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Comparison of the efficacy of various sanitizers and hot water treatment in inactivating inoculated foodborne pathogens and natural microflora on mung bean sprouts

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ABSTRACT

The microbiological safety of seed sprouts has been a concern in recent years following several reports of foodborne outbreaks associated with the consumption of seed sprouts. The frequent occurrence of outbreaks suggests the need for effective decontamination of seed sprouts. In this study, various decontamination methods including acidic electrolyzed water (AEW) (75 ppm available chlorine, ORP 1150 mV, pH 2.8, 180 s), acidified sodium chlorite (ASC) (1200 ppm, pH 2.3, 180 s), cetylpyridinium chloride (CPC) (2%, 180 s), ozonated water (2 ppm, 180 s), trisodium phosphate (TSP) (10%, pH 12.6, 180 s) and hot water (70 °C, 20 s) were evaluated for their efficacy against inoculated pathogens and natural mircoflora on mung bean sprouts. Results showed that the hot water treatment reduced the microbial population by 4.19, 4.35, 4.81 and 4.37 log CFU/g in *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and natural microflora on mung bean sprouts, respectively. On the other hand, chemical sanitizer treatments using AEW, ASC, CPC, ozonated water and TSP resulted in less than 2-log reduction in the same bacterial strains. However, hot water treatment also caused detrimental impact on the color and firmness of the bean sprouts after treatment or during storage for 4 days at 4 and 25 °C. Nevertheless, the present results indicate that, compared to the chemical sanitizers, hot water treatment has a better potential for postharvest control measure to improve the microbiological safety of raw mung bean sprouts. However, this study suggests that the method needs to be further modified to better retain the physical quality of raw mung bean sprouts. These findings may help to expand the limited pool of information on the efficacy and feasibility of decontamination treatments on sprouts.

1. Introduction

Seed sprouts are widely consumed in various parts of the world today. They often appeal to the health conscious consumers as they are high in nutritional value and low in calories (NACMCF, 1999; Taormina, Beuchat, & Slutsker, 1999). Sprouts can be consumed on their own or as a mixture with other vegetables. These are often consumed raw or are lightly cooked in a variety of dishes. The most commonly consumed seed sprouts worldwide are alfalfa, mung bean and radish sprouts.

Consumption of raw seed sprouts has been implicated in foodborne outbreaks across several countries (Watanabe et al., 1999; Winthrop et al., 2003; Yang et al., 2013). Most of these outbreaks were caused by seeds contaminated with foodborne pathogens such as *Salmonella* spp. and *Escherichia coli* O157:H7 (Fu et al., 2001; USFDA, 2001; Winthrop et al., 2003). Sprout seeds may become contaminated with pathogens through various routes such as contaminated soil, irrigation water, and manure (Yang et al., 2013). Additionally, favorable temperature and availability of water during the sprouting process promote the growth of many types of bacteria (Fu et al., 2001; Taormina et al., 1999).

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) recommends that, prior to consumption, sprout seeds should be treated with any method capable of effecting a 5-log reduction of pathogens (NACMCF, 1999). Extensive studies have been carried out to determine the effectiveness of various methods

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including irradiation, ultraviolet light, pulsed electric or magnetic fields, high pressure, heat and chemical sanitizers in eliminating pathogenic bacteria on sprout seeds (USFDA, 2001). Treatment of sprout seeds with 20,000 ppm calcium hypochlorite was successful in achieving a 5-log reduction of pathogens on sprout seeds (Beuchat, Ward, & Pettigrew, 2001; Fett, 2002). However, the treatments based on hypochlorite or alternative sanitizing chemicals such as peroxyacetic acid or hydrogen peroxide only reduce the pathogen counts on sprout seeds rather than completely eliminating them (Brooks et al., 2001; Wessinger & Beuchat, 2000). This is of great concern as even low counts of surviving pathogens (<0.1 CFU/g) on the seeds may grow to high levels during the sprouting process to cause foodborne diseases (Holliday, Scouten, & Beuchat, 2001). The failure of sanitizing treatments on seeds caused the multistate outbreak of *S.* Muenchen that was associated with consumption of raw alfalfa sprouts, despite the fact that the seeds for sprouting were pretreated with calcium hypochlorite (Proctor, Hamacher, Tortorello, Archer, & Davis, 2001). Hence, it is necessary to implement other control measures during postharvest processing to minimize the risk of foodborne outbreaks.

Chlorine is one of the most commonly used chemical sanitizers for fresh produce including sprout production. Relatively high concentrations of chlorine (50–200 ppm) are recommended to decontaminate sprouts during postharvest processing (NACMCF, 1999). However, chlorine-based sanitizers are a growing environmental and health concerns as pollutive and carcinogenic compounds such as trihalomethanes are produced as by-products of chlorination (Beuchat, Nail, Adler, & Clavero, 1998). In addition, the study conducted by Singh, Chandra, Agarwal, and Babu (2005) showed that only 1.6-log reduction in total pathogen count was achieved when wheat sprouts were treated with 20,000 ppm calcium chloride for 15 min, indicating the inadequacies of chlorine treatment. Thus, there is a need for alternative decontamination methods to eliminate foodborne pathogens on sprouts, preferably with lesser environmental and health impacts.

