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Combination of microbubbles with oxidizing sanitizers to eliminate *Escherichia coli* and *Salmonella* Typhimurium on Thai leafy vegetables

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ABSTRACT

Microbubbles (MB) technology was applied in a washing process a few studies have been done with food materials, particularly to reduce the microbial contamination on leafy vegetables. This study determined the effectiveness of MB (Ø ~ 50–70 μm) combined with three sanitizers acidic electrolyte water (AEO, 20 and 40 mg/L, ORP 910–1010 mV, pH 2.7–3.1), chlorine dioxide (ClO_2, 3 and 5 mg/L, ORP 550–680 mV, pH 7.1–7.5), and sodium hypochlorite (NaOCl, 40 and 80 mg/L, ORP 900–990 mV, pH 6.5–6.7) in order to inactivate *Escherichia coli* and *Salmonella* Typhimurium on artificially contaminated sweet basil (*Ocimum basilicum* Linh) and Thai mint (*Mentha cordifolia* Opiz.). Although air MB alone did not possess antimicrobial activity, washing with MB combined with the two oxidizing sanitizers (NaOCl and AEO) for 5 min resulted in an effective reduction in *S.* Typhimurium and *E. coli* on sweet basil and Thai mint with 2–3 log reductions (99.2–99.8%). Washing vegetables with MB and NaOCl at a concentration of free chlorine of 40 mg/L NaOCl or 20 mg/L AEO yielded the best results in killing *S.* Typhimurium with 1.21–1.90 and 0.67–2.25 log reductions, respectively. In addition, the reduction of *E. coli* and *S.* Typhimurium on sweet basil appeared to be higher than on Thai mint. Differences in surface roughness may assist the bubbles and sanitizers to detach bacterial cells and therefore increase the washing efficacy. Furthermore, applying sanitizers in washing solution was a powerful means of killing planktonic *E. coli* and *S.* Typhimurium in the wash water and preventing cross-contamination in the washing process.

1. Introduction

Fine bubbles are defined as small bubbles with a diameter range in the micro and nanoscale (10–0.1 μm) so-called micro/nano bubbles. Microbubbles (MB) make up part of fine bubbles, but generally the MB diameter range is between 10 and 50 μm. These bubbles decrease in size and finally disappear under water (Takahashi, 2005; Tsuge, 2014). The application of microbubbles and nanobubbles research has progressed rapidly in many fields. Bubble technology has been applied in food industries in Japan for more than a decade, despite most applications being in waste water treatment. The potential of bubbles technology in food, agriculture and fishery applications has been studied, but few applications in food processing have been directly reported.

The use of bubble technology in food washing has been studied (Himuro, 2007; Ikeura, Kobayashi, & Tamaki, 2011; 2013). Washing is a crucial step in food processing to reduce the number of microorganisms on food. Outbreaks have been traced to fresh fruits and vegetables due to unsanitary and poor practices in washing, while the amount of research on the efficiency of different methods to reduce the microbial load of fresh produce has increased (Parish et al., 2003). Fresh produce may be exposed to pathogens and contamination in the processing steps from the farm to the consumer (Gil, Selma, López-Gálvez, & Allende, 2009) and the contamination may become the source of foodborne diseases. Thus, washing with sanitizers is required to remove these foodborne

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pathogens.

The main pathogens of particular concern are *Salmonella* spp. and *Escherichia coli* O157:H7 (Warriner, Huber, Namvar, Fan, & Dunfield, 2009). In London in 2005, 32 out of 244 herbs imported from non-EU countries were inspected, and *Salmonella* contamination was found on four varieties of basil grown in Thailand. Later in 2006, 5 out of 298 fresh herbs in the UK were found to be contaminated with *Salmonella,* including coriander, curry leaves and holy basil grown in India and Thailand (Elviss et al., 2009).

Washing with water or sanitizing agents primarily removes dirt and soil and washes out pesticide residues (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Sapers, 2014). Washing also reduces the microbial load on the surface of incoming produce (Fan, Niemira, Doona, Feeherry & Gravani., 2009). However, the washing process can transfer microbial contaminants to the wash water and then to other uncontaminated raw materials (Gil et al., 2009). Adding a sanitizing agent to the wash water can significantly reduce the population of planktonic bacterial cells and thus reduce the risk of cross-contamination (Sapers, 2014).

Washing methods and washing solutions are not all effective (Olaimat & Holley, 2012). The success of washing depends on many factors for example, the type of commodity, the type of sanitizer, the target microorganism, and the washing system. The challenge for industry is to choose the appropriate sanitizing treatments to reach pathogens on the surface and in subsurface areas of fresh produce in an active form, while not compromising the sensory quality of the fresh produce.

In this research, MB were generated by pressurized dissolution or dissolved air floatation (Terasaka, Hirabayashi, Nishino, Fujioka, & Kobayashi, 2011). The bubbles were generated by allowing air to dissolve in water, then applying pressure (3–4 atm) and then immediately flushing the water through a nozzle inside the water tank. The reduction in pressure causes the supersaturated air to be released in the expelled water in the form of bubbles (Parmar & Majumder, 2013; Terasaka et al., 2011).

