic extracts (PEs) from clove (CE), oregano (OE), artichoke stem (ASE) and walnut shells (WSE) were evaluated as antimicrobial zein film components. Films containing PAs between 1 and 4 mg/cm^2 inhibited all pathogens while EOs between 1 and 4 mg/cm^2 and CE between 4 and 8 mg/cm^2 inhibited pathogens except *P. syringae*. The most potent films were obtained by using GA against *E. amylovora* and *P. syringae*, VA against *E. carotovora,* and CA, THY or CAR against *X. vesicatoria.* The addition of phenolic compounds into films increased the porosity of films. The phenolic containing films also become more flexible and lost their brittleness. This study is important in that it prepared the basis of using edible antimicrobial coatings in outdoor applications on infected tree stems, soil surfaces and agronomy tools or in classical fruit and seedling coating applications to control bacterial contamination or spoilage.

1. Introduction

The use of edible biopolymeric materials and natural antimicrobial compounds in antimicrobial packaging provides a promising alternative method to inhibit the growth of pathogenic and spoilage microorganisms in food and to increase safety and quality of food products. Thus, extensive studies have been conducted in the recent years to develop edible films and coatings from biopolymers such as zein, whey proteins, soy proteins, chitosan, alginate, carrageenan, pullulan, cellulose and its derivatives (Gniewosz et al., 2014; Joerger, 2007; Mendes de Souza, Fernández, López-Carballo, Gavara, & Hernández-Muñoz, 2010; Rojas-Grau et al., 2007; Zhong, Cavender, & Zhao, 2014). Different natural antimicrobials including phenolic extracts, essential oils, bacteriocins and antimicrobial enzymes have been incorporated into edible films to obtain antimicrobial packaging materials (Alboofetileh, Rezaei, Hosseini, & Abdollahi, 2014; Appendini & Hotchkiss, 2002; Atares, Bonilla, & Chiralt, 2010; Benavides, Villalobos-Carvajal, & Reyes, 2012; Del Nobile, Conte, Incoronato, & Panza, 2008; Gómez-Estaca, López de Lacey, López-Caballero, Gómez-Guillén, & Montero, 2010; Li, Yin, Ynag, Tang, & Wei, 2012; Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010; Salgado, López-Caballero, Gómez-Guillén, Mauri, & Montero, 2012).

The antimicrobial packaging targets mainly the inhibition of human pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* O157:H7, *Pseudomonas fluorescence* and *Salmonella* sp. in food (Du et al., 2009; Han, 2005; Kanmani & Rhim, 2014; Shakeri, Shahidi, Beiraghi-Toosi, & Bahrami, 2011; Ünalan, Arcan, Korel, & Yemenicioğlu, 2013). The antimicrobial packaging could also target food spoilage yeasts and molds and non-pathogenic spoilage bacteria such as *Bacillus* spp. and *Lactobacillus* spp. (Kraśniewska et al., 2014; Manso, Cacho-Nerin, Becerril, & Nerìn, 2013; Mecitoglu et al., 2006). However, there are no studies in the literature to employ antimicrobial edible coating technology for the inhibition of bacterial plant pathogens. The percent crop spoiled by the plant pathogens change between 10% and 16% of the total crop grown in the world (Chakrabarty & Newton, 2011). Thus, severe use of toxic chemicals to prevent economic losses in orchards and fields is a widespread problem (Pimentel, 2002). As a novel approach the edible antimicrobial

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coatings containing natural active compounds could be applied in the orchards for coating of contaminated tree stems, and in the fields on plants, soil surfaces or agronomy tools and equipment to suppress infections caused by bacterial plant pathogens without using toxic chemicals. Such an application could help suppression of diseases like Bacterial canker mediated by some pathovars of *Pseudomonas syringae* and causes important damages in the stems and leaves of *Prunus* (plums, cherries, apricots and peaches) trees. Different authorities including the Royal Horticultural Society advises the application of copper-based chemicals like Bordeaux mixture (originally a fungicide) on three stems to control Bacterial canker (https:/www.rhs.org.uk.). However, the Bordeaux mixture obtained by mixing copper-sulfate with lime is a hepatotoxin persistent in the soil and it leads to documented health problems in farm workers (Bolan et al., 2014; Dixon, 2004; Mackie, Müller, & Kandeler, 2012).

