



Effect of temperature on chlorine dioxide inactivation of *Escherichia coli* O157:H7, *Salmonella* typhimurium, and *Listeria monocytogenes* on spinach, tomatoes, stainless steel, and glass surfaces

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ABSTRACT

The objective of this study was to evaluate how treatment temperature influences the solubility of ClO_2 gas and the antimicrobial effect of ClO_2 gas against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on produce and food contact surfaces. Produce and food contact surfaces inoculated with a combined culture cocktail of three strains each of the three foodborne pathogens were processed in a treatment chamber with 20 ppmv ClO_2 gas at 15 or 25 °C under the same conditions of absolute humidity (11.2–12.3 g/m³) for up to 30 min. As treatment time increased, ClO_2 gas treatment at 15 °C caused significantly more ($p < 0.05$) inactivation of the three pathogens than treatment at 25 °C. ClO_2 gas treatment at 25 °C for 30 min resulted in 1.15 to 1.54, 1.53 to 1.88, and 1.00 to 1.78 log reductions of the three pathogens on spinach leaves, tomatoes, and stainless steel No.4, respectively. ClO_2 gas treatment at 15 °C for 30 min caused 2.53 to 2.88, 2.82 to 3.23, and 2.37 to 3.03 log reductions of the three pathogens on spinach leaves, tomatoes, and stainless steel No.4, respectively. Treatment with ClO_2 gas at 25 °C for 20 min resulted in 1.88 to 2.31 log reductions of the three pathogens on glass while > 5.91 to 6.82 log reductions of these pathogens occurred after 20 min when treated at 15 °C. Residual ClO_2 levels after gas treatment at 15 °C were significantly ($p < 0.05$) higher than those at 25 °C. The results of this study can help the food processing industry establish optimum ClO_2 gas treatment conditions for maximizing the antimicrobial efficacy of ClO_2 gas.

1. Introduction

Chlorine dioxide (ClO_2) has emerged as a promising non-thermal sanitizing technology in recent years (Bhagat et al., 2010). ClO_2 is a strong oxidizing agent, and functions as a selective oxidant by a one-electron transfer mechanism where it attacks electron-rich centers in organic molecules and is reduced to the ClO_2^- ion (Hoehn et al., 1996). The mechanism of inactivation by ClO_2 is oxidative attack on cell membrane proteins and enzymes and increased membrane permeability (Aieta and Berg, 1986). Also, it penetrates the cell membrane and damages proteins and enzymes within the cell (USDA, 2002). Studies on application of ClO_2 gas to fresh produce, such as blueberries (Sun et al., 2014), spinach (Neal et al., 2012; Park and Kang, 2015), potatoes (Wu and Rioux, 2010), oranges (Bhagat et al., 2011), tomatoes (Bhagat et al., 2010; Trinetta et al., 2013), lettuce (Mahmoud and Linton, 2008), mung bean sprouts (Prodduk et al., 2014), carrots (Sy et al., 2005), and cantaloupe (Mahmoud et al., 2008) have been reported. Also, the antimicrobial effect of ClO_2 gas against pathogens on food contact

surfaces such as stainless steel (Vaid et al., 2010; Trinetta et al., 2012), wood, plastic (Han et al., 2003), polyvinyl chloride, and glass (Li et al., 2012; Morino et al., 2011) have been evaluated. However, most of these studies only evaluated the antimicrobial effect of ClO_2 gas against several foodborne pathogens on various sample surfaces according to gas concentration and treatment time.

The antimicrobial efficacy of ClO_2 gas is affected by intrinsic factors such as sample surface characteristics (roughness and hydrophobicity) and extrinsic factors such as gas concentration, treatment time, and relative humidity (RH) (Han et al., 2001; Park and Kang, 2017). Especially, Park and Kang (2016) reported that residual ClO_2 on produce surfaces increased with increasing RH and the amount of residual ClO_2 on produce is correlated with the level of inactivation of pathogens. Also, Park and Kang (2017) reported that surface hydrophobicity is a more important factor relative to bacterial inactivation by ClO_2 gas than is surface roughness. Water contact angles of selected produce and food contact surfaces were highly negatively correlated with log reductions of foodborne pathogens: that is, the more hydrophobic the

