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Efficacy of lactic acid in reducing foodborne pathogens in minimally processed lotus sprouts

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ABSTRACT

Fresh lotus sprout is easily browned and perishable due to microbial growth and degradation. Therefore, browning and foodborne pathogen have become the most serious problems. The objective of this study was to investigate the effect of lactic acid (0.25%, 0.5%, 1% and 2% (v/v)) on reduction of foodborne pathogens, such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*, in lotus sprouts. Tap water and sodium hypochlorite (200 mg/l) were used as control treatments. Results indicated that tap water caused a slight reduction (<0.5 log) in the microbial loads. The sodium hypochlorite treatment led to 1.3 log reductions of the microbial population. When treated with 0.5% and 2.0% lactic acid solutions, 1.5 and 2.3 log reductions were achieved, respectively. The effectiveness of lactic acid treatment increased with the increase of lactic acid concentration. Results showed that the lactic acid treatment at 0.5% or higher was effective to reduce foodborne pathogens in lotus sprouts. The L^* values of samples treated with lactic acid decreased slightly during storage. Furthermore, the lactic acid treatment contributed to slow accumulation of red color on lotus sprouts, which was more effective than sodium hypochlorite treatment to reduce the discoloration of lotus sprouts. These results indicated that lactic acid can be used to improve the color and safety of minimally processed lotus sprouts.

1. Introduction

China is the largest producer of Lotus (*Nelumbo nucifera gaertn*) in the world. Fresh lotus sprout is a seasonal vegetable available only from April to September in China. It has excellent flavor and is considered as a highly sought-after culinary item. However, with a high water content (>90% in weight), the fresh lotus sprout tends to become brown and perishable due to metabolism, physical damage and microbial growth. Therefore, minimally processed lotus sprouts could be a good choice to prolong its shelf-life to meet ever-increasing demands of consumers for high quality, fresh, nutritious, and conveniently prepared vegetables (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).

However, there has been an increase in the frequency of foodborne disease outbreaks associated with consumption of fresh produce. Vegetables contain nutrients necessary for rapid growth of foodborne pathogens such as *Escherichia coli*, *Salmonella*, *Listeria* and *Yersinia* (Alegre, Abadias, Anguera, Usall, & Viñas, 2010; Issa-Zacharia, Kamitani, Morita, & Iwasaki, 2010; Issa-Zacharia, Kamitani, Tiisekwa, Morita, & Iwasaki, 2010; Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). *E. coli* O157:H7 is a major foodborne pathogen that can cause illnesses, such as hemorrhagic colitis and hemolytic uremic syndrome (Ibrahim, Salameh, Phetsomphou, Yang, & Seo, 2006; Smigic et al., 2009; Velazquez, Barbini, Escudero, Estrada, & de Guzman, 2009). *Listeria monocytogenes* has caused much concern to the food industry, since it has been associated with large-scale outbreaks (Byelashov et al., 2010).

Different washing chemicals have been investigated to determine their efficacy in the inactivation of pathogens on vegetables (Oms-Oliu et al., 2010; Velazquez et al., 2009). Among them, solutions of 50–200 ppm chlorine (sodium hypochlorite) are widely used at commercial scale for washing fresh cut produce (Beuchat, Nail, Adler, & Clavero, 1998; Ruiz-Cruz et al., 2007). However, the efficacy of sodium hypochlorite is limited due to the formation of toxic by-products in the presence of organic matter as a result of chlorination (Abadias, Alegre, Usall, Torres, & Viñas, 2011; Beuchat, 2000; López-Gálvez et al., 2010). Currently, consumers are paying more attention not only to the risk of foodborne pathogens but also to the safety of artificial chemical preservatives that are used to

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control these foodborne pathogens (Ibrahim, Yang, & Seo, 2008; López-Gálvez et al., 2010).

The effect of organic acids on reducing populations of microorganisms on fresh vegetables has been explored, while lactic acid with different concentrations (from 0.2% to 2%) has shown promising results against common pathogens such as *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* and *Yersinia enterocolitica* (Akbas & Olmez, 2007; Huang & Chen, 2011; Ibrahim et al., 2008; Sagong et al., 2011; Tajkarimi & Ibrahim, 2011; Uyttendaele, Neyts, Vanderswalmen, Notebaert, & Debevere, 2004; Velazquez et al., 2009). The inhibiting effect of weak organic acid is based on their pKa, antimicrobial activity of their non-dissociated form, and specific effects of each acid (Giannuzzi & Noem, 1996; Velazquez et al., 2009).

Up to now, most research on lotus sprout has been directed to prevent its browning and softening (Du, Fu, & Wang, 2009; Li et al., 2010). Little information is available regarding the microbiological quality. Effective methods for assuring the microbiological safety of minimally processed lotus sprout have not been extensively explored. Therefore, the main objective of this study was to assess the effectiveness of lactic acid on *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated on minimally processed lotus sprouts, and compare its efficacy with tap water and sodium hypochlorite solution. In addition, the color of minimally processed lotus sprout was determined to evaluate the antibrowning effect of lactic acid on lotus sprouts during storage at 4 °C.

