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Short communication

Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries

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Abstract

The effect of aqueous chlorine dioxide (ClO_2) on controlling foodborne pathogens, yeasts, and molds on blueberries was studied. Five pathogens were spot-inoculated on the skin of blueberries. A sachet containing necessary chemicals for generation of ClO_2 was used to provide 320 ppm of ClO_2 in 7.5 l of water. The efficacy of different concentrations (1, 3, 5, 10, and 15 ppm) of ClO_2 and various contact times (10 s; 1, 5, 10, 20, 30 min; and 1 h and 2 h) were studied. ClO_2 was most effective in reducing *Listeria monocytogenes* (4.88 log cfu/g) as compared to the other pathogens. *Pseudomonas aeruginosa* was reduced by 2.16 log cfu/g after 5 min when treated with 15 ppm of ClO_2. Relatively short treatment time was more effective in reducing *Salmonella Typhimurium* than longer treatment time for most concentrations. The highest reduction (4.56 log cfu/g) of *Staphylococcus aureus* was achieved with 15 ppm of ClO_2 for 30 min. When treated for 2 h with 5 ppm of ClO_2, *Yersinia enterocolitica* was reduced by 3.49 log cfu/g. Fifteen ppm of ClO_2 reduced natural yeasts and molds by 2.82 log cfu/g after 1 h. Concentrations of ClO_2 decreased over time. When exposed to blueberries, ClO_2 concentrations were further reduced, showing significant degradation.
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1. Introduction

Bacterial foodborne outbreaks have been associated with raw fruit and vegetable products (Burnett and Beuchat, 2001). Bacterial pathogens isolated from raw vegetables or fruits include *Aeromonas*, *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Saureus*, *Shigella*, *Staphylococcus*, *Vibrio chlolerae*, *Yersinia enterocolitica*, and so on (Beuchat, 1998). A cabbage-associated outbreak of listeriosis has been documented (Schlech et al., 1983). There are reports of human salmonellosis linked to cantaloupe and sprouts produced from alfalfa seeds imported to the United States (Beuchat, 1998). *Staphylococcus aureus* has been detected on raw produce (Abdelnoor et al., 1983) and ready-to-eat vegetable salads. Seven percent of carrot samples obtained from eating establishments in France were reported to contain serotypes of *Yersinia* that may be pathogenic to humans (Catteau et al., 1985). *Pseudomonas*, yeasts, and molds are concerns due to their contribution to spoilage of fruits and vegetables.

Although bacterial foodborne illnesses have not been linked to consumption of fresh or raw blueberries to date, blueberries are vulnerable to bacterial contamination in the field due to hand harvesting, field packing, and non-composted manure fertilization (if it is used). In industries, many produces including blueberries are washed or sprayed with chlorinated water containing 50–200 ppm of active chlorine to reduce microorganisms (Brackett, 1992; Cherry, 1999). However, chlorine may not be effective in reducing microorganisms on fruits and vegetables at high concentrations (Brackett, 1992; Zhang and Farber, 1996; Taormina and Beuchat, 1999). Indeed, some chemical by-products formed when chlorine is used for reducing microorganisms in food processing are considered as mutagenic or carcinogenic (Richardson et al., 1998).

Researchers have focused on chlorine dioxide (ClO_2) as an alternative sanitizer since it has 2.5 times the oxidation capacity of chlorine and is less reactive to organic

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compounds (Benarde et al., 1967; Richardson et al., 1998; Beuchat et al., 2004; Han et al., 2004; Lee et al., 2004; Sy et al., 2005). Most of the investigators working on sanitization use gaseous ClO_2 because it offers greater penetration than aqueous ClO_2 and is therefore considered more effective in reducing microorganisms on fruits and vegetables (Han et al., 2001; Lee et al., 2004). In addition, aqueous ClO_2 treatment may result in residual water which may promote mold growth on fresh-market blueberries if residual water is not further dried off. For applications in the food industry, however, use of gaseous ClO_2 may be limited because gaseous ClO_2 treatment must be conducted in a firmly and safely sealed chamber (Lee et al., 2004), high concentrations of the gas are potentially explosive (EPA,1999), and numerous mechanical devices or steps are necessary to handle ClO_2 gas as well as to provide precise concentrations for sanitization (Han et al., 2004; Lee et al., 2006).

