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Decontamination of iceberg lettuce by some plant hydrosols

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ABSTRACT

Hydrosols are byproducts obtained by steam distillation of plant materials. In this study, six hydrosols obtained from thyme (*Thymus vulgaris* L.), summer savory (*Satureja hortensis* L.), rosemary (*Rosmarinus officinalis* L.), salvia (*Salvia officinalis* L.), sideritis (*Sideritis canariensis* L.), oregano (*Origanum onites* L.) and bay leaf (*Laurus nobilis* L.) were used for decontamination of fresh-cut iceberg lettuce inoculated with *Salmonella enterica* subsp. *enterica* serovar. Typhimurium, *Listeria monocytogenes* and *Escherichia coli* O157:H7. Volatile composition of the hydrosols and sensorial properties of hydrosol-treated lettuce were also investigated. Thyme and summer savory hydrosols achieved ~3–4 log reductions on all bacterial strains tested while significant ($P < 0.05$) reductions were obtained by all hydrosol treatments depending on the treatment time (0, 20, 40 or 60 min). Thymol and 1,8 cineole were the most abundant volatile constituents of the hydrosols, likely affecting their antibacterial activity. Hydrosol-treated samples especially with bay leaf and sideritis were generally accepted by the panelists. This study confirmed that plant hydrosols could be successfully used as sanitizing agents for fresh-cut lettuce to provide their microbiological safety without causing deep sensorial defects on the products.

1. Introduction

Consumers have been demanding minimally processed foods in recent years due to their healthier perception and the increasing negative perception of use of additives in foods and food processing (Kroyer & Hegedus, 2001). By this way, fresh-cut industry has tremendously grown in last two decades. Investigations have shown that abundance of fresh fruit and vegetables that are rich in minerals, vitamins, and phytochemicals in diet promotes a healthy life and decreases risk of diseases (Artés & Allende, 2005; Nou & Luo, 2010). Fresh-cut lettuce has also been one of the most frequently demanded commodities depending on the increasing popularity of salad bars containing fresh-cut lettuces (Allende et al., 2007). Since leafy vegetables such as lettuce grow in a natural environment, they may frequently be contaminated with bacterial foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica* subsp. *enterica* Typhimurium and *Staphylococcus aureus* (Islam, Doyle, Phatak, Millner, & Jiang, 2004; Nou & Luo, 2010; Wachtel, Whitehand, & Mandrell, 2002). Unit operations including trimming, core removal, cutting or slicing, washing, drying and packaging that are conducted for fresh-cut production may also be source of contamination (King, Magnuson, Török, & Goodman, 1991). It is well established that the first way of preventing contamination of fresh produce with pathogenic/spoilage bacteria is performing of good agricultural practices. On the other hand, convenient and hygienic processing conditions are also necessary during harvesting and transportation to provide safety of the product (Taormina et al., 2009). Among the unit fresh-cut lettuce preparation operations, washing is the most critical step to reduce/eliminate pathogens from the plants (Tornuk et al., 2011). However, water washing is not effective itself for extending shelf life of the product by decontamination. Therefore, several sanitizers are used in fresh-cut industry. Chlorine based chemicals are the most common sanitizers with their low cost and proven effectiveness against pathogens (Erkmen, 2010; Gil, Selma, López-Gálvez, & Allende, 2009). However, chlorine has been blamed for formation of carcinogenic byproducts including trihalomethanes and haloacetic acids by interaction of chlorine with natural organic materials. In addition, chlorine use contributes to the environmental pollution via production of increased levels of wastewater requiring high amounts of biological oxygen for recovery. Based on these problems concerning chlorine chemicals, their use was

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prohibited in some countries (Ölmez & Kretzschmar, 2009). Consumers also demand a more natural processing of fresh-cut fruit and vegetables in recent years. Up to date, a number of physical and chemical fresh-cut disinfection methods have been tested in order to eliminate use of chlorine from fresh-cut industry and decrease high water requirement as well as providing high decontamination efficiency. Although ozone is effective against many kinds of bacteria and fungi at low concentrations, it may cause physical damage on surface of fresh-cut vegetables (Kim et al., 2006). Efficiency of organic acids that is another group of disinfectant used for fresh-cut decontamination depends on the type and acidity level of the acid. Biopreservation of fresh-cut fruits with lactic acid bacteria is another method tested for disinfection of foodborne pathogens (Allende et al., 2007; Trias, Bañeras, Badosa, & Montesinos, 2008). In spite of reduction on foodborne pathogens in ranging levels from 1 to 2 logs by lactic acid bacteria, its adaptation into fresh-cut food industry is very difficult. Hydrosols are known as byproducts that are obtained by hydrodistillation of aromatic plant materials. They contain a trace amount of essential oils and other water soluble compounds (Tornuk et al., 2011). Besides their low cost and ease of production, they do not carry any health risk. Humans are tolerable to essential oils, therefore they and hydrosols can be used for human consumption safely (Altınterim, Gulec, & Aksu, 2012). Essential oils and other related natural compounds are listed in GRAS status (Cosentino et al., 1999). The knowledge that hydrosols have been consumed as beverage for ancient times also demonstrates their safety for human (Sağdıç, 2003). Efficiency of hydrosol washing on decontamination of several fresh-cut fruits and vegetables has been demonstrated in several previous studies (Gündüz, Gönül, & Karapinar, 2010; Ozturk, Tornuk, Sagdic, & Kisi, 2012; Sagdic, Ozturk, & Tornuk, 2013; Tornuk et al., 2011; Törnük & Dertli, 2015; Tornuk, Ozturk, Sagdic, Yilmaz, & Erkmen, 2014). This study was aimed to reveal role of hydrosols obtained from thyme (*Thymus vulgaris* L.), summer savory (*Satureja hortensis* L.), rosemary (*Rosmarinus officinalis* L.), salvia (*Salvia officinalis* L.), sideritis (*Sideritis canariensis* L.), oregano (*Origanum onites* L.) and bay leaf (*Laurus nobilis* L.) on *S.* Typhimurium, *E. coli* O157:H7 and *Listeria monocytogenes* inoculated to fresh-cut iceberg lettuce as well as sensorial properties of the product. Additionally, volatile compositions of the hydrosols were also analyzed in order to have information about the effect on their antimicrobial activity.

