Food Control 94 (2018) 324–330

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Two-step washing with commercial vegetable washing solutions, and electrolyzed oxidizing microbubbles water to decontaminate sweet basil and Thai mint: A case study

Pitirat Klintham[a,b], Sasitorn Tongchitpakdee[b], Wannee Chinsirikul[c,d], Warapa Mahakarnchanakul[b,*]

[a] The Graduate School, Kasetsart University, Bangkok, Thailand
[b] Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand
[c] National Metal and Materials Technology Center (MTEC), National Science and Technology Development Agency, Ministry of Science and Technology, Pathumthani, Thailand
[d] National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency, Ministry of Science and Technology, Pathumthani, Thailand

ARTICLE INFO

Keywords:
Decontamination
Washing
Surfactants
Microbubbles
Food safety
Vegetables

ABSTRACT

A study was conducted on pre-washing vegetables with four different kinds of surfactant in the washing solution, followed by an acidic electrolyzed oxidizing microbubbles water (AEO-MB) to compare their efficacy on the decontamination of *E. coli* and *S.* Typhimurium on sweet basil and Thai mint. Three commercial surfactants (0.1% v/v) and Tween 80® were applied in pre-washing with a constant rate of shaking at 60 rpm for 3 min, then washed with 40 mg/L of AEO, oxidation reduction potential (ORP) 1130–1180 mV and pH 2.8–3.1, under the continuous generation of MB for 5 min. All surfactants showed no antimicrobial activity but greater decontamination (99.0–99.9%) was observed when two-step washing was applied. Wash water with AEO indicated the absence of microorganisms thus helping to prevent cross-contamination. Our study revealed that pre-washing helped to improve the washing efficacy and to enhance the food safety of sweet basil and Thai mint and the treatment may be suitable for application with household or commercial ready-to-eat produce.

1. Introduction

Food safety problems linked to fresh produce contamination have been repeatedly reported (Kirezieva et al., 2015); the main bacterial pathogens of concern include *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Olaimat & Holley, 2012). The USDA has introduced proper washing of fruits and vegetables in household practices (USDA, 2013) to raise awareness of wholesome and safe food in domestic preparation. Washing of fresh produce has become an important step in food preparation.

Washing helps to remove soil and debris and reduces the microbial load on the surface of the produce, which impacts the product's quality, shelf-life and safety (Herdt & Feng, 2009). Washing vegetables becomes a concern due to the various types of microbial decontamination methods. Many vegetable washing solutions have been commercialized in the market; some it is claimed contain natural ingredients with no synthetic chemicals added. Usually, many commercially washed vegetables contain a number of synthetic active agents. There are reports pertaining to the application of detergent (surfactant or surface active agent) in the wash solution to remove microbes from the surface of fresh produce and to improve the efficacy of washing (Keskinen & Annous, 2011; Ukuku & Fett, 2002). Most surface-active agents are amphipathic molecules that consist of a non-polar hydrophobic portion. The application of the surfactants has been known to be used in the preparation of an emulsion and suspension results in their subsequent stabilization and microemulsion, whilst improving their wetting, spreading and adhesion. The addition of a surfactant alone in washing solution has not always had a significant effect on decontamination (Hassan & Frank, 2003; Keskinen & Annous, 2011). In contrast, some research group reported success in decontamination by a 1–2 log reduction using a combination of surface active agents and sanitizers in the washing process (Soli et al., 2010; Xiao et al., 2011).

Washing with water combined with sanitizing agents has been shown to successfully decontaminate foodborne pathogens (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008). Adding a sanitizing agent to process water can greatly reduce the population of planktonic

* Corresponding author.
E-mail address: warapa.m@ku.ac.th (W. Mahakarnchanakul).

https://doi.org/10.1016/j.foodcont.2018.07.025
Received 11 March 2018; Received in revised form 15 July 2018; Accepted 18 July 2018
Available online 19 July 2018

bacterial cells in the washed water and thus reduce the risk of cross-contamination (Klintham, Tongchitpakdee, Chinsirikul, & Mahakarnchanakul, 2017). Water disinfection becomes critical in fresh produce processing. Proper water disinfection technologies not only efficiently inactivate pathogens on fresh produce but also in the decontaminated process water and, recycling the water helps to reduce wastewater and to conserve the environment (Gil, Gómez-López, Hung, & Allende, 2015).

Electrolyzed oxidizing water (EO) was introduced by Shimizu and Hurusawa in 1992. EO has been an effective solution for the inactivation of microorganisms as well as being environmentally friendly (Gil et al., 2015). Many research groups have studied the efficacy of EO against a variety of microorganisms on several kinds of food products (Kim, Hung, & Brackett, 2000; Liao, Chen, & Xiao, 2007; Hao et al., 2012). EO containing 20–70 mg/L of available chlorine has been proved as an effective disinfectant for fresh-cut vegetables (Cheng, Dev, Bialka & Demirci et al., 2012).

