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Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas ☆

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Abstract

Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas at different concentrations (0.5, 1, 1.5, 3 and 5 mg l^{-1}) for 10 min were studied. A cocktail of three strains of each targeted organism (100 μl) was spotted onto the surface of the strawberries (approximately 8–9 log ml^{-1}) separately followed by air drying, and then treated with ClO_2 gas at 22 °C and 90–95% relative humidity. Approximately a 4.3–4.7 log CFU reduction per strawberry of all examined bacteria was achieved by treatment with 5 mg l^{-1} ClO_2 for 10 min. The inactivation kinetics of *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* were determined using first-order kinetic models to establish *D*-values and *z*-values. The *D*-values of *E. coli*, *L. monocytogenes* and *S. enterica* were 2.6 ± 0.2, 2.3 ± 0.2 and 2.7 ± 0.7 min, respectively, at 5 mg l^{-1} ClO_2. The z-values of *E. coli*, *L. monocytogenes* and *S. enterica* were 16.8 ± 3.5, 15.8 ± 3.5 and 23.3 ± 3.3 mg l^{-1}, respectively. Furthermore, treatment with ClO_2 gas significantly ($p \leqslant 0.05$) reduced the initial microflora (mesophilic, psychrotrophic bacteria, yeasts and molds) on strawberries. Treatment with ClO_2 gas did not affect the color of strawberries and extended the shelf-life to 16 days compared to 8 days for the untreated control.

Keywords: Chlorine dioxide gas (ClO_2); *E. coli* O157:H7; Inactivation kinetics; *L. monocytogenes*; Quality; *Salmonella enterica*; Shelf-life; Strawberries

1. Introduction

Fresh fruits are an important part of the human diet worldwide and consumers continue to eat more fruits partly because of reported health benefits (Beuchat, 1996; Sahari et al., 2004). In the US, consumption of fruit and vegetables increased 27% during 1970–1993 (NACMCF, 1999). Among these fruits, strawberries provide a desirable taste and flavor and account as one of most popular summer fruits (Ford et al., 1997; Moreno et al., 2000; Pelayo et al., 2003; Shamaila et al., 1992; Sturm et al., 2003; Vicente et al., 2002; Zabetakis and Holden, 1997; Van der Steen et al., 2002). Strawberries are a good source for many vitamins, minerals and natural antioxidants, which give them high scavenging activity toward oxygen radicals, protecting tissues from stresses and disease (Ayala–Zavala et al., 2004; Azodanlou et al., 2003; Cao et al., 1996; Kallio et al., 2000; Ke et al., 1994; Wang et al., 1996, 2002; Velioglu et al., 1998; Zheng et al., 2007). However, strawberries have a short postharvest life, mostly due to high metabolic bacterial activities and fungal decay (Aguayo et al., 2006; Jiang et al., 2001; Nunes et al., 1995; Ragaert et al., 2006; Steen et al., 2002; Vargas et al., 2006).

Fruits can serve as a vehicle for many foodborne pathogenic microorganisms (Bean and Griffin, 1990; Beuchat, 1996; Sy et al., 2005). There are many reports about foodborne outbreaks associated with fruits including frozen strawberries contaminated with the hepatitis A virus

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(Behrsing et al., 2003; Flessa et al., 2005). The most frequent bacterial pathogens associated with fresh produce are *Escherichia coli* O157:H7, and *Salmonella* spp. (Bean and Griffin, 1990; Beuchat, 1996; Du et al., 2003).

There are many of forms traditional sanitizing agents, such as chlorinated water, electrolyzed water and hydrogen peroxide that are widely used to wash and decontaminate produce. However, their effects are limited in reducing pathogenic and spoilage bacteria to 3 log CFU or lower (Brackett, 1987; Cherry, 1999; Koseki et al., 2004; Ukuku, 2004; Yu et al., 2001). To ensure the safety of fresh produce, there is a continual need to identify a highly effective sanitation treatment that is suitable for industrial use. The Food and Drug Administration is currently recommended a 5 log reduction for pathogenic bacteria on produce (FDA, 1995).

