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# Bactericidal Effects of Fresh-Cut Vegetables and Fruits after Subsequent Washing with Chlorine Dioxide

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**Abstract.** Fresh-cut vegetables and fruits are easily contaminated by microbes during food processing. Further, browning reactions in fruits and vegetables are regarded as a serious problem that occurs during handling, processing and storage. Hence, the use of bactericide is a critical to assuring the microbiological safety and quality of food products at various stages of the supply chain. Chlorine dioxide ($ClO_2$) is a strong oxidizing agent and a safe bactericide, and generates only a small amount of trihalomethans (THMs) as a byproduct. This study mainly evaluates the bactericidal efficacy of $ClO_2$ and sodium hypochlorite solution (NaOCl) for six kinds of fresh-cut vegetables and fruits (cucumber, lettuce, carrot, apple, tomato and guava). The samples were dipped an washed in 50, 100 and 200 ppm bactericide solution for 20 minutes and stored for 0, 1, 2, 4 and 7 days, then checked for microbial content (total bacterial, *coliform*, *E. coli*, yeasts and molds count) and browning, respectively. The results indicated that 100 ppm $ClO_2$ solution can reduce 3.5-4.0 log CFU/g ($p < 0.05$) in total bacterial and *coliform* counts on lettuce, carrot and tomato which is better than sodium hypochlorite solution. The flowing washing treatment is better than dipping treatment. As for the browning test, the apple slices treated with 50 ppm $ClO_2$ solution showed anti-browning effects, but carrot slices treated with 200 ppm $ClO_2$ solution displayed a fading effect. Gas chromatography-mass spectrometry (GC-MS) analysis also showed that using a 200 ppm $ClO_2$ bactericide solution for 20 minutes, results in 12.85 ppb residuary THMs which is lower than NaOCl treatment (142 ppb residuary THMs). The treatment of $ClO_2$ solution for fresh-cut vegetables and fruits also conform to the Taiwan environment protection regulation, which calls for THMs counts lower than 80 ppb.

**Keywords:** fresh-cut vegetables, chlorine dioxide, bactercide, trihalomethans (THMs).

## 1. Introduction

Applications of disinfectant in food industry is an important tool in killing microorganisms and ensuring food quality for food preservation, shelf-life extension, equipment sterilization, elimination of undesirable flavor produced by bacteria during storage and food shipping [1]. Much attention is now being paid to the safety of vegetable and fruit products. The production of fresh-cut vegetables and fruits usually involves cleaning, trimming, coring, slicing, shredding, washing, centrifugal drying and packaging [2]. The cutting increases the area of injured tissue available for microbial proliferation, and tissue disruption by cutting results in elevated respiration, thus promoting rapid deterioration [3]. The microorganisms can also be located in irregularities on the vegetable surface [4], strongly attached underneath it [5] and form biofilms [6]. Their shelf-life is also greatly reduced during mechanical processing [8]. For these reasons, the disinfection and sanitation of water, food products and food-processing equipment have used halogen-containing disinfectants such as chlorine or iodine [9].

Chlorine dioxide ($ClO_2$) is one of the disinfectants that is currently used to control microbiological growth in the food industry [8]. The U.S. Environmental Protection Agency (EPA) has approved the use of $ClO_2$ as a disinfectant for potable water treatment with a maximum of a 1 ppm chlorite ion in the treated water [10]. 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 $ClO_2$ [11, 12]. Chlorine dioxide ($ClO_2$) has received attention as a decontaminant for vegetables, because its efficacy is

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less affected by pH and organic matter and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites [13]. It has recently been reported that $ClO_2$ can prolong the shelf-life of carrots [14]. However, some reports showed that the potentially carcinogenic byproducts generated during chlorination in the presence of organic material, such as: trihalomethanes (THMs), chloroform, and chlorophenols carry a health risk because they are potentially mutagenic [15]. Of particular concern in seafood treatment is the formation of chloroform from the reaction of chlorine with humic acid [9].

In this work, $ClO_2$ and sodium hypochlorite solution (NaOCl) were evaluated for bactericidal efficacy on six kinds of fresh-cut vegetables and fruits (cucumber, lettuce, carrot, apple, tomato and guava). The second experiment was to evaluate the ability of immersion in solutions with various concentrations of $ClO_2$ to inhibit browning in the six previously-mentioned fruits and vegetables. The third experiment consisted of comparing fruits and vegetables treated and untreated with $ClO_2$ for the effects of potentially carcinogenic byproducts generated by GC-MS.

