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Evaluation of chlorine dioxide as an antimicrobial against *Botrytis cinerea* in California strawberries

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ABSTRACT

Strawberries are highly sensitive to deterioration by microbial decay after harvest and *Botrytis cinerea* is one of the most common diseases that infect them. Previous work has reported successful extension of strawberry shelf life through the use of chlorine dioxide (ClO_2) gas treatments. In this study, California strawberries purchased at retail stores were treated with continuously generated ClO_2 gas over concentrations ranging from 0.01 to 5.0 mg/L for durations ranging from 7 to 1000 min to determine how ClO_2 gas impacts fungal mortality and fruit quality as a function of treatment concentration, treatment duration, and ClO_2 absorption by the fruit. Repeated measures and Gompertz models were used to infer on the efficacy of the ClO_2 gas, and a novel method was used to quantify the ClO_2 absorption by fresh produce. It was found that ClO_2 gas treatments have minimal effect at delaying the onset or growth rate of *Botrytis cinerea* for treatments followed by incubation at 4 or 22 °C even when corrected for natural variability of *Botrytis cinerea* presence among batches of strawberries, indicating that treatments are not sufficient for shelf-life extension.

1. Introduction

Food spoilage is both a sustainability and a commercial issue because visible mold and undesirable odors lead to consumer rejection, which in turn causes significant economic losses and food waste. According to Kantor et al. (1997), the annual edible food available to the U.S is reduced by about 27% due to food spoilage and waste at retail and consumer levels and fruits and vegetables are one of the main categories responsible for food losses and waste (Kantor, Lipton, Manchester, & Oliveira, 1997). This percentage is especially significant in the context of a life cycle assessment (LCA) study for food systems where input factors, including energy, water irrigation, packaging and transportation, are considered (Heller & Keoleian, 2000; Peano, Girgenti, Giuggioli, & Bounous, 2012). It is such a relevant issue that the Environmental Protection Agency (EPA) and the United State Department of Agriculture (USDA) both raise awareness about food waste management and assist entities of the U.S food chain to meet their food-waste goals through initiatives such as "EPA's Food Recovery Challenge" and "U.S. Food Waste Challenge".

Within the food production chain, fruits and vegetables may be contaminated at different stages since they are exposed to bacteria, parasites, viruses, and fungi/molds through multiple sources including insects, irrigation water or rain, soil, air, manure based fertilizers, manual handling by workers during the harvest and packaging process, food processing facilities and transportation (Brackett, 1994; Wei et al., 1995; Yuk, Bartz, & Schneider, 2006). All these factors directly influence their mode of decay and the time to reach the end of shelf life. Specifically, mold spoilage of food results from a biological process that begins with contamination by fungal spores which germinate and extend and into hyphae to form a visible mycelium over time resulting in the end of the product's shelf life (Dantigny, Marín, Beyer, & Magan, 2007). Mold growth is affected by environmental factors such as water activity (a_w), pH and temperature, and also by food constituents like proteins, carbohydrates, lipids and organic acids (Dagnas & Membre 2013; Garcia, Ramos, Sanchis, & Marín, 2009). Optimum conditions for mold growth depend on the type of microorganism, but in general, food spoilage will occur more rapidly at temperatures above 25 °C and a_w above 0.85 as long as the food system is contaminated with

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spores and that those spores are able to germinate (Dantigny, Guilmart, & Bensoussan, 2005; Gougouli & Koutsoumanis, 2012). Therefore, to improve the quality of fruits and vegetables, and to help prolong their shelf life, it is necessary to both understand the behavior of fungal growth and to identify methods that effectively reduce the population of microorganisms. Researchers have proposed models to describe microorganism population growth as a function of time, and to simulate and predict the behavior of different types of fungi growth at different environmental conditions and at different growth stages (germination and hyphae extension) such as logistic, the Gompertz and the Baranyi models (Dantigny et al., 2006, 2007; Declerck et al., 2001; López et al., 2004).

Understanding how molds behave and what factors influence their growth could, among others, help identify suitable sanitation technologies that would effectively reduce the fungi population that cause food spoilage. Chlorine dioxide (ClO_2) gas for example, has been extensively studied for its proven efficacy as a potent sanitizer and disinfectant capable of reducing populations of pathogenic microorganisms that compromise food safety (Abdul-Raouf, Beuchat, & Ammar, 1993; Benarde, Snow, & Olivieri, 1967; Costilow, Uebersax, & Ward, 1984; Du, Han, & Linton, 2002; Han, Linton, Nielsen, & Nelson, 2000; Han, Sherman, Linton, Nielsen, & Nelson, 2000; Netramai, 2011; Reina, Fleming, & E.G. Humphries, 1998; Wang et al., 2014). Some organizations have established limits for the exposure of food to ClO_2 such as the U.S Food and Drug Administration who recommends a maximum of 3 mg/L in water for sanitizing fresh produce (FDA, 2012), the United States EPA that allows levels of 0.8 mg/L and 1.0 mg/L for ClO_2 and chlorite in drinking water, respectively (EPA, 2006) while the Food and Agricultural Organization/World Health Organization (FAO/WHO) considers safe a daily consumption of body weight per day of 0.03 mg/kg of chlorite and 0.01 mg/kg of chlorate (Gómez-López et al., 2009).