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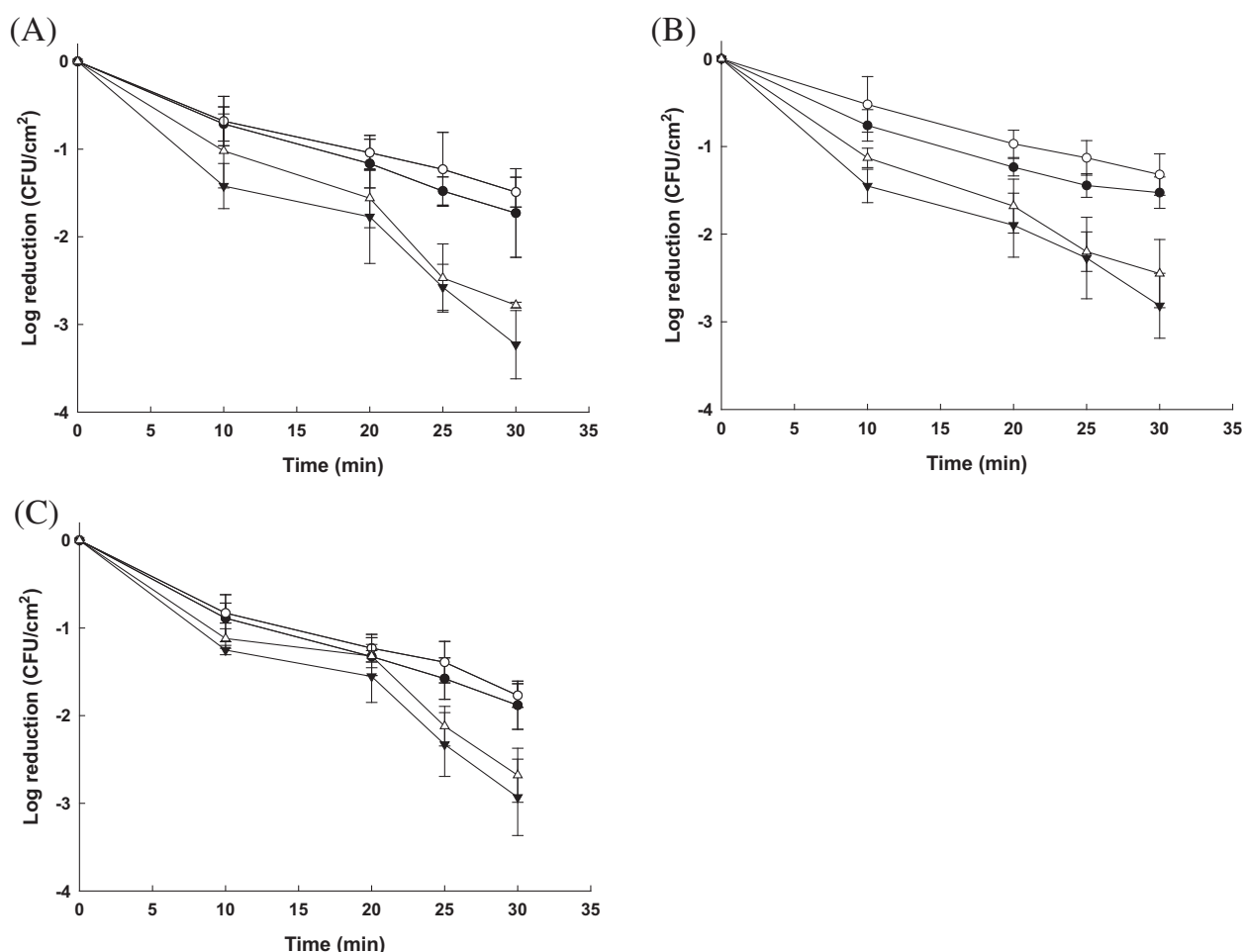


Fig. 1. Log reduction of *E. coli* O157:H7 (A), *S. Typhimurium* (B), and *L. monocytogenes* (C) on tomatoes treated with 20 ppmv ClO₂ gas at 15 °C (○, total cells; ●, healthy cells) and 25 °C (△, total cells; ▼, healthy cells). Each point represents the mean for triplicates, and error bars show standard errors.

surface, the lower the reduction of the three pathogens. Differences in reduction levels according to hydrophobicity may be due to different levels of hydration of each sample surface. As is well known, ClO₂ gas has high solubility in water, so it acts similar to aqueous ClO₂ for inactivating microorganisms (Linton et al., 2006).

Treatment temperature may be an important factor affecting antimicrobial efficacy of ClO₂ gas because it could affect ClO₂ gas solubility and reactivity simultaneously. In the case of aqueous ClO₂, inactivation efficacy generally increases with increasing treatment temperature (Taylor et al., 1999; Vicuña-Reyes et al., 2008). However, there have been no studies considering the influence of treatment temperature on the inactivation efficacy of ClO₂ gas. Although Han et al. (2001) evaluated the correlations between temperature (5 to 25 °C) and RH (55 to 95%), absolute humidity (AH) should be used to compare the effect of different treatment temperatures on the microbial inactivation of ClO₂ gas. The objective of this study was to determine how ClO₂ gas treatment temperature influences the antimicrobial effect of ClO₂ gas against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on produce and food contact surfaces.

2. Materials and methods

2.1. Bacterial strains and culture preparation

Three isolates each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S. Typhimurium* (ATCC 19585, ATCC 43971, and DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC

15313) obtained from the bacterial culture collection of the Food Safety Engineering Laboratory at Seoul National University (SNCC; Seoul, South Korea) were used in this study. All three strains of *E. coli* O157:H7 were human feces isolates. *S. Typhimurium* ATCC 43971 was derived from an existing strain. *L. monocytogenes* strains ATCC 19111, 19,115, and 15,313 were isolated from poultry, human, and rabbit subjects, respectively. All strains of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* were cultured individually in 10 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) at 37 °C for 24 h and harvested by centrifugation at 4000 ×g for 20 min at 4 °C. The final pellets were resuspended in sterile buffered peptone water (BPW; Difco), corresponding to approximately 7.0–8.0 log CFU/ml. Then, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes*.

2.2. Sample preparation and inoculation

Spinach and tomatoes were purchased from a local market (Seoul, South Korea), washed in running water, then dried in a laminar flow biosafety hood (22 ± 2 °C) for 1 h before experiments to remove surface moisture. Spinach and tomatoes used in this study were previously screened to ensure no presumptive *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes*-like colonies were recovered from un-inoculated samples. Spinach leaves and tomato surfaces were cut into 5 × 2 cm pieces, and the upper side of spinach leaves and outer surface of tomatoes were wiped with clean tissue paper (Kimtech Science Wipers, Yuhan-

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