## 2. Materials and Methods

### 2.1. Sample preparation

The six kinds of vegetables and fruits used in the experiments were cucumber (*Cucumis sativus* L.), Iceberg lettuce (*Lactuca sativa var. capitata* L.), carrot (*Daucus carota* L.), apple (Fuji apple), tomato (*Lycopersicon esculeutum Mill.*) and guava (*P. guajava* L.). They were purchased from a local market in Pingtung City, in southern Taiwan. The samples were stored at 7 °C and were processed within one day. The samples were manually shredded into pieces 1 mm thick, and were washed with tap water, chlorine dioxide and sodium hypochlorite at ambient temperatures (20-24 °C) for 20 minutes. Sample slices were washed with tap water, chlorine dioxide and sodium hypochlorite at concentrations of 50, 100 and 200 ppm, respectively, and assessed for bactericidal effects, GC-MS and color.

### 2.2. Description of the apparatus

The self-designed electrolysis equipment consists of a raw material tank, a $ClO_2$ electrolyzed cell, a NaOH collecting tank, a vacuum device, a $ClO_2$ collecting tank, and a cooling water unit. The internal structure and reaction of the electrolysis cell is shown in Fig. 1. Saturated saline and sodium hypochlorite were directed and mixed in the electrolyzed cell using a direct current (100-125 Amp, 7-8 V); electrolyzed temperature were maintained at 65-75° C, and the electrolyzed fluid flow rate was 50 ml/min. Both the electrolyzed cell and the $ClO_2$ collecting tank temperatures were controlled by the cooling water unit. The NaCl was electrolyzed into $NaClO_2$. The reaction equation is rendered as follows:

$NaCl + 2\ H_2O \rightarrow NaClO_2 + 2\ H_2\uparrow$ (1)

Meanwhile, the $NaClO_2$ was further electrolyzed and the $ClO_2^-$ was attracted by cathode and $H_2O$ was attracted by anode to release $H_2$ (Fig. 2). The reaction equations are written as follows:

Cathode: $ClO_2^- \rightarrow ClO_2\uparrow + e^-$ (2)

Anode: $H_2O + e^- \rightarrow OH^- + 1/2\ H_2$ (3)

Overall: $2\ NaClO_2 + 2\ H_2O \rightarrow 2\ ClO_2 + 2\ NaOH + H_2\uparrow$ (4)

$NaCl + NaClO_2 + 4\ H_2O \rightarrow 2\ ClO_2 + 2\ NaOH + 3\ H_2\uparrow$ (5)

The resultant $ClO_2$ was sucked out and collected into 10 ° C pure water in the collecting tank and the waste was collected in a waste tank. The oxidation/reduction potential (ORP) and pH of $ClO_2$ solutions were measured using an ORP/pH meter (Seven Easy ORP/pH meter, Kaohsiung, Taiwan).

### 2.3. Bacteriological analyses

Bacteriological analyses were carried out in triplicate on 10 g of raw dried samples that were blended with 90 mL of sterile water as described in the bacteriological analytical manual. Pour plates were prepared from 10-fold dilutions in potato dextrose agar (PDA, Difco™, Sparks, MD, USA) for mold and yeast counts, and plate count agar (PCA, Difco™) for bacterial counts, *coliform* and *E.coli*. Counts were made after incubation at 35 °C for 48 h.

### 2.4. Color assessment

The degree of browning, resulting from both enzymatic and non-enzymatic browning reactions, was mainly determined by the induced color change for different conditions. The Hunter $L^*a^*b^*$ values of samples were obtained using a color difference meter (CDM-08, Laiko, Tokyo, Japan). Three replicates were used for each sample treatment. The L* value was used as an indicator of brightness, *a** to indicate chromaticity on a green (-) to red (+) axis (the higher the *a** value, the closer it is to green); and *b** to indicate chromaticity on a blue (-) to yellow (+) axis (the higher the *b** value, the closer it is to yellow). However, it is also useful to convert the numerical values of *L**, *a** and *b**, into a white index (WI) value. The highest value of the WI represents the lowest degree of browning which was defined as follows:

