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Reduction of *Dickeya chrysanthemi* on fresh-cut iceberg lettuce using antimicrobial sachet containing microencapsulated oregano essential oil

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ABSTRACT

An antimicrobial sachet releasing vapor of oregano essential oil (EO) was developed and its effectiveness as an antimicrobial packaging system for fresh-cut iceberg lettuce was evaluated. Oregano EO was microencapsulated with polyvinyl alcohol (oregano EO/PVA ratio: 3/5 (w/w)) using a spray-drying technique. The sachet containing oregano microcapsules was incubated with iceberg lettuce at 20 °C and 85% RH for five days. During this storage, the reduction efficiency against growth of *Dickeya chrysanthemi*, molds and yeasts (MY), and total mesophilic aerobic bacteria (MAB) on the surface of iceberg lettuce was investigated. Oregano EO released from the sachet inhibited the growth of *D. chrysanthemi*, resulting in 3.9 log CFU/5 pieces reduction over five days storage at 20 °C. The volatile oregano EO also showed significant growth inhibitory effects against MY and total MAB that each was significantly reduced by 2.1 log CFU/5 pieces and 1.5 log CFU/5 pieces, respectively. The texture and color characteristics of the iceberg lettuce were not affected by the release of oregano EO vapor. These results may be useful for the development of antimicrobial packaging systems that are intended to increase the microbiological safety and to extend the shelf life of fresh products.

1. Introduction

During the last decades, the demand for minimally processed and fresh-cut products has been increasing because of their convenience and health benefits. However, fresh-cut vegetables have been recognized as one of the main problematic foods because it is difficult to preserve them in fresh state. Fresh-cut vegetables are more susceptible to physiological and microbiological deterioration because cells are destroyed and exudates are released those are rich in minerals, sugar, vitamins, water, and other nutritious compounds. These nutrients and storage conditions may lead to proliferation of microorganisms on freshly cut vegetables, resulting in postharvest losses and quality reduction (Schofield, DeEll, Murr, & Jenni, 2005). Deterioration caused by pectolytic bacteria is the major cause of postharvest losses of perishable products worldwide (Aysan, Karatas, & Cinar, 2003).

Dickeya chrysanthemi (*Erwinia chrysanthemi*) is a Gram-negative bacillus that causes maladies to freshly cut and fresh vegetables (Aysan et al., 2003). *D. chrysanthemi* is a pectolytic plant pathogen and can cause a soft rot disease by its enzymes during the plant cell wall degradation. For example, proteases and pectinases produced by *D. chrysanthemi* cause the soft rot disease during its growth on various plant species (Lee & Yu, 2006; Nazerian, Sijam, Ahmad, & Keshavarz, 2011).

In general, decontamination of fresh vegetables is based on the application of chlorine, although there is industrial demand for natural alternative disinfectants (Gutierrez, Bourke, Lonchamp, & Barry-Ryan, 2009). The growing concern about potentially harmful synthetic additives as well as benefits of natural ingredients (low toxicity to mammals and fewer environmental effects) have led to wide public acceptance of the latter (Paranagama, Abeysekera, Abeywickrama, & Nugaliyadde, 2003).

The use of plant essential oils (EOs) in food system seems

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attractive because they hold promise as natural food preservatives, with a wide spectrum of antimicrobial activity and the potential for control of foodborne pathogens and spoilage bacteria (Gutierrez et al., 2009). EOs are classified as GRAS (generally recognized as safe) by the Food and Drug Administration (FDA) of USA and as food additives by the European agencies. The use of EOs in food preservation, however, remains limited mainly because of their intense aroma.

Antimicrobial packaging is one of the innovative technology concepts of active food packaging that inhibits or retards proliferation of undesirable microorganisms in foods, thus extending the shelf-life of the product (Muriel-Galet, López-Carballo, Gavara, & Hernández-Muñoz, 2012). Therefore, several attempts have been tried for developing antimicrobial packaging systems. One of them, microencapsulation incorporated into polymeric materials can be applied to entrap natural compounds, like EOs, to be used in food packaging, resulting in slow release onto the food surface (Nazzaro, Orlando, Fratianni, & Coppola, 2012). For another, addition of sachets containing antimicrobial agents is one of the successful packaging systems (Appendini & Hotchkiss, 2002), even though there is a risk of accidental ingestion of the sachets by consumers (Suhr & Nielsen, 2005). Antimicrobial activity of sachet containing microencapsulated EO has been studied by several researchers (Han, Patel, Kim, & Min, 2014; Medeiros, Soares, Polito, Sousa, & Silva, 2011; Pires et al., 2009; Seo et al., 2012), however, limited data exist regarding the reduction of plant pathogen.

Thus, the main objectives of this study were to develop an antimicrobial sachet containing oregano microcapsules to be used in the packaging of fresh-cut iceberg lettuce and to characterize the antimicrobial effect of this packaging against *D. chrysanthemi* and other food spoilage flora. In addition, the quality attributes of fresh-cut lettuce after antimicrobial sachet treatment and storage were evaluated for the purpose of evaluating its potential use as natural food packages.

