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Short communication

# Chlorine dioxide as water disinfectant during fresh-cut iceberg lettuce washing: Disinfectant demand, disinfection efficiency, and chlorite formation

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ABSTRACT

The use of chlorine dioxide ($ClO_2$) as water disinfectant during fresh-cut iceberg lettuce washing was studied concerning three key issues: i) disinfectant demand, ii) disinfection efficiency, and iii) production of chlorite ($ClO_2^-$). Standardized process water (SPW), a watery suspension of iceberg lettuce, was used as model water system. In SPW (chemical oxygen demand (COD) 1130 mg $O_2$/L, 4 °C, pH 7) the chlorine demand was more than 10 times higher than the $ClO_2$ demand. Dosing 5 mg/L $ClO_2$ (0.40 mg/L residual after 2 min contact time) inactivated the total psychrotrophic plate count (TPC), including molds, to undetectable levels (>3 log reduction) after 2 min, and reduced *Escherichia coli* (*E. coli*) to undetectable levels (>5 log reduction) within 3 min. A dose of 70 mg/L chlorine (free chlorine residual of 1.7 mg/L after 2 min) was unsuccessful to inactivate molds to undetectable levels. About 77% of the consumed $ClO_2$ was reduced to $ClO_2^-$ due to reaction with the SPW. Concerning disinfectant dose, $ClO_2$ is more efficient than free chlorine for disinfecting fresh-cut lettuce wash water. However, data on the transfer of $ClO_2^-$ and chlorate ($ClO_3^-$) to the lettuce is needed before $ClO_2$ can be recommended as wash water disinfectant.


## 1. Introduction

Large scale processing of fresh-cut "ready to eat" lettuce involves cleaning, trimming, coring, slicing, washing, drying and packaging. Washing removes dirt, pesticides, microorganisms and exudate, and pre-cools the product. Because the water use is considerable, the water is recirculated and used to wash large batches of lettuce (Gil, Marin, Andujar, & Allende, 2009). A spread of pathogenic microorganisms from one lettuce leaf to another can occur *via* the wash water, i.e. cross-contamination. Spoilage microorganisms accumulate in the wash water (as they are readily present on the produce) and can be transferred in large numbers to lettuce leafs, a process that increases with product-to-water ratio. Washing with disinfectants does not succeed in completely removing microbial contamination from produce. However, cross-contamination *via* wash water can be virtually completely eliminated by wash water disinfection (Van Haute, Tryland, Veys, & Sampers, 2015).

$ClO_2$ is a possible alternative to chlorine as lettuce wash water disinfectant. $ClO_2$ oxidizes but contrary to chlorine, it does not chlorinate and as such, chlorinated disinfection by-products (DBPs) are not significantly produced. If chlorinated DBPs are produced, this can be attributed to the presence of excess chlorine due to sub-optimal $ClO_2$ generator performance (Richardson, Plewa, Wagner, Schoeny, & DeMarini, 2007). Studies in municipal wastewater show that the chlorination demand is higher than $ClO_2$ demand, resulting in a lower necessary $ClO_2$ dose to maintain a disinfectant residual (Gordon & Rosenblatt, 2005; Van Haute, Sampers, Jacxsens, & Uyttendaele, 2015). The lower demand of $ClO_2$ has not been confirmed in fresh produce wash waters however. Contrary to these advantages, when $ClO_2$ oxidizes water matrix constituents it is reduced in large part to $ClO_2^-$. $ClO_3^-$ and chloride are formed to lesser extent (Hua & Reckhow, 2007). $ClO_2^-$ is a regulated DBP in the United States (1 mg/L) (USEPA, 2009). $ClO_2^-$ and $ClO_3^-$ can cause anaemia in some animals and high levels are harmful on thyroid function (Hebert et al., 2010).

Three key issues related to assessing the feasibility of $ClO_2$ disinfection of lettuce wash water were studied: i) the disinfectant

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demand, ii) wash water disinfection efficiency, and iii) the production of $ClO_2^-$ due to reaction of $ClO_2$ with water matrix constituents.

## 2. Materials & methods

### 2.1. Standardized process water

The outer leaves of the iceberg lettuce (*Lactuca sativa*) were removed. The leaves were cut and 67 g of cut salad was put in a stomacher bag to which 200 mL tap water was added. The mixture was homogenized for 2 min, and subsequently this mixture was diluted to obtain SPW with approximately the desired COD, after which the COD was determined.

### 2.2. Physicochemical measurements

COD was measured according to the small-scale sealed tube method (LCK 514; Hach; Germany); turbidity with a 2100AN turbidimeter (Hach, USA). Free chlorine (NaOCl stock, BRAND) was determined using the DPD colorimetric (4500-Cl-G) method (Eaton, Clesceri, Rice, & Greenberg, 2005). A $ClO_2$ stock solution (0.1 w/w % $ClO_2$) was produced by a $ClO_2$ generator (Legio Zon type CDLa, Prominent, Norway) which mixes $NaClO_2$ and HCl. The $ClO_2$ concentration and $ClO_2^-$ concentration were measured using the amperometric (4500-$ClO_2^-$E) method (Eaton et al., 2005).

### 2.3. Bacterial analysis

TPC was determined with the pour plate method on Water plate count agar (Oxoid, England) (5 days, 22 °C) and *E. coli* with the pour plate method, using *E. coli* 2 agar (Bio-rad; France) (24 h, 37 °C).

