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Influence of surface properties of produce and food contact surfaces on the efficacy of chlorine dioxide gas for the inactivation of foodborne pathogens

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ABSTRACT

The objective of this study was to evaluate the influence of surface properties of produce and food contact surfaces on the antimicrobial effect of chlorine dioxide (ClO_2) gas against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. The hydrophobicity of the selected surfaces was evaluated by water contact angle measurements. White light scanning interferometry (WLSI) was used to acquire surface roughness values of each surface. Produce and food contact surfaces inoculated with foodborne pathogens were treated with 20 ppmv ClO_2 gas for 5, 10, and 15 min. As treatment time increased, different levels of inactivation of the three pathogens were observed among the samples. Contact angles of produce and food contact surfaces were highly and negatively correlated with the log reduction of all three pathogens. There were generally weaker correlations between the roughness values of sample surfaces and microbial reduction compared to those between hydrophobicity and microbial reduction. The results of this study showed that surface hydrophobicity is a more important factor relative to bacterial inactivation by ClO_2 gas from the surface than is surface roughness. Also, the existence of crevices with features of similar size to the pathogen cell was more important than the R_a and R_q values in the inactivation of pathogens.

1. Introduction

Foodborne illnesses which result in considerable morbidity, mortality, and economic costs are a global concern (Stein et al., 2007; Tauxe, Doyle, Kuchenmuller, Schlundt, & Stein, 2010). As the consumption of produce has increased due to increasing awareness of its health benefits, produce has become one of the major sources of foodborne outbreaks accounting for 13% of outbreaks during 1990–2005 (DeWaal & Bhuiya, 2007; Uyttendaele, Jacxsend, & Van Boxstael, 2014). Food contact surfaces can also contribute to cross-contamination due to the presence of pathogens on these surfaces and increase the risk of outbreaks (Gibson, Crandall, & Ricke, 2012; Nyachuba, 2010). Therefore, efficient sanitization of both food and food contact surfaces must be ensured to reduce microbiological hazards. Chlorine dioxide (ClO_2) gas has emerged as one of the most effective sanitizers for the food industry in recent years (Bhagat, Mahmoud, & Linton, 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, Rosenblatt, & Gates, 1996). It is well known that gas concentration, treatment time, relative humidity (RH), and temperature can affect the antimicrobial effect of ClO_2 gas, and especially, the combination of gas concentration and RH shows a synergistic effect (Han, Floros, Linton, Nielsen, & Nelson, 2001).

Surface properties also can influence bacterial inactivation from a surface (Wang, Feng, Liang, Luo, & Malyarchuk, 2009). Surface hydrophobicity, surface roughness, and surface constitutional characteristics could describe surface properties (Wang et al., 2009). Surface hydrophobicity relates to surface structures and surface chemical composition, and influences surface hydration (Vacheethasanee et al., 1998). Also, surface hydrophobicity can influence the distribution and attachment of bacteria on surfaces (Harkes, Feijen, & Dankert, 1991; Syamaladevi et al., 2013). Surface roughness has been known to influence bacterial attachment to and removal from a surface (Faille et al., 2000; Jullien, Bénézech,

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Carpentier, Lebret, & Faille, 2002). Surface roughness increases the physical surface area for bacterial colonization and can protect bacteria from shearing forces (Groosh, Bozec, & Pratten, 2015; Katsikogianni & Missirlis, 2004). The influence of surface hydrophobicity and surface roughness on microbial inactivation has been reported by several studies. Wang et al. (2009) reported that a positive linear relationship between surface roughness values and residual bacterial populations was observed after washing treatments with acidic electrolyzed water, peroxyacetic acid, and sterilized deionized water. Increasing the surface roughness appeared to induce lower inactivation of *Listeria innocua* on food packaging materials (Ringus & Moraru, 2013). Conversely, Fernandes et al. (2014) observed that roughness and hydrophobicity of the fruit surface did not affect the efficiency of sanitation treatments on removal of *Salmonella* Typhimurium. Jullien et al. (2002) indicated that it was difficult to link the hygienic status of stainless steel to surface roughness values. These inconsistent results suggest that the effect of surface properties on microbial inactivation is a complex phenomenon.

To date, there have been no studies investigating the influence of surface properties of produce and food contact surfaces on the inactivation efficacy of ClO_2 gas. Also, comparative data for different produce and food contact surfaces subjected to the same treatment are not readily available. The objective of this study was to examine how surface properties (hydrophobicity and roughness) of produce and food contact surfaces influence the antimicrobial effect of ClO_2 gas against *Escherichia coli* O157:H7, *S.* Typhimurium, and *Listeria monocytogenes*.