While less research has focused on studying its effect on spoilage molds and bacteria, the few results found in the literature are promising and worth exploring. For example, Spotts and Peters (1980) investigated the effect of ClO_2 on fungi for the control of d'Anjou pear decay and found that ClO_2 did not affect the germination of *Botrytis cinerea* when concentrations lower than 5 mg/L were used even when held for 10 min, but that ClO_2 was effective when pears were treated with 10 mg/L concentration for 10 min. Roberts and Reymond (1994) studied the percentage spore mortality of four fungal species after *in vitro* exposure to different ClO_2 concentrations and times and found that *Botrytis cinerea* was one of the most resistant species, sensitive only to high concentrations of 3 and 5 μg/mL within 1 min of exposure. This information seems to suggest i) that contrary to what has been documented for pathogenic microorganisms, it has been challenging to reduce populations of *Botrytis cinerea* using treatments with low concentrations of ClO_2 and ii) that despite Spotts et al. and Roberts et al. agreement on the concentration of the effective treatment (5 mg/L), the difference in exposure times (1 *versus* 10 min) implies that the biological subject where *Botrytis cinerea* is present has an effect on the efficacy of ClO_2 treatments. Strawberries are a unique fruit with irregular shape, seed-studded surfaces, and high organic acid content (Han, Selby, Schultze, Nelson, & Linton, 2004; Kallio, Hakala, Pelkkikangas, & Lapveteläinen, 2000). Moreover, their shelf life is short due to susceptibility to rot-causing pathogens and fast ripening due to high respiration rates (Kim, Kim, Lim, Jang, & Song, 2010). Sy, McWatters, & Beuchat (2005) found that yeast and mold populations (not specified what type of population) on strawberries were significantly reduced when treated with gaseous ClO_2 at concentrations of 8 mg/L for 120 min. More recently, Aday, Buyukcan, and Caner (2012) found that treatments with ClO_2 on strawberries are capable of extending the shelf life of the fruit; and Vardar, Ilhan, & Karabulut (2012) found that the incidence of decay of strawberries by *Botrytis cinerea* was significantly reduced by treatments with ClO_2 (1500 and 2000 $\mu L L^{-1}$) with little impact on sensorial characteristics. However, as a result of ClO_2 treatments, it and its by-products may persist in the fruit (Wise et al., 2012) requiring knowledge of the quantities of these chemical compounds, particularly in the case of fruits and vegetables because they are often consumed raw. Therefore, a holistic approach to assess the feasibility of use of ClO_2 as a sanitizing technology for reducing *Botrytis cinerea* on strawberries should focus on its efficacy, impact on quality, and ClO_2 consumption by the strawberries. The main goal of this study was to determine the efficacy of ClO_2 gas in the delay of onset of *Botrytis cinerea*, the most common microorganism responsible for spoilage of strawberries, meanwhile quantifying the concentration range that does not produce visual damage to strawberries. The specific objectives of this work were to (*i*) expose strawberries to different ClO_2 treatments to determine its efficacy while maintaining the color and moisture content of the fruit, (*ii*) to quantify total ClO_2 absorption by the fruits and (*iii*) to correlate total ClO_2 absorption with concentration and exposure time.

2. Materials and methods

2.1. Selection of California strawberries

California strawberries were purchased from a local supermarket and were harvested approximately 1-week prior to experiments and shipped in refrigerated trucks to Michigan. There were differences between strawberry batches in terms of the suppliers and cultivars based on size and shape of the berries, but appropriate control populations from each batch are compared. All strawberries were thoroughly inspected before use. Only fruits similar in size and color that were free of bruises and other damage were chosen and were randomly assigned to treatments. Raw strawberries were weighed and conditioned at $22 \pm 2\,^{\circ}C$ for an hour prior to treatments in order to avoid condensation during treatments and to maintain a constant temperature inside the treatment chamber.

2.2. Design of experiments

A set of three completely randomized block designs (CRBD) (hereafter experimental designs) with appropriate controls for each design were conducted in order to determine appropriate ClO_2 sanitizing treatments on California strawberries for delaying the growth of *Botrytis cinerea* without affecting the visual quality of the fruit in terms of bleaching and desiccation of the tissue or calyx. Table 1 shows the different treatments in pairs of concentration and time of exposure selected for each experimental design. The order of the treatments was randomized to account for variability as treatments were performed sequentially.

The first experimental design consisted of a 10-point response surface design covering low and high ClO_2 concentrations for short and long periods of time. Intentionally, combinations of high concentration with long exposure times, and low concentrations with short exposure times were excluded because it was anticipated that in the first case the quality of the produce would be significantly diminished (*e.g.*, excessive bleaching and desiccation), and that in the second case the sanitizing effect of the gas would be minimal. Exposures consisted of 12 strawberries per condition, with randomized replicates of some conditions, for a total of 132 berries treated and a control was removed at each time