2. Materials and methods

2.1. Bacterial strain and culture conditions

D. chrysanthemi ATCC 27388 was obtained from the American Type Culture Collection (ATCC). The bacterial strain was grown in potato dextrose broth (PDB; Difco, Sparks, MD, USA) at 30 °C, and potato dextrose agar (PDA) plate was prepared with PDB supplemented with 1.5% Bacto agar (Difco) for cell enumeration and vapor diffusion assay.

2.2. Preparation of EOs

Oregano (*Origanum vulgare*) EO was purchased from Neumond-Düfte der Natur GmbH (Raisting, Bayern, Germany). EOs of red thyme (*Thymus vulgaris*), white thyme (*Thymus vulgaris*), clary sage (*Salvia sclarea*), sage (*Salvia officinalis*), mint (*Mentha* spp.), cinnamon (*Cinnamomum zeylanicum*), rosemary (*Rosmarinus officinalis*), garlic (*Allium sativum*), clove (*Syzygium aromaticum*), pine (*Pinus rigida*), eucalyptus (*Eucalyptus globulus*), orange (*Citrus sinensis*), geranium (Pelargonium graveolens), cypress (*Cupressus sempervirens*), ginger (*Zingiber officinale*), tea tree (*Melaleuca alternifolia*), and fennel (*Foeniculum vulgare*) were provided by Scentpia Co., Ltd. (Bucheon, Korea).

2.3. Antimicrobial activity assay of EOs in vapor phase against *D. chrysanthemi*

The antimicrobial activity of 18 EOs against *D. chrysanthemi* was tested using an inverted petri plate method (Singh, Singh, Rao, & Sharma, 2002). Eighteen milliliters of molten PDA were poured into Petri dish plates and they were air-dried in a laminar flow hood at room temperature to solidify them. *D. chrysanthemi*, in the exponential growth phase (OD_{600}, 0.4), was harvested and serially diluted after suspending in sterile 0.1% peptone buffer. Further, 100 μl of each suspension was surface-plated on PDA; this amount corresponds to ~4.0 log CFU/mL. Then, 10 μl of each pure EO was soaked in a sterilized filter paper discs (10 mm diameter; Advantec Toyo Kaisha, Ltd., Tokyo, Japan) and placed at the inner surface of the lid of the petri plate and placed in inverted position. To avoid eventual evaporation of the EOs, the petri dishes were sealed using sterile laboratory parafilm and incubated at 30 °C for 24 h.

2.4. Preparation of oregano EO microcapsules

Five grams of polyvinyl alcohol (PVA) (MW 22,000, Daejung Chemical & Metals Co., Siheung, Korea) were dissolved in 100 mL distilled water at 85–90 °C for 10 min and kept at room temperature for 10 min. Emulsions were prepared by adding oregano EO to the PVA solution at ratio of 3:5 (oregano EO:PVA) with Tween 20 at the ratio of 0.7% (w/v) in the total emulsion. The mixture was then homogenized at a rotational speed of 8000 rpm for 3 min with a homogenizer (model SR30, mtop-Korea, Seoul, Korea) (Paramita, Furuta, & Yoshii, 2010). Next, this emulsion solution was microencapsulated using spray drying. The spray drying process was performed in a laboratory scale spray dryer (Büchi mini spray dryer B290, Oldham, UK) with a 1.5-mm diameter nozzle. The emulsion solution was fed into the main chamber through a peristaltic pump. The operation conditions of spray drying were as follows: (1) inlet air temperature of 130 ± 2 °C; (2) outlet temperature of 85 ± 2 °C; (3) feed flow rate of 3 mL/min; (4) drying air flow rate of 73 m^3/h. Finished powder (oregano microcapsules) was stored in a hermetically sealed bottle at 4 °C until analysis.

2.5. Analysis of encapsulation efficiency

Ten milligrams of oregano microcapsules were dispersed in 10 mL of distilled water, followed by the addition of 10 mL of acetone to dissolve the wall (PVA) and core (oregano EO) materials. A 10 mL aliquot of hexane was then added to the mixture and kept for fast solvent extraction in a water bath at 45 ± 5 °C for 5 min. The flavor content of the organic phase was analyzed by gas chromatography flame-ionization detector (GC-FID: Agilent 7890A, Agilent Technologies Inc., Wilmington, DE, USA) equipped with HP-5 Capillary GC columns (30 m × 0.32 mm inner diameter, 0.25 μm film thickness; Agilent Technologies Inc., Palo Alto, CA, USA). The detector and injection temperatures were set to 320 and 250 °C, respectively. The oven temperature was increased from 50 °C for 2 min to 260 °C for 5 min at a rate of 5 °C/min. The carrier gas was nitrogen, with a split ratio of 1:2. Peaks areas were recorded and calculated using the Agilent software (Hewlett Packard, Agilent Technologies Inc.).

2.6. Preparation of sachets containing oregano EO

A roll paper was purchased from Hana paper mall Co. (70 μm thickness; Namyangju, Korea). Two sides of the roll paper were composed of the paper and the polypropylene-coated material with thermal-adhesive properties. The roll paper was used to prepare sachets (paper sachet, 5 × 10 cm). Oregano microcapsules were placed in paper sachets and sealed with an impulse bag sealer (SK-310; Cheung-il Co., Seoul, Korea) immediately before use in experiments.