2. Material and methods

2.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19114, ATCC 19115) were obtained from the bacterial culture collection of the Food Safety Engineering Laboratory at Seoul National University (SNCC; Seoul, South Korea). Stock cultures were stored at −80 °C in 0.7 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) and 0.3 ml of 50% glycerol. For all experiments, working cultures were streaked onto tryptic soy agar (TSA) and incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Sample preparation

Produce and food contact surfaces were selected which represent different surface characteristics (surface hydrophobicity and roughness) to ensure effective correlation analysis. Carrots, kale, cabbage, spinach, apples, tomatoes, and green bell peppers were purchased from a local market (Seoul, South Korea) and stored at 7 °C. These produce were washed in running water and dried in a laminar flow biosafety hood (22 ± 2 °C) for 1 h before experiments to remove surface moisture. Produce used in this study were previously screened to ensure no presumptive *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes*-like colonies were recovered from uninoculated samples. Produce surfaces were cut into 5 × 2 cm pieces and the surface of produce was wiped out by clean tissue paper (Kimtech Science Wipers, Yuhan-Kimberly Inc., Seoul, South Korea) to remove juice from produce. Food contact surfaces tested included Teflon (Gongguone, Goyang-si, South Korea), silicon (Jun Sangsa, Seoul, South Korea), rubber (Chehyung, Seoul, South Korea), polyvinyl chloride (PVC) (Kahee, Incheon, South Korea), type 304 stainless steel (SS) with 2B or No.4 finish (Ian industry, Ansan-si, South Korea), and glass (Corning Inc., NY, USA). These materials were cut into coupons (5 × 2 cm), immersed in 70% ethanol for 20 min, and rinsed with distilled water. After washing, coupons were dried in a laminar flow biosafety hood (22 ± 2 °C) for 1 h to remove surface moisture. Each coupon was used only once in order to avoid any variation due to surface changes.

2.3. Surface hydrophobicity measurement

The hydrophobicity of produce and food contact surfaces was evaluated by water contact angle measurements. Water contact angle was measured by the sessile drop method using a contact angle goniometer (DSA 100, KRUSS, Germany) equipped with a camera. Small drops (3 μl) of distilled water were deposited onto the produce and food contact surfaces described above using a microliter syringe and a 0.5-mm diameter needle at room temperature (22 ± 2 °C). Contact angle measurements were conducted for less than 30 s to avoid changes in the tested surface. Ten data points were taken for each sample ($n = 10$).

2.4. Surface roughness measurement

White light scanning interferometry (WLSI) was used to acquire surface roughness values of produce and food contact surfaces. A glass surface was coated with platinum by ion sputtering to obtain a reflective surface. Samples were directly mounted on the stage of a noncontact three-dimensional surface profiler (NanoView-E1000, NanoSystem, Daejeon, South Korea). Topographic images of 125 × 95 μm areas were acquired from each sample. Height profiles were expressed in the topographic images (3D) by the color scale. The R_a (arithmetic mean roughness) and R_q (root mean squared roughness) values were calculated from 10 scan areas (125 × 95 μm) of each sample using a software package (NanoMap Version 2.5.17.0; NanoSystem).

2.5. Culture preparation and sample inoculation

All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were cultured individually in 10 ml of TSB at 37 °C for 24 h, harvested by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in sterile distilled water, corresponding to ca. 10^7–10^8 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of all test strains.

Prepared produce and food contact surface coupons were placed on aluminum foil in a laminar flow hood, and 0.1 ml of culture cocktail was inoculated onto each sample by depositing droplets with a micropipettor at 14–16 locations. After inoculation, samples were dried in a laminar flow biosafety hood for 1 h at 22 ± 2 °C.

2.6. ClO_2 gas treatment

ClO_2 gas treatment was conducted in a treatment system described previously (Park & Kang, 2015). ClO_2 gas was prepared using a ClO_2 gas generating system and generated ClO_2 gas was introduced into the polyvinyl chloride treatment chamber (length × width × height, 0.7 × 0.5 × 0.6 m). The concentration of ClO_2 gas in the treatment chamber was continuously monitored and controlled using a ClO_2 gas transmitter (ATi F12, Analytical Technology, U.K.). A humidifier (H-C976, Osungsa, Changwon-si, South Korea) was used to control RH in the treatment chamber. A thermohygrometer (YTH-600, Uins, Seoul, South Korea) was used to measure RH and temperature in the treatment chamber.

Inoculated samples were placed in the treatment chamber and covered with a plastic lid. Samples were treated with 20 ppmv ClO_2 gas for 5, 10, and 15 min at 22 ± 2 °C. The RH of the treatment