2. Material and methods

2.1. Bacterial culture and inoculum preparation

Salmonella enterica subsp. *enterica* serovar. Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 7644 and *Escherichia coli* O157:H7 ATCC 33150 were used as bacterial cultures in this study. These bacterial cultures were obtained from Erciyes University, Food Engineering Department Food Microbiology Laboratory, Kayseri, Turkey. Frozen stock cultures were activated before the use for 24 h at 37 °C in Nutrient Broth.

2.2. Preparation of plant hydrosols

Dried leaves of Lamiaceae family: (Thyme (*Thymus vulgaris* L.), summer savory (*Satureja hortensis* L.), rosemary (*Rosmarinus officinalis* L.), salvia (*Salvia officinalis* L.), sideritis (*Sideritis canariensis* L.) oregano (*Origanum onites* L.)) and Lauraceae family: bay leaf (*Laurus nobilis* L.) samples were used as plant materials. These plants were purchased from a local spice market in Kayseri, Turkey. Hydrosols were obtained as following the method of Sağdıç (2003). Approximately 100 g of each of ground plant material was placed into a flask (2 L) with 1 L of hydrodistilled water (1:10 w/v) for 2 h with a Clevenger apparatus (Ildam, Turkey). After hydrodistillation, essential oil was separated through the cooling tunnels. Hydrosols were kept in sterile bottles at 4 °C until use.

2.3. Volatile compounds analysis of plant hydrosols

Volatile compounds analysis of plan hydrosols was run on an Agilent 7890A gas chromatograph system (Agilent, Avondale, USA) coupled to a mass selective detector (Agilent Technologies, Agilent, Avondale, USA) and HP-5MS column (0.2 mm × 50 m, film thickness 0.25 μm) according to the procedure of Tornuk et al. (2011). The compounds adsorbed by the fibers were desorbed from the injection port for 60 min at 40 °C in the splitless mode. The oven temperature was held at 40 °C for 5 min, and then heated to 125 °C at 3 °C/min, and further increased from 125 °C to 230 °C at 5 °C/min, finally at 230 °C/min it was held for 5 min. The carrier gas was helium with a flow rate of 1.0 mL/min. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley 7, Flavor 2 and HPCH 1607 GC/MS Libraries. The peak areas were used directly to give the percentage composition of the hydrosols by dividing the area of each peak by the total area under all of the peaks.

2.4. Preparation of iceberg lettuce sample and inoculation process

Fresh iceberg lettuces were purchased from a local supermarket in Kayseri, Turkey and stored at 4 °C until use. Firstly, iceberg lettuces were washed with cold tap water for 5 min to remove undesired residues and reduce native microbial load and cut into pieces (approximately 2 g each) with a sterile knife. These iceberg lettuce pieces were used in the experiments. Dip inoculation method was used as inoculation method. In this study, initial inoculation concentration was approximately 10^6 cfu/mL for *S.* Typhimurium and 10^5 cfu/mL for both *L. monocytogenes* and *E. coli* O157:H7. Shredded iceberg lettuces (100 g each) were immersed into the inoculum solutions (sample:inoculum ratio 1:5 w/v) and shaken for 2 min to distribute the inoculum homogenously and then kept in a biosafety cabinet for 1 h at 24 ± 2 °C (Tornuk et al. 2011).

2.5. Washing of iceberg lettuce samples

Washing of inoculated iceberg lettuce pieces was carried out by immersing shredded the samples (50 g) in the sterile bottles containing 100 mL of each sanitizing hydrosol (Thyme, sideritis, rosemary, salvia, summer savory, oregano and bay leaf) for 0, 20, 40 and 60 min. Control samples were immersed in sterile tap water. Bottles were covered following the addition of samples and subjected to continuous gently shake during the treatment period.

2.6. Enumeration of bacteria

At the end of the hydrosol treatment, 10 g of iceberg lettuce sample was transferred into sterile stomacher bag and mixed with 90 mL of sterile maximum recovery diluent solution (Merck, Germany) and, then was shaken vigorously by a stomacher for 2 min. The solution (1 mL) was serially diluted in test tubes containing 9 mL of sterile maximum recovery diluent solution. According to spread plate technique, decimal dilutions of samples were drop plated to Brilliant-Green Phenol-Red Lactose Agar acc. to KAUFFMANN (Merck, Germany), Oxford Listeria Agar (Merck, Germany) and Sorbitol MacConkey Agar (Merck, Germany) for enumeration of *S.* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7, respectively. Then the plates were incubated for 24 h at 37 °C and colonies were counted.