Bubbles technology was widely introduced in 2005. In Japan, different research applications of microbubbles (MB) and nanobubbles (NB) have been investigated for environmental, industrial, agricultural, medical and food industry applications (Tsuge, 2014). MB are small bubbles with diameters in the range 10–50 μm which decrease in size with time and finally disappear in water (Parmar & Majumder, 2013; Takahashi, 2005), while NB are even smaller bubbles with a diameter less than 200 nm (Takahashi, 2014). The differences in the quantity and size of the bubbles, MB or micro-nano bubbles (MNB), may contribute to the appearance, physical properties and texture of the food; therefore, the application of this technology in food processing could be used to obtain new characteristics in the products. Such application extends to control over the growing and pre-postharvest physiological properties of fresh produce and the sterilization of food or equipment but little research has been reported. The results from our previous study on a single step washing using MB (diameter 50–70 μm) with sanitizers reduced the levels of *E. coli* on sweet basil and Thai mint by 27–79%, while the decrease was less especially on Thai mint (Klintham et al., 2017).

The objective of this research was to improve washing efficacy by applying a two-step washing procedure for the decontamination of microorganisms on sweet basil and Thai mint. The design is intended to be applied for home use and could be extended to the industrial scale to achieve greater food safety in the fresh-cut industry. The investigation was undertaken using two-step of washing with surface active agents and AEO using MB to decontaminate two distinct foodborne pathogens (*E. coli* and *S.* Typhimurium) on sweet basil and Thai mint. Surviving pathogens were determined for samples of both the washed vegetables and the wash water. The properties of the washing solution included the oxidation reduction potential (ORP) value, the concentration of available chlorine and the pH, which were observed throughout the washing process.

2. Materials and methods

2.1. Vegetable sample preparation

Fresh sweet basil (*Ocimum basilicum* Linh.) and Thai mint (*Mentha cordifolia* Opiz.) were used as leafy vegetable models. The vegetables were purchased from the wholesale fresh market in Pathum-Thani, Thailand. Visibly damaged and wilted portions were discarded. Uniform leaves were sorted in terms of size and stem. The sweet basil and Thai mint samples were cut to a length of 25 and 20 cm, respectively, from spears using a clean stainless steel knife; then the samples were washed with tap water to reduce the soil and debris before being drained and left in a biological safety cabinet model Microflow class II Advance (Astec Microflow, Bioquell, UK) for 15 min, followed by packing in polyethylene (PE) plastic bags and storing at 12 ± 2 °C. Before inoculation, vegetable samples were tested for the natural presence of *E. coli* and *Salmonella* spp. and none were detected. The spread plate technique was conducted on selected MacConkey agar (Merck Chemical, Germany) for *E. coli* and on xylose lysine deoxycholate (XLD) agar (Merck Chemical, Germany) for *Salmonella* spp. Background flora were enumerated using a standard plate count on agar (Merck Chemical, Germany). Vegetables were subjected to wash treatments on the day of preparation.

2.2. Bacterial cultures

Pathogenic strains of *E. coli* TISTR 780 (ATCC 8739) and *S.* Typhimurium TISTR 292 (ATCC 13311/NCTC 74) were tested as representatives of the pathogenic strains in vegetables. Cultures were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathumthani, Thailand. *E. coli* TISTR 780 was isolated from human feces, while *S.* Typhimurium TISTR 292 was also a patient-isolated strain.

2.3. Inoculum preparation and inoculation procedure

Activated cells were cultured individually in 9 ml of tryptic soy broth (Merck Chemical, Germany) then incubated at 37 °C for 24 h and subcultured over two consecutive days. On the third day, 18–20 h cultures were used as the working inoculum with the final bacterial concentration at 7–8 log CFU/ml. Four ml of bacterial inoculum (*E. coli* or *S.* Typhimurium) were added into 369 ml of 0.1% w/v peptone water and mixed thoroughly. The solution was then poured onto 200 g of vegetable in a PE bag (40 × 50 cm) and manually shaken every 2 min over a 20 min period. Before washing, the contaminated vegetables were dried in a biological safety cabinet with an air flow for 20 min. The initial population on the artificially contaminated vegetables was 6–7 log CFU/g (before washing).

2.4. Washing conditions

Samples of 100 g of artificially contaminated vegetables were used in each washing treatment. The pre-washing was done by submerging samples in water with or without 0.1% v/v commercial washing solutions, then continuously shaking for 3 min at 60 rpm (Major Science Funny Shaker, GIBTHAI, Thailand). Testing involved three commercial vegetable washing agents that were available in the markets of Thailand: Liquid cleanser® (S1), St. Andrews® (S2), Safeguard veggies wash® (S3). In addition the laboratory surfactants Tween80® (S4) was tested. Table 1 shows the main components and active ingredients of the tested washing agents.

Fig. 1a shows the two-step washing procedure. The ratio of vegetable sample and washing solution was 1:40 (100 g of vegetable: 4000 ml of washing solution). The second vegetable wash was done using AEO-MB solution for 5 min. AEO with available chlorine 40 mg/L was used as the medium for generating MB and this washing medium was produced using an electrolysis water generator model ROX-10WA-E (Hoshizaki Electric, Japan). The MB water was generated using an MB generator model Sumizumi II (Science & Technology Service Co., Ltd, Thailand) (Fig. 1b). The average MB diameter was measured using image analysis with a light microscopy technique according to Klintham et al. (2017). The MB diameter (Ø) was ~50–70 μm (Fig. 1c).

2.5. Microbial enumeration

Microbiological enumeration was carried out on each 100 g sample of sweet basil or Thai mint before washing and after two-step washing. A random sample of 25 g of vegetable was transferred into a sterile stomacher bag containing 225 ml of 0.1% w/v peptone water and the contents were pummeled and serial dilutions (1:10) were carried out. Additionally, 10 ml of the wash water before washing, after pre-washing and after second washing were enumerated for surviving