$$WI = 100 - ((100 - L)^2 + a^2 + b^2)^{1/2}). \quad (6)$$

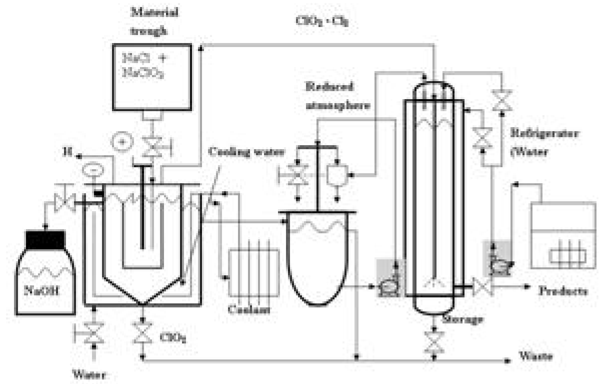


Fig. 1. Self-designed $ClO_2$ electrolyzed equipment.

## 2.5. GC-MS analysis

The GC-MS analysis system (GC-MS, Hewlett-Packard 6890 SERIES II, Japan) was used to further determine the structure of the sample extracted by different methods. The injected temperature was 200 °C, the detected temperature was 260 °C and was raised at a rate of 5 °C /min to test. The nebulizer gas was mixed with nitrogen and collided in argon which was then ionized by a high electric field for double collision. It was monitored by electron spray (ES) ionization for qualitative and quantitative analyses.

## 2.6. Statistical analysis

Analysis of variance (ANOVA) was used to investigate the significance of the influence and confidence of the processing parameters on the performance. A regression analysis of the experimental data was obtained using SAS® 8.2 (Statistical Analysis System, Cary, NC, USA). Duncan's multiple-range test was used to compare the difference between means at a probability level of < 0.05.

# 3. Results and Discussion

## 3.1. Bactericidal effect of $ClO_2$

The study utilized electrolysis to produce chlorine dioxide of better than 99% purity that was applied to fruits and vegetables washing to test bactericidal ability. The results are shown in table1, the original total bacteria counts of fresh-cut cucumber, lettuce, carrots, apples, tomatoes and guava were 6.41, 6.58, 6.48, 6.23, 6.97, and 6.30 log (CFU / g), respectively. After washing with tap-water their total bacteria counts still ranged from about 5.30 to 6.48 log (CFU / g). These levels fail to meet health regulation standards of less than 5 log (CFU / g) values. The study used concentrations of 50 ppm, 100 ppm, 200 ppm of both chlorine dioxide and sodium hypochlorite to wash the fruits and vegetables; both substances have bactericidal effects better than tap-water washing. With the concentrations of chlorine dioxide and sodium hypochlorite increased that their total bacteria counts showed significant reductions ($p < 0.05$). In samples treated with 200 ppm sodium hypochlorite, the total bacteria counts decreased to values of less than 5 log (CFU / g), yet chlorine dioxide treated with only 100 ppm achieved the same objective. The results of the *coliform* test in six fruits and vegetables (cucumber, lettuce, carrots, apples, tomatoes, and guava) are shown in table 2. The original *coliform* counts of fresh-cut cucumber, lettuce, carrots, apples, tomatoes and guava were 4.89, 4.26, 4.30, 4.46, 4.62 and 4.30 log (CFU / g), respectively. After washing with tap-water their total bacteria counts were about 3.52 to 4.62 log (CFU / g), which fails to meet health regulation standards of less than 3 log (CFU / g) values in *coliform* counts. The bactericidal effect of chlorine dioxide was the better than others. 100 ppm chlorine dioxide reduced to less than 3 log (CFU / g) values and even achieved complete sterilization in lettuce, carrots, and tomatoes. Similar results were achieved in the *E. coli* test (Table 3) for the six fruits and vegetables (cucumber, lettuce, carrots, apples, tomatoes, and guava). 100 ppm chlorine dioxide reduced to less than 3 log (CFU / g) values and even achieved complete sterilization for yeasts and molds in tomatoes.

