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Inactivation kinetics of *Listeria monocytogenes* and *Salmonella enterica* serovar *Typhimurium* on fresh-cut bell pepper treated with slightly acidic electrolyzed water combined with ultrasound and mild heat

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The goal of this study was to enhance the antimicrobial effect of slightly acidic electrolyzed water (SAEW) through addition of synergistic treatment with ultrasound (US) and mild heat treatment in order to improve the microbial safety of fresh-cut bell pepper. To evaluate the synergistic effects, the Weibull model was used to mathematically measure the effectiveness of the individual and combined treatments against *Listeria monocytogenes* and *Salmonella Typhimurium* on the pepper. The combined treatment (SAEW+US+60 °C) resulted in the T_R values of 0.04 and 0.09 min for *L. monocytogenes* and *S. Typhimurium*, respectively, as consequence of the minimum value. Subsequently, texture analysis was carried out to test the potential effect on quality of the samples due to the involved mild heat and ultrasound treatment. When compared to the control, there was no significant change ($p \geq 0.05$) in the texture (color and hardness) of the samples that were treated by 1 min of the combined treatment (SAEW+US+60 °C) during storage at 4 °C for 7 days. This combined treatment achieved approximately 3.0 log CFU/g reduction in the two pathogens. The results demonstrate that the involved hurdle factors which are ultrasound and mild heat achieved the synergistic effect of SAEW against the two pathogens. According to the results of texture analysis, 1 min of SAEW+US+60 °C is the optimal condition due to without negative influence on the quality of the samples during the storage. The optimal condition shows the enhanced antimicrobial effect of SAEW and enables to improve microbial safety of fresh bell pepper in food industry as a consequence of hurdle approach.

1. Introduction

The demand for fresh vegetables has been increasing rapidly over the last few decades due to changes in consumer eating habits, health concerns, and convenient foods (Huxley et al., 2004; Ajlouni et al., 2006; Martinéz-Sánchez et al, 2006; Vandekinderen et al., 2009; Chemat et al. 2011; Fava et al., 2011; São et al., 2014a). As a result of the increased consumption, the microbial safety of these products becomes a major challenge for the food industry due to foodborne pathogens commonly present in the products.

Chlorine (Tomás-Callejas et al., 2012), hydrogen peroxide (Alexander et al., 2012), ozone (Alexandre et al., 2011), and organic acids (Huang and Chen, 2011) are chemical sanitizers which are frequently used for pre-treatment in minimally processed fruits and vegetables in order to minimize the populations of microorganisms and to extend their shelf-life. However, the use of some sanitizers, such as chlorine, is forbidden in several European countries due to the potential formation of by-product, which is harmful to environment as well as to human health. Slightly acidic electrolyzed water (SAEW), a type of electrolyzed water with a pH value of 5.0–6.5, is regarded as an effective antimicrobial agent in the recent decades. This sanitizer contains a high concentration of hypochlorous acid (HOCl) which mainly cause antimicrobial effect (Cao et al., 2009; Nan et al., 2010). Owing to low concentration of available chlorine, this sanitizer is capable of reducing the denaturation of the surface of fresh produce and the potential damage to human health and the environment. The significant effectiveness of antimicrobial activity of electrolyzed water against pathogenic bacteria has also been reported by several published reports (Koide et al., 2009; Issa-Zacharia et al., 2010; Rahman et al., 2010; Ding

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et al., 2011; Zhang et al., 2011; Forghani and Oh, 2013a, 2013b; Mansur et al., 2015). However, the results from these studies showed that the SAEW treatment alone was not able to completely decontaminate the microbes. This might be due to the adherent microorganisms in hidden places that is difficult to be decontaminated by aqueous sanitizer.

Ultrasound, a form of energy generated by sound waves (>16 kHz) (Jayasooriya et al., 2004), is regarded as an environmental friendly antimicrobial agent in food industry. Ultrasonic cleansing baths possessing tank size range from 10 to 2500 L are commonly applied for cleaning materials in food industry and this type of ultrasound usually operates at approximately 40 kHz at fixed levels of acoustic energy density (AED) (Awad, 2011). AED is determined in term of the sound energy per unit volume. It has been reported that antimicrobial efficacy was not remarkable when ultrasound performed alone (Ajlouni et al., 2006; Piyasena et al., 2003), but it is capable of improving the antimicrobial effectiveness of aqueous sanitizers to reduce the number of bacteria on food (Rodgers and Ryser, 2004; Huang et al., 2006; Cao et al., 2010; Sagong et al., 2011; São José and DantasVanetti, 2012). Further studies are, therefore, necessary to improve the bactericidal efficiency via combination of sanitizer and another physical method, such as ultrasound and mild heat.

