Food Microbiology 36 (2013) 40–45

Contents lists available at SciVerse ScienceDirect

Food Microbiology

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

Hurdle enhancement of slightly acidic electrolyzed water antimicrobial efficacy on Chinese cabbage, lettuce, sesame leaf and spinach using ultrasonication and water wash

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ARTICLE INFO

Article history:
Received 3 January 2013
Received in revised form
6 March 2013
Accepted 2 April 2013
Available online 16 April 2013

Keywords:
Slightly acidic electrolyzed water
Fresh vegetables
Microbial reduction
Hurdle approach
Escherichia coli O157:H7
Listeria monocytogenes

ABSTRACT

Slightly acidic electrolyzed water (SAEW) is well known as a good sanitizer against foodborne pathogens on fresh vegetables. However, microbial reductions from SAEW treatment are not enough to ensure produce safety. Therefore, it is necessary to improve its antimicrobial efficiency by combining it with other appropriate approaches. This study examined the microbicidal activity of SAEW (pH 5.2–5.5, oxidation reduction potential 500–600 mV, available chlorine concentration 21–22 mg/l) on Chinese cabbage, lettuce, sesame leaf and spinach, four common fresh vegetables in Korea under same laboratory conditions. Subsequently, effects of ultrasonication and water wash to enhance the sanitizing efficacy of SAEW were studied, separately. Finally, an optimized simple and easy approach consisting of simultaneous SAEW treatment with ultrasonication (3 min) followed by water wash (150 rpm, 1 min) was developed (SAEW + US–WW). This newly developed hurdle treatment significantly enhanced the microbial reductions compared to SAEW treatment alone, SAEW treatment with ultrasonication (SAEW + US) and SAEW treatment followed by water wash (SAEW–WW) at room temperature (23 ± 2 °C). Microbial reductions of yeasts and molds, total bacteria count and inoculated *Escherichia coli* O157:H7 and *Listeria monocytogenes* were in the range of 1.76–2.8 log cfu/g on different samples using the new hurdle approach.

1. Introduction

Fresh vegetables are an essential part of the diet of people around the world and their consumption is increasing rapidly due to their health effects and changes in people lifestyles (Huxley et al., 2004; Ajlouni et al., 2006). The trend to consume fresh produce including vegetables has become even stronger over the last few decades (López-Gálvez et al., 2009). However, along with the increase in fresh vegetables consumption, concerns about the safety of consumers have risen, as presence of spoilage bacteria, yeasts and molds and pathogens is common in these foods (Zhang and Farber, 1996; Seymour et al., 2002).

Despite the extensive advances in food safety regulations and food processing methods, vegetables have been implicated in many foodborne disease outbreaks caused by a variety of pathogen microorganisms (Farber and Peterkin, 1991; Roever, 1998; Elizaquível and Aznar, 2008). The fact that lettuce (Mermin and Griffin, 1999), spinach (CDC, 2007), cucumber (WHO, 2011) and other vegetables have been reported to cause foodborne diseases frequently, clearly proves that the present commercial sanitizing operations are not enough to assure produce safety. Therefore, the complete inactivation or removal of pathogens from the fresh produce is still a challenge to the food industry (José and Dantas Vanetti, 2012) and developing new sanitization approaches as well as improving the present techniques is necessary. Some examples of the present sanitization techniques which have been used or studied for fresh produce sanitization are: irradiation (Chaudry et al., 2004), different types of electrolyzed water (Al-Haq et al., 2005), warm water (Klaiber et al., 2005), chlorine dioxide (Huang et al., 2006), ultrasound (Ugarte-Romero et al., 2006), acidified sodium chlorite (Ruiz-Cruz et al., 2007), ozone (Alexandre et al., 2011), organic acids (Huang and Chen, 2011) and hydrogen peroxide (Alexander et al., 2012). Also combinations of these methods have been studied in seek of higher efficiency (Koseki et al., 2004; Ukuku et al., 2005; Yuk et al., 2006; Park et al., 2009; Zhou et al., 2009). Despite all these methods are available for sanitizing fresh produce, their efficacies vary and none are able to ensure a complete elimination of microbes without compromising sensory quality.

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http://dx.doi.org/10.1016/j.fm.2013.04.002

Slightly acidic electrolyzed water (SAEW), is a type of electrolyzed water with a pH value of 5.0–6.5 that contains a high concentration of hypochlorous acid (HOCl) and its antimicrobial effect, mainly caused by the presence of HOCl has been proved (Cao et al., 2009; Nan et al., 2010). It is generated by electrolysis of a dilute hydrochloric acid (HCl) and/or NaCl solution in a non-membrane electrolytic cell (Honda, 2003). SAEW has the advantage of possessing antimicrobial activity with low available chlorine, resulting in reduced corrosion of surfaces and minimization of the potential damage to human health and the environment. Therefore, there is growing interest in new applications for the bactericidal activity of SAEW in the food industry as an environmental friendly sanitization method (Issa-Zacharia et al., 2010; Nan et al., 2010; Koide et al., 2011).