Although the application of edible coatings in orchards and fields is a novel approach, the application of edible films for coating of fresh fruits and vegetables is a well-known practical process used to reduce their respiration rates and senescence (Park, 1999). For a successful fruit and vegetable coating application, the gas permeability characteristics of the coating material and the product respiration rate should be compatible. This helps to obtain the "modified atmosphere effect" that forms by reduction of fruit or vegetable respiration rate under reduced O_2 and elevated CO_2 atmospheres and to extend the shelf life of the coated product (Park, 1999; Rojas-Graü, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009). The biopolymers like cellulose, casein, zein, soy proteins and chitosan are frequently applied for fruit coating due to their desired gas permeability characteristics and other characteristics such as being odorless, tasteless and transparent (Lin & Zhao, 2007; Rojas-Graü et al., 2009). The application of zein as a fruit coating material attracts a particular interest since zein is the major co-product of the oil industry and rapidly growing bio-ethanol industry. The zein is also one of the rare hydrophobic proteins and it gives excellent coatings with good gas and moisture barrier properties (Lin & Zhao, 2007). Moreover, the zein films provide an effective delivery system for different natural active compounds including phenolic compounds (Arcan & Yemenicioğlu, 2011, 2014). Thus, the zein coatings have been successfully applied on different fruits including apples (Bai, Baldwin, & Hagenmaier, 2002), pears (Scramin et al., 2011), mangoes (Gol & Rao, 2014) and tomatoes (Zapata et al., 2008) to delay their ripening process and to reduce their moisture loss during storage. However, no studies have been conducted to design antimicrobial edible zein fruit coatings specifically against bacterial plant pathogens so far. The natural antimicrobial coatings could also be applied to control postharvest spoilage of root vegetables like cold stored potatoes spoiled largely by specific bacterial plant pathogens (Mills, Platt, & Hurta, 2006; Wood, Miles, & Wharton, 2013). Such an antimicrobial coating application could also be beneficial for tubers separated as seedling and it might reduce the disease problems in the field without using classical chlorine based potentially toxic and odorous chemicals. Thus, the aim of the present study is to adopt the principles of antimicrobial edible packaging for inhibition of major bacterial plant pathogens such as *Erwinia amylovora*, *Erwinia carotovora*, *Xanthomonas vesicatoria* and *P. syringae* which cause different plant diseases and great economic losses in fruits and vegetables at preharvest and postharvest stages (Hao & Brackett, 1994; Mills et al., 2006). For this purpose, edible zein films were incorporated with different antimicrobial phenolic acids, and phenolic rich essential oils and plant extracts. The use of natural phenolic agents in edible films has become increasingly popular since these compounds are not only potent antimicrobials, but they also have different bioactive effects on human including antioxidant, anticancerogenic, antidiabetic and antihypertensive activities (Basgedik, Aysel, & Nurdan, 2014; Moure et al., 2001; Wojdyło, Teleszko, & Oszmiański, 2014). In the literature, the effects of different phenolic rich essential oils on fungal plant pathogens and development of antifungal edible fruit coatings for postharvest decay control have been studied (Sivakumar & Bautiata-Banos, 2014). However, there are only few studies related to antimicrobial potential of phenolic compounds on bacterial plant pathogens. For example, the inhibitory effects of phenolic compounds towards growth of *Xylella fastidiosa*, a plant pathogen that causes diseases in different crop species, has been reported by Maddox, Laur, and Tian (2010). Luzzatto et al. (2007) found that the use of different plant defense activators with phenolic compounds contributes to increased resistance against soft-rot pathogen *Pectobacterium carotovorum*. Mohana and Raveesha (2006) reported the antimicrobial effects of *Caesalpinia coriaria* (Jacq.) Willd extracts on *Xanthomonas* pathovars. However, the present study was the first one in the literature which investigated the potential application of natural phenolic antimicrobials and edible film technology against major bacterial plant pathogens. This work made a contribution to increase use of antimicrobial edible coatings not only for fruit coating, but also for coating of soil surfaces and agronomic tools, tree stems, and seedlings.

2. Materials and methods

2.1. Materials

Zein, GA, CA, VA, CAR, THY, EUG, and CIT used in film making were obtained from Sigma Chem Co. (St. Louis, MO). Glycerol and ethanol were purchased from Merck (Darmstadt, Germany). Nutrient broth and buffered peptone water were obtained from Oxoid Ltd. (Hampshire, United Kingdom). Nutrient agar used in antimicrobial tests was obtained by adding 1.4% agar (Applichem, Darmstadt, Germany) in nutrient broth prepared according to the user's manual. All the other chemicals were reagent grade.

2.2. Bacterial cultures

The four plant pathogenic bacteria; *E. carotovora* (RK-EC-462), *X. vesicatoria* (RK-XCV-110C), *E. amylovora* (RK-EA-228) and *P. syringae* (P.syr-RK-453) were kindly provided by Assoc. Prof. Recep Kotan from the Faculty of Agriculture at Atatürk University, Turkey.

2.3. Preparation of plant extracts

The extraction of phenols from plant materials was carried out according to Chun, Vattem, Lin, and Shetty (2005) with slight modification. Plant materials (1–5 g) (dry mortar crushed oregano, clove and walnut shells and chopped fresh artichoke stems) were placed into a beaker containing 100 mL of ethanol (60%) and the extraction was carried out at room temperature under continuous magnetic stirring for 24 h. The mixture was then centrifuged at 9000 rpm for 14 min. After that the supernatant was collected and concentrated in a rotary evaporator working under vacuum at 100 mbar and 40 °C. The concentrated extract was then lyophilized to obtain dry PE powder. The PEs suitable for film making were selected depending on their minimum inhibitory concentration (MIC) on plant pathogens.

2.4. Antimicrobial activity of plant extracts

The MICs of PE were determined in broth medium using 96-well microplates. A stock solution of each PE was prepared in nutrient broth at a concentration of 41 mg/mL and then series of two-fold