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Microbial load reduction of sweet basil using acidic electrolyzed water and lactic acid in combination with mild heat

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ABSTRACT

Sweet basil has been used worldwide as an ingredient for several cuisines. However, it can be contaminated with pathogens; especially *Escherichia coli* and *Salmonella* spp. are mainly introduced by poor hygiene during harvesting, cleaning, packaging and distribution. This study aimed to study the efficacy of lactic acid (LA) and acidic electrolyzed water (AEW) against mesophilic bacteria on sweet basil. In addition, a combination treatment was also investigated, using mild heat with the selected sanitizer to disinfect sweet basil inoculated with *S.* Typhimurium and *E. coli*. The 2% LA treatment showed high efficiency in microbial decontamination, and mesophilic bacteria were reduced by about 3 log CFU/g. Additionally, the decontamination efficacy of LA increased when combined with mild heat. The use of 2% LA at 50 °C showed the highest microbial reduction in sweet basil, in which mesophilic bacteria, *S.* Typhimurium, and *E. coli* were reduced by 4.62, 3.80 and 3.61 log CFU/g, respectively. These findings indicate that LA can be more effective than AEW in disinfection and provides greater efficiency when combined with mild 50 °C heat.

1. Introduction

Sweet basil (*Ocimum basilicum* L.), a member of the Lamiaceae or Labiatae family, is a leafy green herb widely used as a food ingredient around the world because of its own unique scent. Sweet basil is a good source of vitamin A, vitamin C, minerals, and phenolic compounds associated with antioxidant capacity (Nguyen, Kwee, & Niemeyer, 2010). Commonly, sweet basil can be consumed as raw or added to cooked food as additional ingredient, depending on culinary practices (Elviss et al., 2009). Fresh produce are contaminated by microorganisms on the surfaces from many sources, such as soil, water, wild animals, birds, and insects. Processing by harvesting, washing, cutting, packaging, and shipping could also create additional contamination. Fresh produce that is rich in essential nutrients and water can provide ideal conditions for the growth of microorganisms, including foodborne pathogens and spoilage microorganisms (Tirawat et al., 2010; Trias, Bañeras, Badosa, & Montesinos, 2008). There are great concerns about microbiological hazards in leafy vegetables, as well as in fresh herbs. Leafy vegetables have consistently been associated with outbreaks of gastrointestinal illnesses (FAO/WHO, 2008). *Escherichia coli* and *Salmonella* spp. are the most frequently found pathogenic microorganisms in sweet basil. *E. coli* can attach well to leafy structures, which makes it difficult to remove these cells from the surfaces (Bermúdez-Aguirre & Barbosa-Cánovas, 2013; López-Gálvez, Allende, Selma, & Gil, 2009). In general, fresh water or fertilizer used in farming can lead to *Salmonella* spp. contamination of fresh produce (Hanning, Nutt, & Ricke, 2009). In 2006 and 2007, two outbreaks in UK, Denmark, the Netherlands and the US were associated with *Salmonella* spp. contaminated fresh sweet basil (Pakalniskiene et al., 2009; Pezzoli et al., 2008). In the European Union, fresh herbs such as sweet basil, holy basil, mint and coriander must be examined for *E. coli* and *Samonella* spp. contamination before being imported (EC, 2012).

Washing is a most important step in postharvest handling since it helps to remove from the fresh produce surface contaminants such as soil, sewage, and feces that are full of microorganisms. Although water may partly remove microorganisms on the surface in a mechanical way, the addition of chemical sanitizers into the washing water is advisable to reduce the microbial load and to

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extend the shelf-life of freshly cut produce (Rahman, Ding, & Oh, 2010; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009). Adding chlorine in the washing water is a conventional method for microbial decontamination of food products, and in the processing lines of food industries. However, the use of chlorine has been related with the formation of carcinogenic chlorinated by-products such as chloramines and trihalomethanes (Abreu, Beirão-da-Costa, Gonçalves, Beirão-da-Costa, & Moldão-Martins, 2003). In some European countries, including Germany, the Netherlands, Switzerland and Belgium, its use for washing fresh-cut produce has been banned (Alegria et al., 2009). Therefore, new sanitizers free of carcinogenicity are required in disinfection of fresh produce.