According to the pre-treatment associated with aqueous sanitizers in minimally processed foods, the dipping time is regarded as a critical factor in processing of sanitizing. Several studies have been reported that ultrasound and mild heating can enhance the bactericidal efficiency of electrolyzed water through reducing the dip time (Rahman et al., 2011; Xie et al., 2012; Forghani and Oh, 2013a, 2013b). Until now there is no report on development of inactivation model to evaluate the combined bactericidal effects of SAEW and ultrasound treatment with mild heat on bell pepper. The aims of this study were to (a) evaluate the synergistic bactericidal effects of SAEW and ultrasound at 40, 50, and 60 °C for *Listeria monocytogenes* and *Salmonella* adherent cells to the surface of fresh-cut bell pepper; and to (b) estimate the inactivation kinetics of the pathogens via the Weibull model to obtain the optimized condition of the combination approach.

2. Materials and methods

2.1. Strains

The three strains of *L. monocytogenes* (ATCC 19114, ATCC 19116, ATCC 19118) and two strains of *Salmonella Typhimurium* (ATCC 14028, ATCC 26926) used in this study were obtained from the Department of Food Science, University of Georgia (Griffin, GA, USA). For preparation of the inoculum, each suspension (0.1 ml) of the stock cultures was individually transferred into 10 ml of tryptic soy broth (TSB; Becton Dickinson Diagnostic Systems, Sparks, Maryland; BD) and incubated at 37 °C for 24 h. When the cultures reached the late stationary phase, the cells were harvested by centrifugation (3000 g, 10 min, 4 °C) and the pellets were then washed, resuspended in 10 ml of 0.1% sterile peptone water (PW, Difco, MD, USA)and adjusted to make a final cell concentration of approximately 9.0 log CFU/ml. Each strain was mixed to construct *L. monocytogenes* and *S. Typhimurium* culture cocktails. The cocktails were used in the following experiments. The cell count of the mixed culture was confirmed by plating 0.1 ml of appropriately diluted cocktail onto TSA plates and incubating the plates at 35 °C for 24 h.

2.2. Inoculation of samples

Fresh bell pepper was purchased from a local retail store in Chuncheon, South Korea, and then aseptically transported to the laboratory within 2 h. Prior to starting the experiment, bell pepper samples were aseptically cut into pieces of 10 ± 0.2 g with similar size (30 × 30 mm) using a sterile knife. The external surface of samples were then inoculated by pipetting 0.1 ml of approximately 9.0 log CFU/ml with *L. monocytogenes* and *S. Typhimurium* suspensions. To inoculate evenly the samples, each suspension (0.1 ml) was deposited on the surface with droplets at 20 minimum locations (Ding et al., 2011). After that, the inoculated samples were aseptically dried in a laminar flow hood for 30 min to guarantee sufficient attachment of the bacteria on the samples. An initial pathogen level of approximately 6.0 log CFU/g can be obtained after this procedure.

2.3. Preparation of slightly acidic electrolyzed water (SAEW)

To standardize the water that is used to produce SAEW, the water characteristics such as pH, ORP and water hardness were estimated and shown in Table 1. Water hardness was measured using a total hardness test kit (Model HA-71A, Hach Company, Loveland, CO, USA) by drop count titration method according to the manufacturer's instructions. The SAEW (pH: 5.0–5.2, oxidation reduction potential (ORP): 930–950 mV, and available chlorine concentration (ACC): 28–30 mg/l) were produced by electrolysis of a diluted hydrochloric acid (6% HCl) in a chamber without membrane using an electrolysis device (BC-360, Cosmic Round Korea Co. Ltd., Seongnam, Korea) at a setting of 2.5 A and 22.8 V. The pH and ORP of the SAEW solution were determined by a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, N.J.) bearing pH and ORP electrodes. The ACC was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). After production, the SAEW was stored in polypropylene containers until use.

2.4. Experimental procedure

A complete factorial design (2 × 2 × 3 × 3) was carried out to evaluate synergistic effect of SAEW, ultrasound (US), and mild heat (MH) treatment (40, 50, 60 °C) at dip time of 1, 3, 5 min against *L. monocytogenes* and *S. Typhimurium* on the bell pepper. The thermo-sonication treatments were performed using a sterile bench-top ultrasonic cleaner (JAC-4020, KODO Technical Research Co., Ltd., Hwaseong, Gyeonggi-do, South Korea) at a fixed frequency of 40 kHz and acoustic energy density (AED) of 400 W/l at different temperatures (23, 40, 50, and 60 °C). The ultrasound device (rectangular tank, 721 × 451 × 297 mm) was filled with 6 L of SAEW prior to the experiment, and ten samples were processed at a time. After each factorial experiment, SAEW was removed and the tank was sterilized by ultraviolet light irradiation for 10 min, serially rinsed with 70% ethanol and left to dry.

2.5. Microbiological analyses

After sanitizing, the treated samples without mild heat were immediately placed into the stomacher bags (Nasco Whirl-pak, Janesville, WI) containing 90 ml of 0.1% sterile BPW, while the heat-treated samples were immediately placed into the bags

Table 1
Physicochemical properties of the process water.

Electrolyte	6% HCL
Water hardness (mg/l)	47 ± 2
pH	6.53–6.75
Oxidation reduction potential (min./max.)	565/591