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Short communication

A simple instrument-free gaseous chlorine dioxide method for microbial decontamination of potatoes during storage

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ABSTRACT

An instrument-free gaseous chlorine dioxide (ClO_2) method to control microorganisms on potatoes during storage was developed. Gaseous ClO_2 was generated by combining an equal amount of impregnated sodium chlorite and activating acids in a sachet without using any solution or equipment. After activation by mixing, the sachet was placed in the application area. The decontamination efficiency of ClO_2 on natural microbiota including total microorganisms, yeasts and molds, and inoculated *Pseudomonas aeruginosa* on potatoes was investigated. Different treatments using 2, 3, and 4 g of materials and various time intervals (2.5 and 5 h) to generate 16, 20, 24, 30, 32, and 40 mg/L of ClO_2 were evaluated. The results were effective for natural microbiota, showing over a 5 log CFU/potato reduction with a 4 g treatment after 5 h. For *P. aeruginosa*, there was almost a 6 log CFU/potato reduction after 5 h of the 4 g treatment. The lowest treatment tested (2 g at 2.5 h) showed reductions of 1.7, 1.9, and 2.3 log CFU/potato for total microorganisms, yeasts and molds, and *P. aeruginosa*, respectively. Gaseous ClO_2 did not affect the overall visual quality of the potato. The residue of ClO_2 decreased to <1 mg/L after 14 days for each treatment, indicating ClO_2 dissipates naturally over time.

1. Introduction

In the United States, harvested potatoes are stored for several months to provide a year-round supply. However, many potato farmers lose potatoes during storage due to the length of the storage time and microbial contamination. During harvesting, dirt, organic matter, and microbial pathogens may be introduced to potatoes (Suslow, 1997). Additionally, potatoes may become damaged and susceptible to microorganisms such as yeasts, molds, and bacteria. When in storage, bacteria and fungi that may be introduced during harvesting may contaminate a number of the potatoes. As the potatoes are in such close contact during storage, the infection may spread throughout the entire storage facility. Different types of natural microbiota may become problematic to potatoes. These include soft rot coliforms: *Erwinia carotovora* ssp. *carotovora, E. carotovora* ssp. *atroseptica*, and *Erwinia chrysanthemi* (Perombelon, 2002); pectolytic saprophytic bacteria: *Pseudomonas aeruginosa*, oomycete: *Pytophthora infestans* (Yanta and Tong, 2009); and fungi: *Fusarium* spp., *Pythium ultimum*, *Sclerontinia sclerotiorum* (Olsen et al., 2003; Yanta and Tong, 2009).

Tubers with a severe infection may not only be useless, but may result in ruining potatoes in storage. Mild and underdeveloped problems with yeasts, molds, and other infections will most likely worsen. Since these storage diseases are not curable, finding ways to limit pathogens from spreading to healthy tubers is important. The challenge, however, is due to the close proximity of the potatoes during storage (Olsen et al., 2003). Therefore, an efficient disinfection process becomes important for inactivating pathogenic and spoilage bacteria, fungi, viruses, cysts, and other microorganisms (Suslow, 1997).

Chlorine dioxide (ClO_2) is one of the disinfectants, which is being used increasingly to control microbiological growth in a number of different industries. The U.S. Environmental Protection Agency (EPA) has approved the use of ClO_2 as a disinfectant for potable water treatment with a monitoring requirement of a 1-ppm limit to chlorite ion in the treated water (U.S. Federal Register, 2000). The U.S. Food and Drug Administration (FDA) has also approved the use of ClO_2, with less than 5% impurity, as a bactericidal agent in poultry processing water at a level of up to 3 ppm residual ClO_2 (U.S. Federal Register, 1995).

Chlorine dioxide is a water-soluble, strong oxidizing agent with an oxidation potential 2.5 times higher than that of chlorine; and is less affected by pH and organic matter (Beuchat et al., 2004; Han et al., 2004). Research has shown that both the aqueous and gaseous phases of ClO_2 are effective sanitizing agents

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(Benarde et al., 1967; Richardson et al., 1998; Beuchat et al., 2004; Han et al., 2004; Lee et al., 2004; Wu and Kim, 2007). However, gaseous ClO_2 may be more effective when applied in equal concentrations as it offers greater penetration than aqueous ClO_2 and may be able to penetrate small spaces which the liquid form cannot reach (Pharmaceutical Technology, 2005; Wu and Kim, 2007). In addition, aqueous ClO_2 treatment may fog potato storage rooms or result in residual water and unwanted condensation of ClO_2 on the surfaces of potatoes other than where the rot is located. Traditionally, the generation of chlorine dioxide is inconvenient and relatively expensive due to the need for on-site instruments such as applicators, generators, isolators, or the reaction with acid and liquid. Recently, a simple, inexpensive, fast release Z-Series™ product was developed for generating gaseous ClO_2 (ICA TriNova, LLC, Forest Park, GA). The Z-Series fast gas release works by combining an equal amount of sodium chlorite and activating acids in a sachet without adding any solution. Consequently, gaseous ClO_2 can be activated on site in an easy to use sachet. After activation, the sachet is then placed in the application area. Different concentrations of the desired gas may be generated without the need for expensive equipment. With this fast gas release method, less ClO_2 is needed than with a chlorine treatment. The application of this method as a gaseous sanitizer to reduce natural microbiota and *P. aeruginosa* on potatoes during storage has not been reported. Therefore, the objective of this study was to develop a simple, instrument-free gaseous ClO_2 method that could effectively control natural microbiota (bacteria, yeasts and molds) and inoculated *P. aeruginosa* on potatoes during storage.