### 2.4. Water reconditioning experiment

Doses of 20–70 mg/L free chlorine (from 3 or 0.3 w/w % stock) were added to 100 mL SPW at 4 °C. Doses of 4–10 mg/L $ClO_2$ (from 0.1 w/w % stock) were added to 100 mL SPW at 4 °C. The SPW was gently mixed during the experiment. The $ClO_2$ experiments were conducted in recipients that were shielded from light. TPC was sampled after 2 min contact time. Samples for microbial analyses were immediately quenched by addition of 200 mg/L sodium thiosulfate. The conversion of $ClO_2$ to $ClO_2^-$ was assessed by dosing 5, 6, 7.5 and 10 mg/L $ClO_2$ in SPW (COD 1130 mg $O_2$/L), measuring the $ClO_2$ and $ClO_2^-$ in the stock solution, and determining the $ClO_2$ decay and $ClO_2^-$ formation.

The *E. coli* inactivation potential of $ClO_2$ was also studied in SPW. *E. coli* ATCC 25922 were grown in nutrient broth (Oxoid, France) for 24 h at 37 °C. The *E. coli* cells were washed twice in phosphate-buffered saline by centrifugation and subsequently added to the SPW to obtain about 6 log CFU *E. coli* /mL. *E. coli* was sampled after 1, 2, 3, 4, and 5 min.

## 3. Results & discussion

### 3.1. Disinfectant demand of $ClO_2$ compared to free chlorine in fresh-cut lettuce wash water

To achieve a disinfectant residual of 1 mg/L after 2 min of contact time with the SPW (1130 mg $O_2$/L), about 68 mg/L of free chlorine was needed, whereas 7.5 mg/L $ClO_2$ sufficed (Table 1). This confirms the considerably higher free chlorine demand than $ClO_2$ demand in lettuce wash water. The extent of disinfectant demand depends on the water matrix and the disinfectant. Most of the knowledge concerning disinfectant demand comes from municipal wastewater studies. Reactivity with (organic) water matrix constituents seems to increase in the following order: peracetic acid < $ClO_2$ < free chlorine < ozone (Van Haute et al., 2015; Veschetti et al., 2003). However, for vegetables wash water, the decay of $ClO_2$ has never been compared with that of other disinfectants. As the same model system has been used in previous studies, i.e. SPW from butterhead lettuce (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013) and iceberg lettuce (this study) to study chlorine, and SPW from iceberg lettuce to study peracetic acid (Van Haute et al., 2015) and hydrogen peroxide (Van Haute et al., 2015), a comparison can be made with $ClO_2$. The results confirm that $ClO_2$ is less stable than PAA in SPW and considerably more stable than chlorine and hydrogen peroxide.

### 3.2. Disinfection efficiency of $ClO_2$ compared to free chlorine in fresh-cut lettuce wash water

The fecal indicator *E. coli* was reduced >5 log after 3 min with 5 mg/L $ClO_2$ in SPW (COD 1130 mg $O_2$/L), while at lower COD this reduction was achieved after 1 min with 4 mg/L $ClO_2$ (Table 2). The reduction of *E. coli* with 4 mg/L in SPW (COD 1130 mg $O_2$/L) was more than 3 log after 1 min, but was not further reduced with longer contact time (Table 2), presumably due to complete degradation of the $ClO_2$ residual (Table 1). Dosing 5 mg/L $ClO_2$ reduced

**Table 2**
Log reduction of *E. coli* after addition of 4 mg/L $ClO_2$ and 5 mg/L $ClO_2$ to SPW inoculated with *E. coli* (5.7 ± 0.4 log CFU/mL) after different contact times in water with different COD-levels (625; 734; or 1130 mg $O_2$/L) at 4 °C (n = 3).

| COD (mg $O_2$/L) | $ClO_2$ (mg/L) | 1 min | 2 min | 3 min | 5 min |
|---|---|---|---|---|---|
| 625 | 4 | >5 | >5 | >5 | >5 |
| 734 | 4 | >5 | >5 | >5 | >5 |
| 1130 | 4 | 3.3 ± 0.8 | 4.0 ± 1.0 | 4.0 ± 1.0 | 3.5 ± 0.4 |
| 625 | 5 | >5 | >5 | >5 | >5 |
| 734 | 5 | >5 | >5 | >5 | >5 |
| 1130 | 5 | 2.7 ± 1.3 | 2.4 ± 1.6 | >5 | >5 |

**Table 1**
Disinfectant residuals and log reductions of TPC after 2 min of exposure to different doses of free chlorine or $ClO_2$ to SPW (COD: 1130 mg $O_2$/L; Turbidity: 24 NTU; pH: 7.1; temperature: 4 °C; TPC contamination: 3.0 ± 0.2 log CFU/mL) (n = 3).

| Dose (mg/L) | Free chlorine residual (mg/L) | Log reduction | Dose (mg/L) | $ClO_2$ residual (mg/L) | Log reduction |
|---|---|---|---|---|---|
| 20 | <0.1 | <0.1 | 4 | <0.1 | 2.2 ± 0.5 |
| 30 | <0.1 | <0.1 | 5 | 0.40 | >3 |
| 50 | <0.1 | 0.2 ± 0.1 | 6 | 0.72 | >3 |
| 60 | <0.1 | ND[a] | 7.5 | 1.05 | >3 |
| 67 | 0.70 | ND | 10 | 2.68 | ND |
| 68 | 0.98 | ND | | | |
| 70 | 1.68 | 2.2 ± 0.2[b] | | | |

[a] Not determined.
[b] Only molds were observed on the plates (mold confirmation when filamentous, "hairy" colony morphology was seen and hyphae observed through light microscopy).