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Influence of temperature and organic matter load on chlorine dioxide efficacy on *Escherichia coli* inactivation

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ABSTRACT

Postharvest losses of fresh vegetables are mostly caused by microbial infections. Furthermore, contamination of raw-consumed fruits and vegetables with human pathogens may lead to serious foodborne illness. Washing with tap water reduces the total number of produce surface attached microorganisms by only 1–2 log. Addition of sanitizers to washing waters can help to effectively reduce the microbial load. However, some sanitizers are less successful than initially assumed and/or may negatively affect produce quality. During recent years, efficiency of chlorine dioxide (ClO_2) applications was tested as an alternative sanitation agent for fresh produce. Most published studies, however, were planned and performed under laboratory conditions, i.e. in tap water with a low chemical oxygen demand (COD). Thus, they do not necessarily reflect the practical conditions in processing lines, where effluent produce sap may largely increase COD. Factors such as pH, temperature and load of organic matter may influence the actual biocidal efficiency of ClO_2. Successful technical implementations, thus, require information about the relationship between efficiency of ClO_2 and influencing factors. Here, a comprehensive evaluation of the influence of temperature on the effectiveness of ClO_2 treatment (ClO_2 concentrations = 0, 2, 4, 6, 8 and 10 mg L^{-1}) on *Escherichia coli* (starting load: 5 10^5 cfu mL^{-1}) and the influence of organic matter load on the effective ClO_2 concentration in lettuce washing water is provided. Decreasing the temperature of the processing water (from 15 °C to 2 °C) resulted in a reduced and delayed reduction of *E. coli* counts. At 15 °C, not at 2 °C, a significant reduction of *E. coli* was found after 2 min. At a ClO_2 concentration of 3 mg L^{-1}, microbes were significantly and completely inactivated at 15 °C after 0.5 min. In contrast, *E. coli* loads were significantly, but only incompletely reduced at 2 °C after 1 min. The increase in COD from 250 mg L^{-1} to 1000 mg L^{-1} by the addition of respective amounts of organic matter resulted in a pronounced increase in the ClO_2 demand.

1. Introduction

During recent years, the production of minimally processed fruits and vegetables has increased worldwide. In addition, growth rates of consumption of fresh-cut produce have been estimated as 10–20% per year (Rico, Martin-Diana, Barat, & Barry-Ryan, 2007; Siddiqui, Chakraborty, Ayala-Zava, & Dhua, 2011). These products are commonly consumed raw and, thus, bear the risk of contaminations with human pathogens. Indeed, the increasing consumption of fresh-cut produce has been associated with an increase in outbreaks of enteric diseases (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Hygienic standards for the production of so called ready-to-eat produce should, therefore, be very high. In 2011, the German Society of Hygiene and Microbiology (DGHM, Deutsche Gesellschaft für Hygiene und Mikrobiologie) released guidance and warning limits for relevant microbial contamination of packed cut salads (DGHM, 2011). The United States Food and Drug Administration (FDA) published the 'Guide to minimize microbial food safety hazards of fresh-cut fruits and vegetables', and in 2011, the FDA Food Safety Modernization Act was signed into law (FDA, 2008; FDA, 2011). Causes of produce

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contamination can be faecal contamination by animals in the field, application of contaminated irrigation water or insufficient industrial and stuff hygiene (Beuchat, 1996; Brandl, 2006; Suslow et al., 2003).

Production processes of fresh ready-to-eat products are generally simple designed. Fruits and vegetables are sorted, cut, washed and/or sanitized, rinsed, surface-dried and finally packed in plastic bags. To guarantee a high product quality until the end of minimum shelf life, a complete cool chain must be guaranteed. Interruptions in the cool chain normally result in the undesirable growth of microorganisms, which, in turn, promotes early spoilage.

Usually, fresh produce is washed with ice-cooled water. By physically removing attached microorganisms from the produce surface, washing with water alone typically reduces the number of microorganisms only 10–100 fold (Beuchat, 1998; Tomás-Callejas et al., 2012a). Addition of disinfectants can help to improve the biocidal effect of washing and, hence, the microbial status of produce. On the other hand, previous studies have shown that some disinfectants are less effective than expected (Hassenberg et al., 2007; Park, Alexander, Taylor, Costa, & Kang, 2008; Tomás-Callejas, Martínez-Hernández, Artés, & Artés-Hernández, 2011). In addition, they may even result in product damage such as tissue browning or texture changes (Kim, Kwon, Kwon, Cha, & Jeong, 2006; Koseki & Isobe, 2006; Rico et al., 2007). Therefore, the development of new, more effective and gentle techniques is urgently required.