2. Materials and methods

2.1. Bacterial strains

The bacterial cultures of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* strains were obtained from the Food Microbiology Laboratory, College of Food Science and Technology, Huazhong Agricultural University (Wuhan, China). The cultures were preserved at −25 °C with 50% (v/v) glycerin (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) solution at a ratio of 1:1.

2.2. Inoculum preparation

The *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* suspension were prepared by transferring 0.1 ml of each culture to a 10 ml of 0.1% peptone water (PW, Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) and each sample was incubated for 24 h at 37 °C (DNP-9272E, Shanghai Jing Hong laboratory Instrument Co., Ltd, Shanghai, China) and transferred at 24 h intervals. Bacterial cells were obtained by centrifugation (J-E, BACKMAN, California, USA) at 9100 g for 10 min at 4 °C. The supernatant of each bacterial suspension was decanted, while the cell pellet was resuspended in 100 ml of 8.5 g/l sterile physiological saline (Sanshen YM30F, Shanghai Sanshen Medical Instrument Co., Ltd, Shanghai, China). Three selective media were used to enumerate the bacteria. For *E. coli* O157:H7, Sorbitol-MacConkey agar supplemented with Cefixime-Tellurite. For *S.* Typhimurium and *L. monocytogenes*, Xylose Lysine Desoxycholate and Trypticase Soy-Yeast Extract Agar were used, respectively. All selective media were obtained from Qingdao Hope Bio-Technology Co., Ltd (Qingdao, China). The initial concentration was approximately10^9 cfu/ml. The population of each bacteria was confirmed by plating 0.1 ml portions of appropriately diluted suspension on duplicated plates (90 mm) for 24 h at 37 °C.

2.3. Sample preparation

Lotus (*Nelumbo nucifera Gaertn* cv.) sprouts were purchased from a local wholesale market (Wuhan, Hubei, China) on the day of harvest and transported to the laboratory without delay. Prior to experimental studies, tap water was used to remove the impurities (such as mud and withered leaves) from the surface of lotus sprouts. All the samples were submerged in a big container with enough tap water and stored at room temperature over night before processing.

In the second day, top and tail of lotus sprouts were removed and discarded while central parts were cut into 8–10 cm in length (10 ± 0.2 g each) with a stainless steel knife previously disinfested with 70% (v/v) alcohol.

2.4. Sample inoculation

Fresh-cut lotus sprouts (10 ± 0.2 g each, about 8–10 cm in length) were submerged in a container with inocula of each microorganism and gently agitated for 5 min at room temperature to obtain an initial level of above 10^7 cfu/g. Samples were removed and then air-dried in a biosafety chamber (AIRTECH, SW-CJ-1FD, Jiangsu Suzhou Purification Group, Suzhou, China) for 30 min to facilitate bacterial adhesion before exposure to various disinfection treatments. Appropriate selective medium as mentioned above was used to determine the initial population of each pathogen inoculated onto the surface of samples.

2.5. Procedures for treatment

In the study, all treatment solutions were prepared within 1 h before each experiment. Lactic acid (LA) (Shanghai Chemicals Reagent Co., Ltd, Shanghai, China) was dissolved in distilled water to obtain solutions containing 0.25%, 0.5%, 1% and 2% (v/v). Sodium hypochlorite (NaClO) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) was used to prepare the solution at 200 mg/l of free chlorine. NaCl solution (8.5 g/l) was prepared to dilute each bacterial suspension in advance.

As stated above, inoculated samples were individually placed in different washing treatments after removing excess water. During each experiment, two samples were assayed after each treatment for 10 min. Three untreated samples inoculated with *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium were used to determine the initial bacterial loads on the sample surface. Two untreated samples were immersed in tap water and sodium hypochlorite (200 mg/l) for 10 min as control treatments. All samples were gently agitated during the treatment period. After washing, each sample was transferred to a sterile stomacher blender bag (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) to determine the microbial populations.

For storage, samples were packaged after washing treatments by a vacuum packaging machine (0.08 MPa) (Jiesheng, DZQ 400-2D, Suzhou, China) and stored at 4 °C (Haier Pharmacy Refrigerators, Qingdao, China) until microbiological analysis.

2.6. Bacterial enumeration

For enumeration, 25 g of lotus sprout sample was transferred into sterile stomacher blender bags (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China), combined with 225 ml of sterile physiological saline (8.5 g/l NaCl), and shaken vigorously by hand for 1 min. The solution (1 ml) was serially diluted in test tubes containing 9 ml of sterile physiological saline. Then, properly diluted bacterial solutions were transferred to appropriate selective media and then poured to sterile petri dishes. Finally, the solidified plates were incubated for 24 h at 37 °C (DNP-9272E, Shanghai Jing Hong Laboratory Instrument Co., Ltd, Shanghai, China), while colonies were counted following the incubation in the next day.