Many food disinfection methods have been tested in recent years, and microbial inactivation effects by organic acids on fresh vegetables have been reported. Lactic acid (LA) (0.2%–2%) has shown effective results against many pathogens such as *E. coli* O157:H7, *S.* Typhimurium and *Listeria monocytogenes* in fresh produce without affecting the sensory properties (Akbas & Olmez, 2007; Huang & Chen, 2011; Sagong et al., 2011; Uyttendaele, Neyts, Vanderswalmen, Notebaert, & Debevere, 2004; Wang et al., 2013a). The disinfection efficacy of an acid depends on its pKa, on antimicrobial activity of its non-dissociated form, and on specific effects of the acid (Velazquez, Barbini, Escudero, Estrada, & de Guzman, 2009). Acidic electrolyzed water (AEW) has been authorized as a food additive, and its direct use in food is allowed in Japan since 2002 (Issa-Zacharia, Kamitani, Miwa, Muhimbula, & Iwasaki, 2011). Recently, AEW has been increasingly used to decontaminate fresh produce (Koide, Shitanda, Note, & Cao, 2011). AEW has been used effectively to inactivate *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* in lettuce, alfalfa seed sprouts and tomato (Bari, Sabina, Isobe, Umemura, & Isshiki, 2003; Kim, Hung, Brackett, & Lin, 2003; Park, Hung, Doyle, Ezeike, & Kim, 2001). Moreover, the combination of a mild heat treatment (40–50 °C) with some sanitizers, such as chlorine and alkaline electrolyzed water, exhibits higher efficiency in microbial load reduction than cold water washing (Delaquis, Stewart, Toivonen, & Moyls, 1999; Koseki, Yoshida, Kamitani, Isobe, & Itoh, 2004). The use of combination methods could be a means to reduce microbial load efficiently in sweet basil. The objectives of this study were to investigate the efficacy of LA and AEW in reduction of indigenous aerobic mesophilic bacteria on sweet basil. In addition, the use of mild heat in combination with the selected sanitizer on disinfection of *E. coli* and *S.* Typhimurium inoculated on sweet basil was also investigated.

2. Materials and methods

2.1. Collection and preparation of sweet basil

Sweet basils used in this study were purchased from a local farm in Hat Yai, Songkhla, Thailand. Harvesting was carried out by hand with a knife. The samples were transported within 1 h to the Department of Food Technology, Prince of Songkla University. Upon arrival, the defects such as yellowing decay and/or bruised leaves and flowers were removed before use.

2.2. Preparation of sanitizers

AEW was generated by Electrolyzed Water Generator (Labo-SCI) (Shibata Biotechnology Inc., Tokyo, Japan). Sodium chloride solution (0.1%) was transferred into Electrolyzed Water Generator chamber. The solution passed through electrodes in the chamber (100–115 V, 50 W, treatment duration 10 min) and AEW (pH 2.5, 2 L) was collected from the outlet with buckets. LA (2%) and sodium hypochlorite (NaClO: 6%) solutions were freshly prepared before use. All percentages above are given by weight.

2.3. Analysis of AEW

Oxidation-reduction potential and pH were determined directly with a pH/ORP meter (FEP20, Mettler Toledo, Switzerland). Available chlorine concentration was determined with iodometric titration.

2.4. Reduction of mesophilic microorganisms by the sanitizers

Sweet basils were treated with each sanitizer by immersing 100 g of sweet basil branches into 2 L of the sanitizer solution (AEW, 2% LA, or 6% NaClO) at room temperature (RT: 25 °C). Samples treated with tap water were used as controls. At designated times (1, 3 and 5 min), the samples were agitated manually and removed from the treatment solution. Then the treated samples were immersed into 1 L of distilled water for 2 min at RT. After draining the samples on a screen for 2 min, they were subjected to determination of mesophilic bacteria counts (MBC).

MBC were determined by aerobic plate counts (BAM, 2001), as follows. Ten grams of sweet basil was mixed with 90 mL of sterile 0.1% peptone water in a stomacher bag, and the solids were broken down on a Stomacher (400 Circulator; Seward, England). Serial dilutions of this suspension were made in 0.1% peptone water and then spread-plated onto plate count agar (PCA), which was incubated at 35 ± 2 °C for 24 h to determine the mesophilic bacteria loads.

2.5. Effects of LA with mild heat on mesophilic microorganisms

Sweet basil branches (100 g) were immersed in 2% LA at 40 °C or 50 °C for 1 min and then immersed into 1 L of distilled water at RT for 2 min. In an alternative treatment, sweet basil branches were immersed in 2% LA at the RT for 1 min, and then immersed in distilled water at 40 °C or 50 °C for 2 min. The samples were drained for 2 min on a plastic tray prior to determining MBC as described in 2.4.

2.6. Effects of LA with mild heat on pathogenic bacteria

2.6.1. Pathogenic bacteria preparation

E. coli O157:H7 DMST 4212 and *S.* Typhimurium DMST 562 used in this study were obtained from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand (DMST). Both *E. coli* and *S.* Typhimurium were sub-cultured twice in nutrient broth (NB) at 37 °C for 24 h immediately before use as inocula.

Cell collection was by centrifugation (10000 g, 10 min, 4 °C), and each pellet was washed twice with sterile 0.85% NaCl solution, and then resuspended in 5 mL of sterile 0.85% NaCl solution. The inocula were suspended into 1 L of sterile 0.85% NaCl solution with the final cell concentration of approximately 7–9 log CFU mL^{-1}.

2.6.2. Challenging pathogenic bacteria

Sweet basil branches (100 g) were dipped in 1 L of inoculum suspension at RT for 5 min, in a safety cabinet, and then excess solution was drained on a plastic tray. The sweet basil was allowed to stand in the safety cabinet at RT for 30 min. Then the inoculated sweet basil was dipped in 2% LA (50 °C) for 1 min, and then immersed into distilled water (at RT) for 2 min. In an alternative tested, the inoculated sweet basil was immersed in 2% LA at RT for 1 min and then immersed in distilled water (50 °C) for 2 min. After treatment the samples were immediately subjected to analysis of