Aqueous ClO_2 offers several advantages for food sanitization, especially for processing of vegetables and fruits, such as blueberries. A special chamber is not required for the sanitizing process, handling is easier than with gaseous ClO_2, and the liquid may be easily applied to the existing process during washing without modifying subsequent steps. In addition, using an aqueous solution may provide advantages to producers who wish to preserve the appearance of their sanitized produce. Generating ClO_2 traditionally requires either reaction with acid or on-site instrumentation such as an applicator or generator to generate and apply the liquid. Therefore, it is very inconvenient, relatively expensive, and requires technical expertise. For application of ClO_2 in the food industry, simple and inexpensive sanitizing procedures are necessary. Recently, a simple, small, and dry chemical pouch method was developed for generating ClO_2 (Intellectual Capital Associates (ICA) TriNova, LLC, Forest Park, GA). This method is easy to use when compared with machinery systems. Applying this new pouch method as an aqueous sanitizer to reduce microorganisms on produces has not been investigated.

This study was conducted to evaluate the bactericidal effectiveness of aqueous ClO_2 generated by a simple chemical pouch on five foodborne pathogens, *L. monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium*, *S. aureus*, and *Y. enterocolitica* inoculated onto the surface of blueberries. Molds and yeasts that naturally occur on blueberries were also investigated.

2. Methods

2.1. Bacterial strains

A cocktail mixture of two strains for each pathogen was utilized in this study. Each species was studied individually. Two strains of *L. monocytogenes* (ATCC 7644 and ATCC 19115), *P. aeruginosa* (ATCC 9027 and ATCC 10145), *S. Typhimurium* (ATCC 14028 and ATCC 13311), *S. aureus* (ATCC 25923 and ATCC 12600), and *Y. enterocolitica* (ATCC 27729 and ATCC 9610) were obtained from the Pathogenic Microbiology Laboratory in the Department of Food Science and Human Nutrition at University of Maine (Orono, ME), and used to inoculate the surface of blueberries. The pathogens selected are potential contaminants of raw vegetables or fruits (Beuchat, 1998; Rajkowski and Baldwin, 2003). Each strain of *L. monocytogenes*, *P. aeruginosa*, *S. Typhimurium*, *S. aureus*, or *Y. enterocolitica* was cultured in brain heart infusion (BHI; Difco, Becton Dickinson, Sparks, MD) broth at 37 °C for 24 h. Cultures were kept under refrigeration (4 °C) as stock cultures and transferred weekly to maintain viability.

2.2. Preparation of inoculum

One loop of each strain culture was taken from the stock culture in refrigeration, transferred in 5 ml of BHI, and incubated at 37 °C for 24 h. After 24 h, 0.1 ml of the culture was inoculated into 100 ml of BHI in a 250 ml centrifuge bottle, followed by incubation at 37 °C for 24 h. Cells of the strains cultured in each centrifuge bottle were harvested by centrifugation (15,300*g*, 15 min, 4 °C), washed two times with 100 ml of sterile buffered peptone water (BPW; Difco), and resuspended in 10 ml of BPW. Two suspensions of each pathogen were combined with equal populations to give 20 ml of a cocktail mixture for each pathogen. Each cocktail mixture contained approximately 10^8–10^9 cfu/ml of cells. The populations of each strain were approximately the same in the cocktail mixture for each pathogen. Before inoculation on the blueberries, populations of each mixture were determined by serially diluting suspensions in sterile 0.1% peptone water (Difco) and spread plating 0.1 ml on the appropriate selective agar (described in 2.6. Microbial enumeration).

2.3. Inoculation of blueberries

Blueberries (*Vaccinium corymbosum* L.) were purchased at a local grocery store (Bangor, ME), and stored at 4 °C for a maximum of 2 days before use. The berries were placed in single layers on plastic trays. Each layer of blueberries was supported by two glass rods that held both ends of each blueberry so that the middle skin surfaces were not in contact with the plastic trays. Blueberries were inoculated with individual pathogen cultures (two strains for each pathogen). A 150 µl of cell suspension for each pathogen was spot-inoculated on the skin surface of each blueberry using a micropipette. Half (75 µl) of the cell suspension was deposited onto blueberry skin in 12 locations and dried for 2 h in a laminar flow hood. The remaining 75 µl of cell suspension was deposited onto the uninoculated side of the blueberry, and dried for 2 h. The spot inoculation was used because it allows application of known amount of bacteria onto blueberry surfaces regardless of blueberry size. This enables reductions in the

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