Chlorine dioxide gas (ClO_2) is a promising non-thermal technology for reducing pathogenic and spoilage bacteria on fresh produce. To date, limited studies of the powerful effect of ClO_2 for reducing microorganisms have been reported. A 5 log CFU reduction per strawberry of inoculated of *E. coli* O157:H7 and *Listeria monocytogenes* on strawberries were achieved after treatment with 4 mg l^{-1} ClO_2 gas (batch treatment) for 30 min (Han et al., 2004). Meanwhile, a 4.4 log CFU g^{-1} reduction in inoculated *Salmonella* spp. on strawberries was achieved after treatment with 8 mg l^{-1} ClO_2 gas after 120 min (Sy et al., 2005). Studies on other produce showed that treatment of uninjured green peppers with 3 mg l^{-1} ClO_2 gas reduced the population of *L. monocytogenes* by more than 6 log CFU 5 g^{-1} after 30 min (Han et al., 2001). Additionally, Han et al. (2000) found that treatment of uninjured green peppers with 0.6 mg l^{-1} ClO_2 gas reduced the population of *E. coli* by a 7.3 log CFU 5 g^{-1} after 30 min at 22 °C and 90–95% relative humidity. Du et al. (2003) reported that treatment of apples with 3 mg l^{-1} ClO_2 gas for 20 min resulted in a 5.9 log CFU 5 g^{-1} reduction of the population of *E. coli* after 30 min. Treatment with 4 mg l^{-1} ClO_2 gas for 30 min reduced the population of *L. monocytogenes* on apple pulp skin by 6.5 log CFU spotted per site (Du et al., 2002).

However, the inactivation kinetics (or rate of inactivation) of inoculated *E. coli* O157:H7, *L. monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas have not been reported. The main goals of this study were to: (a) determine inactivation kinetics (*D* and *z*-values) for *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* inoculated onto the surface of strawberries and (b) to study the effect of ClO_2 gas on the quality and the shelf-life of strawberries during refrigerated storage.

2. Material and methods

2.1. Strawberries

Strawberries were purchased at a local supermarket the day before each experiment and stored at 4 °C until use. Fresh unblemished strawberry of similar size and weight (25–30 g) were selected. Strawberry stems were removed and the strawberries were washed by dipping them in tap water for 2 min and air drying at 22 °C for 60 min in the biosafety cabinet (Labconco Corporation, Kansas City, Missouri, USA) to remove excessive moisture.

2.2. Bacterial strains and growing conditions

Three different bacteria were used including: (1) a cocktail mixture of *E. coli* O157:H7 (C7927, EDL933 and 204P), (2) a cocktail mixture of *L. monocytogenes* (Scott A, F5069 and LCDC 81-861) and (3) a cocktail mixture of *S. enterica* (*S. enteritidis*, *S. javiana* and *S. montevideo*). These strains were selected based on their prevalence in strawberries, and their ability to survive in strawberries over time. *E. coli* O157:H7 C7927, a human isolate from a cider-associated outbreak and an acid-resistant strain, was provided by Dr. M.P. Doyle (Center for Food Safety, University of Georgia). *L. monocytogenes* Scott A was provided by Dr. L.R. Beuchat (Center for Food Safety, University of Georgia). F5069 and LCDC 81-861 were provided by Dr. Arun Bhunia (Center for Food Safety Engineering, Purdue University). All other bacterial strains were obtained from our personal culture collection. Bacterial strains were grown in trypticase soy broth with 6% yeast extract (Difco, Becton Dickinson) and inoculated at 37 °C for 24 h. Three strains of each bacterium were mixed with an equal volume to give approximately 10^{8-9} CFU ml^{-1}.

2.3. Inoculation of strawberries

A spot inoculation method was used to inoculate the pathogenic bacteria on strawberries (Han et al., 2004). Briefly, 100 μl of each mixture culture was spotted (10 drops) on the surface of strawberries in a biosafety cabinet. Following, the strawberries were air-dried at 22 °C for 1 h (to allow bacterial attachment) in the biosafety cabinet prior to ClO_2 treatments.

2.4. Production of ClO_2 gas and relative humidity

Chlorine dioxide gas was prepared using a CDG technology generator (CDG Technology Inc., New York, USA). Production of ClO_2 was created based on the reaction of 4% chlorine gas (Matheson gas, Montgomeryville, Pa) with sodium chlorite. Afterwards, the ClO_2 gas was allowed to flow through a flask containing 8–10% sodium chlorite solution (Sigma-Aldrich, St. Louis, MO) to scrub chlorine gas residue. Then, the ClO_2 gas was mixed with filtered air for dilution and introduced into the Plexiglas treatment chamber. Flow of ClO_2 was controlled by a regulation valve to maintain the desired ClO_2 concentration in the treatment chamber. The ClO_2 gas content in the treatment chamber was constantly mixed