Ultrasound is a form of energy generated by sound waves of frequencies that are too high (>16 kHz) to be detected by human ear (Jayasooriya et al., 2004). Application of ultrasound as another environmental friendly antimicrobial agent has a long history (Harvey and Loomis, 1929; Hamre, 1948), although its application in food industry is more recent (Garcia et al., 1989; Sams and Feria, 1991; Cao et al., 2010). It has been proved that ultrasound has antimicrobial effect against *Escherichia coli* (Hua and Thompson, 2000; Furuta et al., 2004), *Listeria monocytogenes* (Mañas et al., 2000; Baumann et al., 2005) and other pathogens. This microbial inactivation is mainly attributed to cavitation, a phenomenon that disrupts cellular structure and function (Barbosa-Canovas, 2002; Ugarte-Romero et al., 2006). It has been reported that ultrasound alone does not effectively inactivate bacteria on food (Piyasena et al., 2003) as it does in saline solution or water. However, combined with other treatments it may result in higher lethality (Scouten and Beuchat, 2002; Álvarez et al., 2003; Rodgers and Ryser, 2004). Therefore, there is a need to further examine the function of ultrasound, when combined with a sanitizer for the decontamination of fresh produce.

As mentioned above, SAEW has been extensively studied. But its combination with ultrasound and moreover post treatment water wash has not been reported to the best of authors' knowledge. Moreover, results obtained from different studies on ultrasound combined with other sanitizers are hard to compare, due to the application of different sanitizers, different samples and different laboratory conditions. Also, significant variations in physical parameters such as ultrasound properties, the size and shape of ultrasonic bath, the depth, volume and temperature of the liquid and treatment time make comparisons more difficult.

Hence, the aim of this study was to: (A) evaluate the efficiency of SAEW in the disinfection of four types of most common fresh vegetables in Korea (Chinese cabbage, lettuce, sesame leaf and spinach) under the same laboratory conditions; (B) develop a simple, environmental friendly, easy to perform hurdle approach using SAEW, ultrasound and water wash to maximize decontamination efficacy based on the fact that each hurdle applied may impose an increase in the antimicrobial action (Martín-Belloso and Sóbrin-Lopez, 2011); (C) study the effect of produce type on the sanitization effect of the treatments; (D) study the effect of inoculated bacteria type on the sanitization effect of the treatments using *E. coli* and *L. monocytogenes* as two pathogenic bacteria of the most concern in fresh vegetables (Yang et al., 2003).

2. Materials and methods

2.1. Sample preparation

Chinese cabbage, lettuce, sesame leaf and spinach were purchased from local supermarkets in Chuncheon, South Korea. The outer three or four leaves, damaged parts and the core of Chinese cabbage and lettuce and defected leaves of sesame leaf and spinach were removed and discarded. Fresh leaves were then aseptically trimmed to approximately 10 g pieces (more than one leaf for sesame and spinach), packed in a polyethylene bag and stored at 4 °C to use for the experiment within 24 h.

2.2. Preparation of inocula

The three strains of *E. coli* O157:H7 (B0259, B0273 and B0265) used in this study were obtained from the Department of Food Science, University of Georgia (Griffin, GA, USA). The three strains of *L. monocytogenes* (ATCC 19115, ATCC 19111 and Scott A) were obtained from Gyeonggi-do Research Institute of Health & Environment (Gyeonggi-do, Republic of Korea). These strains were selected due to their pathogenicity, wide spread in the environment or previously reported presence in human outbreaks. Stock cultures of each pathogen were transferred into tryptic soy broth (TSB; Becton Dickinson Diagnostic Systems, Sparks, Maryland; BD) and incubated for 18 h at 35 °C. Following incubation, 10 ml of each culture was sedimented by centrifugation (3000 × g for 10 min at 4 °C), washed and resuspended in 10 ml of 0.1% peptone water (pH 7.2) (BD) to obtain a final cell concentration of approximately 8 log cfu/ml. Subsequently, suspended pellets of each strain of the two pathogens were combined to construct *E. coli* and *L. monocytogenes* culture cocktails. These culture cocktails were used in subsequent experiments. The bacterial population in each cocktail culture was confirmed by plating 0.1 ml portions of appropriately diluted culture on tryptic soy agar (TSA) (BD) plates and incubating the plates at 37 °C for 24 h followed by enumeration.

2.3. Inoculation of samples

To destroy the background microflora, 10 g samples were placed on a sterile perforated tray and treated with UV light (Philips, TUV 15W) by 30 cm distance in a UV cabinet (Entkeimungsschrank, 220 V, Ernst Schuttjun Laborgerotebau, 3400, Gottingen, Germany) for 30 min (15 min for each side) (Singh et al., 2002; Sengun and Karapinar, 2005; Rahman et al., 2011). After applying this treatment, the naturally existing bacterial population was reduced to an undetectable level (with 10 cfu/g detection limit). Subsequently, trimmed leaves were placed on sterile aluminum foil in a biosafety hood. For inoculation, 0.1 ml of each pathogen cocktail (8 log cfu/ml of *E. coli* or *L. monocytogenes*) was applied to the abaxial-side of each leaf surface by depositing droplets at minimum 20 locations with a micropipettor followed by drying in the laminar flow hood for 30 min at room temperature (23 ± 2 °C) to allow for bacterial attachment to the leaf surface (Park et al., 2008; Ding et al., 2011). This procedure resulted in initial pathogen inocula levels of approximately 6 log cfu/g.

2.4. Preparation of SAEW

The SAEW used in this study was 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 electrolyzed high concentration hypochlorous acid solution was diluted with tap water at a flow rate of 4 l/min to produce the final SAEW with a pH of 5.2–5.5, an oxidation reduction potential (ORP) of 500–600 mV and an available chlorine concentration (ACC) of 21–22 mg/l. The SAEW was collected for use approximately 20 min after starting the device when stable amperage was reached. The pH, ORP and ACC of the SAEW solution was measured with a dual-scale pH meter (Accumet model 15, Fisher