In the last few years, the application of chlorine dioxide (ClO_2) was successfully tested for fruit and vegetable disinfection (Chun, Kang, & Song, 2013; Keskinen, Burke, & Annous, 2009; Kim, Lee, & Song, 2007; Pao, Kelsey, Khalid, & Ettinger, 2007). Most of these studies were, however, planned and performed under laboratory conditions in sterile cold tap water; i.e., at low chemical oxygen demand (COD). In the European Union and, hence, in Germany, the highest acceptable COD for tap water is 5 mg_{O2} L^{-1} (EU council directive, 1998; Trinkwasserverordnung, 2001). In other regions of the world, higher COD can be found in tap water, ranging between 8.2 and 45 mg L^{-1} (Narasimha Rao, Dorairaju, Bujagendra Raju, & Chalapathi, 2011). Consequently, the treatment conditions were optimal and did not reflect real practical conditions, where effluent produce sap largely increased COD. In processing lines, an increasing load of organic matter and/or temperature fluctuations during processing largely increased the undesired chemical ClO_2 consumption. Therefore, the aim of the present study was to comprehensively evaluate the effects of practically relevant temperatures (i.e. ice-cold water at 2 °C and cold tap water at 15 °C) and organic matter loads (indicated by COD in the range of 250 mg L^{-1} to 1000 mg L^{-1}) at a standard temperature (20 °C) on the biocidal effectiveness of ClO_2 treatment on human-pathogenic microorganisms, particularly on *Escherichia coli*, in lettuce washing water.

2. Material and methods

2.1. Cultivation of E. coli and determination of microbiological properties

Cells of *Escherichia coli* (DSMZ 1116) were stored in cryo beads (Carl Roth GmbH, Karlsruhe, Germany) at −80 °C. For each test, one cryo bead was added in 5 ml nutrient broth (Carl Roth GmbH, Karlsruhe, Germany) and incubated at 37 °C for 24 h. Subsequently, the optical density (OD) of the bacterial suspensions was measured at a wavelength of 600 nm (BioPhotometer plus, Eppendorf, Hamburg, Germany). According to the optical density, the main culture was inoculated in nutrient broth and shaken at 170 rpm and 38 °C for 18 h to obtain bacterial suspensions of approx. 10^9 cfu mL^{-1}. Cell numbers were determined with a Multisizer™ 3 Coulter Counter® (Beckman Coulter, Krefeld, Germany) and suspensions were diluted according to the required test parameters to yield initial bacterial counts of approx. 10^5 cfu mL^{-1}. A control was always run in parallel to each treatment.

2.2. Preparation of ClO_2 solutions and measurement of ClO_2 concentrations

Chlorine dioxide was produced from the DK-DOX® two-component-process product (Dr. Küke GmbH, Hannover, Germany). According to the instructions, 250 mL of DK-DOX® aktiv (component 1) was activated by mixing it with 3.85 g DK-DOX® component 2. The final product is fully activated at 30 °C, so after a reaction time of 24 h, ClO_2 concentrations were measured photometrically (American Public Health Association, 1992) at wavelength of 510 nm (diethyl-p-phenylenediamine (DPD) method, spectral photometer DR 2800; cuvette test LCK 310, both Hach Lange, Berlin, Germany). The respective concentrations were adjusted by diluting the stock solution with tap water.

2.3. Preparation of artificial washing water and measurement of organic matter content

The effect of the organic matter content on microbial decontamination efficiency of ClO_2 was analysed in artificial iceberg lettuce washing water. To prepare the latter, the core of an iceberg lettuce was cut out and discarded; then, the remaining head was cut into pieces and these finely puréed with a hand-held blender. The purée was pressed through a tea strainer and the juice obtained was separated from the remaining solid matter by filtration (PE filter, pore size 330 μm). Normally, organic matter contents of washing water are expressed by the chemical oxygen demand (COD). Consequently, the COD of the filtrate was measured photometrically (spectral photometer CADAS 200, Dr. Lange, Berlin, Germany; thermostat LT 200 (at 148 °C for 2 h), cuvette test LCK 014, both Hach Lange, Berlin, Germany) and the requested COD adjusted by adding exact volumes of tap water.

2.4. Analyses of effects of temperature and ClO_2 concentration on E. coli inactivation

To analyse the temperature effect on microbial decontamination efficiency of ClO_2, inactivation tests were performed in 50 mL polypropylene tubes (Falcons, Sarstedt, Nümbrecht, Germany). In the tubes, an *E. coli* suspension (approx. 5 10^5 cfu mL^{-1}) and the requested volume of ClO_2 solution (ClO_2 concentrations = 1.0; 1.5 and 3 mg L^{-1}) were added and the tube content was thoroughly blended. The pH of these solutions was generally stable at 7.8 (pH meter Lab 850, SI Analytics GmbH, Mainz, Germany). After 1 and 2 min, respectively, inactivation was stopped by adding an equivalent amount of sodium thiosulfate pentahydrate (AppliChem, Darmstadt, Germany). The tests were conducted at both 2 °C (ice water) and 15 °C (cold tap water). Treatment effects were evaluated by determination of *E. coli* counts with the spread plate method. For this, serial dilutions in Ringer's solution (Merck, Darmstadt, Germany) were created of controls and treated samples, plated on nutrient agar (Carl Roth GmbH, Karlsruhe, Germany) and incubated at 37 °C for 24 h. Every experiment was repeated thrice.

2.5. Analyses of the effects of organic matter content on ClO_2 concentration in washing water

To analyse the effects of organic matter content on chemical consumption of chlorine dioxide dissolved in the washing water, aliquots of lettuce sap were mixed with defined volumes of ClO_2