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A novel gaseous chlorine dioxide generating method utilizing carbon dioxide and moisture respired from tomato for *Salmonella* inactivation

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ABSTRACT

Chlorine dioxide (ClO_2) is an antimicrobial compound used in fresh produce sanitation. Compared to its aqueous form, gaseous ClO_2 has an enhanced antimicrobial effect. This research proposed a novel generating method of $ClO_{2(g)}$ that utilized carbon dioxide and moisture naturally released from tomato during respiration to react with sodium chlorite for $ClO_{2(g)}$ generation. The results showed that the generated $ClO_{2(g)}$ not only effectively reduced the surface-inoculated *Salmonella* by 4 log CFU/fruit to an undetectable level (<5 CFU/fruit) within 24 h, but also did not impact the surface color and firmness of the fruits within the entire storage period (144 h) at 22 °C. The effects of $NaClO_2$ amount (0.05, 0.1 and 0.2 g), CO_2 concentration (7.5 and 15%), RH (45 and 95%), temperature (10, 22 and 35 °C) and pH (4, 5, 7 and 9) on the release of $ClO_{2(g)}$ were evaluated. The D and z values of *Salmonella* under different temperatures were obtained. A sachet system was successfully developed for the practical applications of this novel $ClO_{2(g)}$ generating method.

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1. Introduction

Fresh produce contains various nutrients for health. In recent years, the annual sales and consumption of fresh produce have increased sharply, exceeding 100 billion USD and 300 pounds per capita, respectively (Carolyn, Abebayehu, & Phillip, 2003; Minor & Bond, 2016). However, frequent outbreaks associated with fresh produce have become a significant challenge to the growing industry and a great safety concern to consumers (CDC, 2017a). Among all the fresh produce related outbreaks, *Salmonella* is one of the most commonly involved pathogens (CDC, 2017b). Associated with tomato, it has caused more than 300 reported hospitalizations (including death) between 2006 and 2008 (CDC, 2006; CDC, 2008).

Chlorine dioxide (ClO_2) has been reported to have a great antimicrobial effect against a broad range of bacteria since 1949 (Ridenour & Armbruster, 1949). Its working mechanism is still not clear so far, but could possibly be owning to the oxidative attack on cell membrane proteins and enzymes (Benarde, Snow, & Olivieri, 1967; Mahmoud & Linton, 2008). Gaseous ClO_2 usually has a greater antimicrobial effect than its aqueous solution, due to the enhanced penetrating ability (Han, Linton, & Nielsen, 2001; Prodduk, Annous, & Liu, 2014; Singh, Singh, & Bhunia, 2002). Numerous studies reported the effect of $ClO_{2(g)}$ against *Salmonella* on the surface of fresh produce, including tomato (Mahmoud, Bhagat, & Linton, 2007; Ray, Jin, & Fan, 2013; Sy, Mcwatters, & Beuchat, 2005a, 2005b).

The generation of ClO_2 could be achieved from different reactants. If starting from $NaClO_2$, the first step is to provide it with H^+ (in aqueous solution) to generate chlorous acid ($HClO_2$), which is the precursor for ClO_2 formation. Then via disproportionation reaction, ClO_2 could be generated (USEPA, 1999). For fresh produce (e.g., tomato) applications, many food grade acids can be used. For example, Ray et al. (2013) have tried acetic acid, citric acid, as well as lactic acid and successfully developed a packaging system for $ClO_{2(g)}$, generated from a PLA film blended with $NaClO_2$ and citric acid utilizing the moisture respired from tomato as the reaction

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trigger.

For many fresh produce, not only the moisture but also quite an amount of carbon dioxide (CO_2) is released (Meyer, Anderson, & Bohling, 1973) during the respiration. The released CO_2 could react with moisture to form carbonic acid (H_2CO_3), provide H^+ and react with $NaClO_2$ to generate $ClO_{2(g)}$. Thus, the generation of $ClO_{2(g)}$ could be further simplified, in which only $NaClO_2$ needs to be provided, while utilizing both CO_2 and moisture respired from fresh produce as the reaction triggers. This process not only reduces the costs but also solves the drawback of Ray's system, in which $ClO_{2(g)}$ may be prematurally released from the film due to the triggering of ambient moisture. Since the generation of H^+ from CO_2 and moisture may be a slow process due to the low pK_a of carbonic acid (6.37 at 25 °C) (Haynes, 2010, pp. 4–58), the release of $ClO_{2(g)}$ may also be a gentle process, which effectively inactivates *Salmonella* on the surface, without impacting the sensory qualities, which is a major concern for $ClO_{2(g)}$ applications in the sanitation process of many fresh produce (including tomato) due to its strong oxidizing effect (USEPA, 1999).

In this research, we hypothesized that the CO_2 and moisture released from respiration of tomato may react with $NaClO_2$ to generate $ClO_{2(g)}$ inactivating *Salmonella* on the surface effectively without impacting the sensory qualities. The objective of this research is to test this hypothesis and to develop a simple delivery system for the practical applications of the hypothesis.

