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Efficacy of chlorine dioxide gas against *Alicyclobacillus acidoterrestris* spores on apple surfaces

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Abstract

Alicyclobacillus acidoterrestris is a thermophilic spore-forming bacterium that spoils acidic juices. In the orchard, apples may be contaminated with spores which can potentially grow in the resulting juice and cause spoilage. This study was undertaken to evaluate the efficacy of gaseous chlorine dioxide against *A. acidoterrestris* spores on apple surfaces. *A. acidoterrestris* spores were inoculated onto apple surfaces and were placed at room temperature, in a tightly sealed chamber containing a chlorine dioxide generating sachet, low, medium, or high release, for 30 min, 1, 2, and 3 h. After exposure, surviving spores were enumerated on K agar. Chlorine dioxide treated apples were stored at 4 °C for 7 days to assess the effect on visual quality. Inoculated, untreated apples served as the visual quality control. After exposure to high and medium release sachets for 1 h, spores were reduced to an undetectable level, a 5 $\log_{10}$ reduction; however, visual quality was compromised. After 1, 2, and 3 h of exposure to low release sachets, spore reductions were 2.7, 3.7, and 4.5 $\log_{10}$, respectively. And, after 7 days of storage, there were no significant visual quality differences between the apples exposed to low release sachet for all treatment times when compared to the control. Gaseous chlorine dioxide can effectively reduce viable *A. acidoterrestris* spores on apple surfaces. Due to the efficacy and easy of use, chlorine dioxide gas sachets may be useful to maintain apple quality during storage and shipping.

Keywords: Chlorine dioxide gas; *Alicyclobacillus*; Spore; Apple

1. Introduction

Alicyclobacillus acidoterrestris is a thermoacidophilic spore-forming microorganism reported to grow at pH values between 2.5 and 6.0 (Yamazaki et al., 1996). *A. acidoterrestris* spores survive conventional juice pasteurization procedures, germinate, grow, and cause spoilage (Yamazaki et al., 1996). Spoilage is indicated by medicinal or phenolic off-flavors or odors, and the juice may appear normal or have slight sediment. *A. acidoterrestris* has been implicated in fruit juice spoilage in a variety of countries including the United Kingdom, Germany, Australia, Japan, and the United States (Chang and Kang, 2004), and is considered one of the major targets for quality control of fruit juices and concentrates. Several recent articles have suggested that apples can become contaminated with spores in the orchard as this is a soil-based organism (Orr and Beuchat, 2000; Splittstoesser et al., 1994; Walls and Chuyate, 2000; Wisse and Parish, 1998).

To reduce the possibility of contamination of juice, apples must be washed prior to processing. Traditionally, aqueous chlorine at concentrations between 50 and 200 ppm is used to wash fruits and vegetables, which results in a microbial reduction of less than 2 $\log_{10}$ on fresh fruits and vegetables (Beuchat, 1992; Brackett, 1992). Brackett (1992) observed that there was no significant difference between chlorinated water and tap water for eliminating *Listeria monocytogenes* from fresh produce. A variety of commercial cleansers are available for washing apples, but one study (Kenney and Beuchat, 2002) found that when used according to the manufacturer's instructions, the maximum reduction achieved by commercial apple washing solutions was 3 $\log_{10}$. Sanitizers are even less effective against *A. acidoterrestris* spores, as the resistance of spores to chemical

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sanitizers is well documented (Gorman et al., 1984; Orr and Beuchat, 2000).

The FDA approved the use of aqueous chlorine dioxide for washing fruits and vegetables in 1998 (FDA et al., 1998). Chlorine dioxide is a strong oxidizing agent, having approximately 3.5 times the oxidation capacity of chlorine (Bernarde et al., 1965). Aqueous chlorine dioxide has been shown to be a relatively effective sanitizer for fruits and vegetables (Brown and Wardowski, 1986; Costilow et al., 1984), however, it is possible for microorganisms to be bound to fruit surfaces or protected by the natural surface variation (Han et al., 2001). It has been reported that *E. coli* O157:H7 cells may become trapped in the floral tube wall, lenticels, and damaged cuticle surrounding puncture wounds on apple surfaces, which protects against aqueous disinfection (Burnett and Beuchat, 2002; Kenney et al., 2001). Therefore, it would be advantageous to develop a sanitizing method with greater penetration power.