The current research investigated the application of MB technology in the washing process for the decontamination of microorganisms with regard to enhancing the food safety of Thai leafy vegetables. Sweet basil and Thai mint were selected for the investigation. The bacteria of interest were *Escherichia coli* (*E. coli*) and *Salmonella* Typhimurium (*S.* Typhimurium), which frequently occur in contaminated vegetables (Berger et al., 2010; Warriner et al., 2009). Three types of sanitizer were chosen and their effectiveness at microbial reduction on the vegetables was compared, as well as on microbial reduction in the wash water.

2. Materials and method

The experiment was divided into two parts. First, the characteristic properties of MB were investigated mainly through the measurement of bubble size using different techniques. The second part involved the application of MB in the washing system in combination with three selected sanitizers that have been in recent use in the produce industry.

2.1. Determination of MB diameter using image analysis with light microscopy and a laser diffraction technique

MB in water were generated for 20 min using an MB generator (Model: Sumizumi II, Science & Technology Service Company Limited, Thailand). Ten liters of distilled water was used as the medium. After generating MB for 15 min, 5 μL of MB in distilled water were immediately taken and dropped on concave culture slides (25.4 × 76.2 mm) and covered with a cover glass (22 × 22 mm). The diameters of bubbles were determined using microscopy (Model: BX53, Olympus, Japan). A series of MB photographs was recorded. The average diameters of MBs from the photographs were captured using image analysis; then, each photograph was analyzed using computer software (cellSens software, Olympus, Japan). The diameters of 600 bubbles were averaged and a bubble distribution profile was developed using the SPSS software for Windows, version 12 (SPSS Inc., Chicago, IL. USA).

Another technique to determine MB size used laser diffraction, where measurement was performed according to the modified method of Couto, Nunes, Neumann, and França (2009). The MB size was determined using a particle size analyzer (Model: 2000S, Malvern, UK). This machine is able to measure the diameter of particles ranging from 0.1 to 2000 μm. The calculations are achieved through Mie's complete theory according to the reflection indexes for the liquid and the air. The reflective indexes were fixed at 1.33 for water samples and 1.00 for the air (Couto et al., 2009). The measurement was set at an obscuration of the sample between 2 and 25%. Obscuration is the laser light lost as it passes through the sample. Average diameter and size distribution were analyzed, where the diameter refers to the volume or mass moment mean or the De Broukere mean diameter (Malvern, 1997).

2.2. Application of MB in washing system

2.2.1. Effect of MB combined with three types of oxidizing sanitizers to reduce E. coli and Salmonella Typhimurium on sweet basil and Thai mint

2.2.1.1. Vegetable sample preparation. Fresh sweet basil (*Ocimum basilicum* Linh) and Thai mint (*Mentha cordifolia* Opiz.) were purchased from Ta-laad Thai, the major fresh produce distribution market in Thailand. Fresh vegetables were sorted to provide uniform leaves and size. Visible damaged and wilted portions were discarded. Pre-washing with tap water reduced soil and debris on vegetables, followed by draining on a clean, stainless steel mesh in a bio-safety cabinet (Model: Microflow Advance Biosafety cabinet, Astec Microflow Ltd, UK) for 15 min. Then, all vegetable samples were placed in sealed polyethylene (PE) plastic bags (40 × 50 cm) and stored at 12 ± 2 °C. All treatments were conducted the same day as preparation. Before inoculating samples with tested organisms, all vegetable sample were taken to test the natural presence of *E. coli* and *Salmonella* spp. by selective plating agar. The spread plate technique was used to culture onto MacConkey agar (Merck Chemical, Germany) for *E. coli* and onto xylose lysine deoxycholate agar (Merck Chemical, Germany) for *Salmonella* spp. In addition, the background flora was enumerated by standard plate count agar. Inoculation was done to achieve final number of cells of 6–7 log CFU/g.

2.2.1.2. Bacterial cultures. A pathogenic strain of *E. coli* TISTR 780 (ATCC 8739) and *S.* Typhimurium TISTR 292 (ATCC 13311, NCTC 74) were used as the test strains. Culture was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. *E. coli* TISTR 780 was isolated from human feces and used in antimicrobial preservatives assay. *S.* Typhimurium TISTR 292 was isolated from a patient.

2.2.1.3. Inoculum preparation and inoculation procedure. Activated cells were cultured individually in 9 ml of tryptic soy broth (TSB, Merck Chemical, Germany) and then incubated at 37 °C for 48 h. On the third day, 18–20 h cultures were used as the working inoculum. The final bacterial concentration in inoculum samples was approximately 7–8 log CFU/ml. Four ml of bacterial inoculum (*E. coli* TISTR 780 or *S.* Typhimurium TISTR 292) in TSB was added into 369 ml of 0.1% w/v peptone water before mixing thoroughly. Then, 200 g of vegetable samples (sweet basil or Thai