2. Materials and methods

2.1. Experimental conditions control

Storage temperatures (±1 °C) were maintained in refrigerators (Haier, Qingdao, Shandong, China) or incubators (VWR, Radnor, PA, USA). CO_2 (99.5% purity, Airgas East, Cheshire, CT, USA) was injected into the storage jars (TMs Ball, Daleville, IN, USA). The concentration (±2%) of CO_2 inside the jars was monitored by a CO_2/O_2 detector (Model 650, Mocon, Mineapolis, MN, USA). RH inside the jars were maintained by saturated K_2CO_3 (Fisher Scientific, Pittsburgh, PA, USA) or de-ionized water (Barnstead E-pure water system, Dubuque, IA, USA) solutions. The RH (±5%) inside the jars was monitored by the moisture detector (Model JR900, Anymetre, Guangzhou, China).

2.2. Chlorine dioxide concentration detection

For chlorine dioxide in water solution, it was diluted using de-ionized water to a desirable concentration suitable for UV spectrophotometer (Model 1280, Shimadzu, Kyoto, Japan) measurement. Beer-Lamber law was utilized to calculate the ClO_2 concentration: $A = k \times l \times c$, where A is the absorbance detected by UV, k is the molar absorptivity of the absorber (L/mol × cm) taken as 1150 L/mol × cm at 360 nm for ClO_2 in this research (Hong & Rapson, 1967), l is the length of the light path (cm) and c is the molar concentration of absorber in the solution (mol/L). For chlorine dioxide in headspace, a proper volume of gas was withdrawn using a syringe and slowly injected into a desirable volume of de-ionized water solution for UV measurement. The concentration of $ClO_{2(g)}$ in headspace was calculated back from its detected concentration in water solution.

2.3. Salmonella strains/cocktail preparation

A loopful of each *Salmonella* typhimurium (ATCC 14028 and ATCC 29630) was separately transferred from a −80 °C stock culture to a fresh 10 mL BHI broth (Becton and Dickinson, Sparks, MD). After incubating at 37 °C for 6 h, a loopful of each cell suspension was transferred to another fresh BHI broth (10 mL). After incubating at 37 °C for 24 h, each BHI broth was diluted by peptone water (0.1%, Sigma, St. Louis, MO, USA) and plated on XLT4 agar (Becton and Dickinson, Sparks, MD) to verify the cell concentration. While waiting for the plate counting results, BHI broth were kept in refrigeration (4 °C). Peptone water was then used to adjust each strain to an equal cell concentration. Cell suspension (1 mL) from each strain was then combined to form a cocktail and further diluted to the targeted level.

2.4. Salmonella inoculation and enumeration

For inoculation on XLT4 agar, a BHI broth containing approximately 9 log CFU/mL *Salmonella* was diluted to reach an approximate concentration of 5 log CFU/mL. Then the diluted solution (0.1 mL) was plated on XLT4 agar (the initial population of *Salmonella* on the agar was calculated from the concentration of *Salmonella* in the broth obtained). At different time intervals, the *Salmonella* population on the agar was enumerated. For inoculation on fresh tomato, properly diluted *Salmonella* broth (0.02 mL) was spot inoculated onto the surface of fresh tomatoes (the initial population of *Salmonella* was approximately 4 log CFU/fruit). At different time intervals, three inoculated tomatoes were separately transferred into a sterile plastic bag (Whril-pak, For Atkinson, WI, USA), washed with peptone water thoroughly, and then plated on XLT4 agar to enumerate the *Salmonella* population.

2.5. Fresh tomato selections and pre-treatments

Fresh cherry tomatoes (average weight of approximately 20 g) with similar size and color were purchased from a local farmer's market in New Brunswick, NJ, USA. All tomatoes were washed with de-ionized water and then rinsed with 70% ethanol (Fisher Scientific, Pittsburgh, PA, USA) before use.

2.6. Sensory evaluation of fresh tomato

Surface color of tomato was measured using a colorimeter (Mode CR-200, Konica-Minolta, Ramsey, NJ, USA). Lightness (L), redness (a) and yellowness (b) were obtained and thus Hue value and Chroma value were calculated (Ray et al., 2013). Firmness of tomatoes was measured using a texture analyzer (Mode TA-XT2i, Texture Technologies Corp, Scarsdale, NY, USA). The detailed setting of the apparatus was as follows: 2 mm diameter probe with 10 mm/s penetrating speed was utilized to penetrate a distance of 10 mm into the central top part of tomatoes. A trained sensory panel consist of 12 Rutgers Food Science graduate students (New Brunswick, NJ, USA) was utilized for discrimination sensory tests including surface color (by visual inspection) and firmness (by hand touch) between treatments and controls. Data were collected and analyzed following the standard “paired comparison” methodology (ISO, 1983).

2.7. Sachet preparation

The sachet (2 cm × 1 cm × 0.1 cm) was made from Tyvek (DuPont, Wilmington, DE, USA). 0.1 g of $NaClO_2$ (ACROS, Morris Plains, New Jersey, USA) was placed inside the sachet, and then it was sealed tight ready for use.

2.8. Experimental design and set-ups

Hypothesis 1 was designed to evaluate whether $ClO_{2(g)}$ is able to be generated under conditions mimicking practical tomato storage. The experiments were conducted inside 1 L storage jars (TMs Ball,

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