2. Methods

2.1. Cell suspension and media

Inoculum suspensions were prepared from pure cultures of *P. aeruginosa* cultures (ATCC #10145 and #27853) obtained from the Pathogenic Microbiology Laboratory in the Department of Food Science and Human Nutrition at the University of Maine (Orono, ME). The original source of the strains is American Type Culture Collection (Manassas, VA). Both strains are commonly studied and easily obtained.

Each strain of *P. aeruginosa* was grown in a Brain Heart Infusion broth (BHI, Difco, Becton Dickinson, Sparks, MD) at 37 °C for 24 h. Cultures were kept under refrigeration (4 °C) as stock cultures and transferred weekly to maintain viability. A cocktail mixture made from two strains was utilized in this study. The culture cocktail was made by combining 75 μl of both fresh cultures (a total of 150 μl) and adjusted to approximately 1×10^8 CFU/ml using 0.1% peptone water (Difco), prior to the inoculation of the potato surfaces. The natural microbiota (including total microorganisms as well as yeasts and molds) originally present on the potatoes were also enumerated.

Tryptic soy agar (TSA, Difco) and Dichloran Rose Bengal Chloramphenicol agar (DRBC, Difco) were used to enumerate the total microbial counts and the yeasts and molds counts, respectively. *Pseudomonas* isolation agar (PIA agar; Difco) was used for the inoculated *P. aeruginosa* on the potatoes.

2.2. Sample preparation and inoculation

For the natural microbiota study, six unwashed potatoes were weighed. Four of the potatoes were placed in a desiccator: 2 of a similar size to be used for testing, 1 used for a 14-day visual check, and the other to be used as a residue check. Two of the potatoes were kept outside of the desiccator for controls: 1 used for microbial enumeration and the other for a 14-day visual check. The vacuum tight desiccator (5.5 L, Scienceware, Pequannock, NY) was used as the housing chamber. A small fan was wired (UC Fan, Copal Co., LTD, Malaysia) through the top of the desiccator for circulation of gaseous ClO_2 treatment.

For the *P. aeruginosa* study, 3 potatoes were separately weighed, and washed with tap water, then with 95% ethanol, and finally with sterile distilled water. Cleaned potatoes were then placed under a bio-safety hood and propped up in an upright position in a small beaker under a germicidal light for 2 h, while being continuously rotated for drying. After drying, a total of 150 μl of *P. aeruginosa* cocktail was then inoculated onto 15 locations on the surface of the potato using a micropipette. Potatoes were then left to dry under the hood for another 2–3 h to allow bacterial attachment. The inoculated dried potatoes were then ready for the gaseous ClO_2 study (1 for the control and 2 for ClO_2 treatment).

2.3. Activation of gaseous ClO_2 and treatment of potatoes

Gaseous ClO_2 was generated by combining an equal amount of impregnates of sodium chlorite and activating acids in a sachet (ICA TriNova, LLC, Forest Park; GA), mixing and shaking for activation. Different treatments included the following combinations: 2 g of sodium chlorite and 2 g of acid into a sachet to generate expected concentrations of 16 mg/l after 2.5 h and 20 mg/l after 5 h (low treatment); 3 g of sodium chlorite and 3 g of acid to generate expected concentrations of 24 mg/l after 2.5 h and 30 mg/l after 5 h (medium treatment); and 4 g of sodium chlorite and 4 g of acid to generate expected concentrations of 32 mg/l after 2.5 h and 40 mg/l after 5 h (high treatment). The level of gas decreased rapidly when gaseous ClO_2 contacted potatoes. The expected concentrations were obtained from a releasing profile of gaseous ClO_2 generated over time without treatment of potatoes. The sachet was sealed and placed on the bottom of the desiccator where the potatoes were stored for 2.5 and 5 h treatment times, for each treatment.

2.4. Microbiological enumeration

In the natural microbiota study, the control potato sample (without ClO_2 treatment) was placed in a sterilized sample bag (Whirl Pak: Nasco, Ft. Atkinson, WI) along with 50 ml of peptone water (0.1% w/v). The treated potato samples from the desiccator (after ClO_2 sanitation) were individually wrapped in sterilized sample bags (Whirl Pak: Nasco, Ft. Atkinson, WI), along with 25 ml of peptone water. The potato samples were massaged thoroughly by hand for 2 min. A series of dilutions were prepared for plating. Dilutions of 0.1 ml were spread-plated in duplicate onto TSA (Difco) for total microbial counts. The plates were inverted and incubated at 35 ± 2 °C for 24 h. From the same dilutions, 0.1 ml of the sample was spread-plated in duplicate onto DRBC agar (Difco) for yeast and mold counts. The DRBC plates were inverted and kept at 22 °C for 5 days.

In the *Pseudomonas* study, the inoculated control and treated samples were diluted using the same procedures as outlined in the natural microbiota study above. Samples were spread-plated in duplicate on PIA agar (Difco). The plates were inverted and incubated at 35 ± 2 °C for 24 h, prior to counting.

2.5. Effect of ClO_2 treatment on visual quality versus control

One ClO_2 treated potato from the desiccator after each trial was placed in a sterilized sample bag (Nasco Whirl Pak, Fort Atkinson, WI) and stored at 4 °C for 14 days. One potato (visual control) without ClO_2 treatment was also placed in a Whirl Pack bag after each trial and stored at 4 °C for 14 days. Evaluation of potato visual quality was performed daily. The 9-point hedonic test (1 = dislike