Gaseous chlorine dioxide has been known as a potent sanitizer/disinfectant for over 30 years (Bernarde et al., 1965). Recently, its use in food processing environments has been investigated. Han et al. (1999) reported that 10 ppm gaseous chlorine dioxide could reduce spoilage organisms by 6 $\log_{10}$ on juice tank surfaces. The use of chlorine dioxide has also been tested on foods, including lettuce (Lee et al., 2004). Gaseous chlorine dioxide has been shown to be more effective than aqueous chlorine dioxide at the same concentration against *L. monocytogenes* on the surface of green peppers (Han et al., 2001). Recent publications also indicate that chlorine dioxide gas is effective against the spores of *Bacillus thuringiensis* on a wide variety of surfaces (Han et al., 2003). In this study, the efficacy of easy-to-use chlorine dioxide gas generating sachets against spores of *A. acidoterrestris* on the surface of apples was investigated.

2. Materials and methods

2.1. Bacterial cultures, growth conditions, and spore suspension preparation

Two strains of *A. acidoterrestris* provided by the National Food Processor's Association (NFPA1013 and NFPA1101) were used to produce spores. These strains were isolated from spoiled apple juice. Cells were spread onto Potato Dextrose Agar (PDA, pH 5.6) and incubated at 43 °C until at least 80% of cells had sporulated (6 days) by microscopic examination. Spores of each strain were individually harvested from agar surface by adding 1 mL aliquots of sterile water, swabbing gently with a sterile swab, and collecting the fluid, 3 times per plate. The resulting suspension was centrifuged at 4000 ×*g* for 30 min and washed four times with sterile water. The final suspension was stored at −20 °C until needed.

2.2. Inoculation of apples

Unwaxed Fuji apples were purchased from a local grocery store (Pullman, WA). Apples were inoculated in a biosafety cabinet by depositing a total of 100 μL of a 2 strain spore suspension onto apple skin in 10 locations using a micropipette. Apples were dried for 30 min with the fan running.

2.3. Comparison of spore recovery with buffered peptone water and D/E neutralizing broth

Inoculated apples were subjected to chlorine dioxide gas treatment using low release chlorine dioxide gas sachets ((ICA TriNova, LLC). Sachets were activated with 10 mL sterile water, added to the bucket with an inoculated apple, bucket sealed, and the fan started. Controls were added to the bucket and sealed without a sachet. All treatments were performed at room temperature (22 ± 2 °C). Control and gas treated apples were aseptically removed from buckets at 5, 10, 15, and 30 min. Apples were placed in sterile stomacher bags containing 50 mL of buffered peptone water or D/E neutralizing broth (Difco, Sparks, MD) and massaged vigorously by hand for 1 min to remove spores from the surface. Ten-fold serial dilutions were performed and plated onto K agar composed of yeast extract (2.5 g), peptone (5.0 g), glucose (1.0 g), tween-80 (1.0 g), and agar (15.0 g) per liter and adjusted to pH 3.7 with filter-sterilized 10% malic acid (Orr and Beuchat, 2000). Plates were incubated in an inverted position at 43 °C for 48 h prior to enumeration.

2.4. Chlorine dioxide gas treatment and storage conditions

A 20 L polypropylene bucket was used as a model gas treatment chamber (Fig. 1). A small electric fan (Hankscraft Motors, Inc., Reedsburg, WI) was installed on the lid to facilitate circulation of gas in the chamber. Three types of chlorine dioxide gas sachets (ICA TriNova, LLC) were used: low, medium, and high release. Sachets were activated as previously described, and treatment was performed at room temperature. Treated apples were placed in UV sterilized plastic zip lock bags

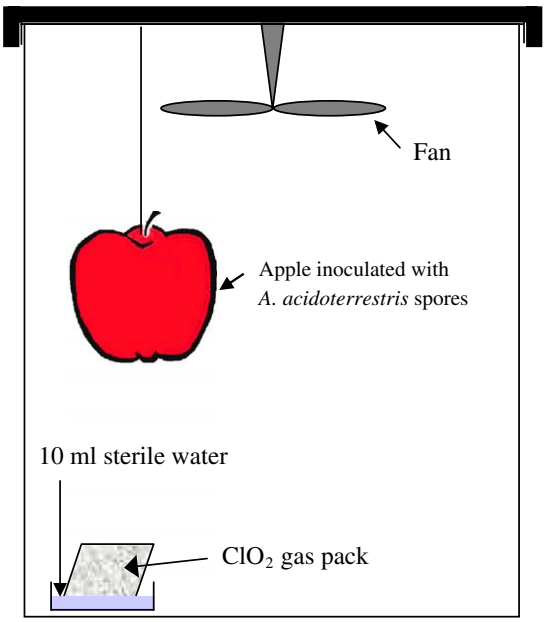

Fig. 1. Diagram of experimental gas cabinet made using a 20 L polypropylene bucket (this figure was adapted from Lee et al., 2004 and revised).