Acidic electrolyzed water (AEW) has demonstrated high effectiveness against several strains of pathogenic bacteria in food due to its low pH, high redox potential and strong oxidizing properties (Kim, Hung, & Brackett, 2000; Len, Hung, Erickson, & Kim, 2000). It was found that AEW treatment for 5 min was able to reduce the populations of *E. coli*, *S.* Enteriditis, and *Listeria monocytogenes* by approximately 7-log units (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). Acidified sodium chlorite (ASC) has been approved for use on animal carcasses and/or food processing equipment by the U.S. Food and Drug Administration (CFR, 2004). Several studies have reported the high efficacy of ASC in reducing the populations of pathogens such as *E. coli* O157:H7 and *Salmonella* spp. on fresh produce (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004; Park & Beuchat, 1999; Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). Cetylpyridinium chloride (CPC) is a quaternary ammonium compound that has a strong antimicrobial property. It was reported that about 5.6-log reductions of *E. coli* O157:H7 and *L. monocytogenes* on chopped lettuce were achieved with 0.2%–0.8% CPC (Johnson & Janes, 2001). Ozonated water is a strong oxidizing agent with demonstrated antimicrobial properties. A previous study reported a 2.53-log reduction of *S.* Typhimurium inoculated on fresh tomatoes when treated with 2 ppm ozonated water for 120 s (Chaidez, Lopez, Vidales, & Campo, 2007). Trisodium phosphate (TSP) is one of the chemical sanitizers that has been extensively used to treat fresh produce. A significant reduction in viable *S.* Montevideo count without any adverse effect on color and brightness was observed in mature green tomatoes treated with TSP (Zhuang & Beuchat, 1996). Hot water is another potent disinfectant widely applied to decontaminate the surface of cantaloupe and sprout seeds (Annous, Burke, Sites, & Phillips, 2013; Pao, Kalantari, & Khalid, 2007). A study on broccoli florets showed that hot water treatment at 55 and 60 °C for 3 min completely eliminated the inoculated *E. coli* and *Salmonella* spp., respectively (Phanida, Varit, Apiradee, Sirichai, & Pongphen, 2010).

As most of the existing studies have only examined the efficacy of decontamination methods on sprout seeds, there is limited information on the efficacy of the methods on sprouts. Therefore, the objective of this study was to compare the efficacy of various decontamination methods including AEW, ASC, CPC, ozonated water, TSP and hot water in inactivating *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. and natural microflora on raw mung bean sprouts. The effect of hot water treatment on the physical and microbiological quality of bean sprouts was also studied during the storage period.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Salmonella spp. (*S.* Montevideo BAA 710, *S.* Newport BAA 707, *S.* Saintpaul ATCC 9712, *S.* Typhimurium ATCC 14028 and *S.* Agona BAA 707) and *L. monocytogenes* (1/2a BAA 679, 1/2b BAA 839, 1 ATCC 19111 and 4b ATCC 13932) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). *E. coli* O157:H7 (EDL 933) was obtained from Dr. Henry Mok, Department of Biological Sciences, National University of Singapore. Bacterial strains were adapted to 100 μg/ml nalidixic acid (Sigma–Aldrich, St Louis, MO, USA) by stepwise increment of nalidixic acid after each transfer of the respective culture. All media used in this study were supplemented with 100 μg/ml nalidixic acid so that these pathogens isolated from inoculated bean sprouts were relatively free from other background bacterial contaminants. Prior to inoculation, the respective pathogenic strains were cultivated in sterile tryptic soy broth (TSB: Oxoid, Hampshire, UK) containing nalidixic acid at 37 °C for 24 h with two consecutive transfers.

2.2. Inoculum preparation

After 24 or 48-h of incubation, 1 ml of bacterial culture was centrifuged (3500 g for 10 min, 4 °C) and the pellet was washed with 1 ml of sterile 0.1% (w/v) peptone water (Oxoid). The washing and centrifugation steps were repeated twice. Harvested pellets were re-suspended in 1 ml of sterile 0.1% peptone water to obtain a final cell density of 10^8 CFU/ml. It is known that the effectiveness of sanitizer is strain dependent (Hora, Kumar, Kostrzynska, Dixon, & Warriner, 2007), thus five *Salmonella* serovars or four *L. monocytogenes* serovars were aseptically combined to produce *Salmonella* or *Listeria* cocktail after washing. On the other hand, only a single strain of *E. coli* O157:H7 was used in this study since pathogenic strains of *E. coli* could not be found in Singapore and its import from other countries is highly regulated.

2.3. Inoculation

The vacuum-packaged mung bean sprouts were purchased from a local supermarket and stored at 4 °C in a refrigerator before each experimental trial. The bean sprouts were retrieved from the refrigerator and left at room temperature for 45 min prior to inoculation. A 400-g portion of bean sprouts was weighed and immersed into 2 L of sterile deionized water containing prepared inoculum/cocktail (approximately 10^5–10^6 CFU/ml). The bean sprouts were thoroughly circulated with a sterile magnetic stirrer for 45 min. After inoculation, the bean sprouts were scooped up with a sterile metal sieve and air dried on two sterile plastic trays in a biosafety cabinet for about 4 h. A 20-g portion of inoculated