Table 1. Bacteria counts of fresh-cut vegetables and fruits treated by different disinfectants

| Treatment | Conc (ppm) | Cucumber log (CFU/g) | Lettuce log (CFU/g) | Carrot log (CFU/g) | Apple log (CFU/g) | Tomato log (CFU/g) | Guava log (CFU/g) |
|---|---|---|---|---|---|---|---|
| Fresh | - | $6.41^{a}$ | $6.58^{a}$ | $6.48^{a}$ | $6.23^{a}$ | $6.97^{a}$ | $6.30^{a}$ |
| Tap-water | - | $5.30^{b}$ | $5.67^{b}$ | $6.00^{b}$ | $6.08^{b}$ | $6.48^{b}$ | $5.90^{b}$ |
| Chlorine dioxide | 50 | $4.15^{e}$ | $3.30^{e}$ | $4.48^{e}$ | $4.78^{f}$ | $4.56^{d}$ | $5.18^{d}$ |
| | 100 | $3.89^{f}$ | $3.08^{f}$ | $3.00^{f}$ | $4.00^{g}$ | $3.04^{g}$ | $4.78^{f}$ |
| | 200 | $3.40^{g}$ | $2.90^{g}$ | $2.15^{g}$ | $3.00^{h}$ | $1.78^{h}$ | $4.00^{h}$ |
| Sodium hypochlorite | 50 | $4.48^{c}$ | $5.51^{c}$ | $6.00^{b}$ | $5.63^{c}$ | $5.65^{c}$ | $5.26^{c}$ |
| | 100 | $4.36^{d}$ | $5.49^{c}$ | $5.30^{c}$ | $5.30^{d}$ | $4.20^{e}$ | $5.00^{e}$ |
| | 200 | $4.15^{e}$ | $5.08^{d}$ | $4.78^{d}$ | $5.08^{e}$ | $3.72^{f}$ | $4.48^{g}$ |

a, b, c, e, f, g, h : Means in column followed by different letters are significantly different ( $p < 0.05$).

Table 2. Coliform counts of fresh-cut vegetables and fruits treated by different disinfectants

| Treatment | Conc (ppm) | Cucumber log (CFU/g) | Lettuce log (CFU/g) | Carrot log (CFU/g) | Apple log (CFU/g) | Tomato log (CFU/g) | Guava log (CFU/g) |
|---|---|---|---|---|---|---|---|
| Fresh | - | $4.89^{a}$ | $4.26^{a}$ | $4.30^{a}$ | $4.46^{a}$ | $4.62^{a}$ | $4.30^{a}$ |
| Tap-water | - | $4.62^{b}$ | $3.54^{b}$ | $3.88^{b}$ | $4.00^{b}$ | $3.83^{b}$ | $3.85^{b}$ |
| Chlorine dioxide | 50 | $3.21^{e}$ | $2.00^{f}$ | $1.00^{f}$ | $2.04^{f}$ | $ND^{d}$ | $2.00^{f}$ |
| | 100 | $2.71^{g}$ | $ND^{g}$ | $ND^{g}$ | $1.89^{g}$ | $ND^{d}$ | $1.83^{g}$ |
| | 200 | $2.46^{h}$ | $ND^{g}$ | $ND^{g}$ | $1.53^{h}$ | $ND^{d}$ | $1.41^{h}$ |
| Sodium hypochlorite | 50 | $3.40^{c}$ | $3.00^{c}$ | $3.61^{c}$ | $3.70^{c}$ | $1.00^{c}$ | $3.48^{c}$ |
| | 100 | $3.32^{d}$ | $2.81^{d}$ | $3.11^{d}$ | $3.16^{d}$ | $ND^{d}$ | $3.30^{d}$ |
| | 200 | $3.11^{f}$ | $2.15^{e}$ | $2.30^{e}$ | $2.60^{e}$ | $ND^{d}$ | $2.82^{e}$ |

a, b, c, e, f, g, h : Means in column followed by different letters are significantly different ( $p < 0.05$)

Table 3. E. coli counts of fresh-cut vegetables and fruits treated by different disinfectants

| Treatment | Conc (ppm) | Cucumber log (CFU/g) | Lettuce log (CFU/g) | Carrot log (CFU/g) | Apple log (CFU/g) | Tomato log (CFU/g) | Guava log (CFU/g) |
|---|---|---|---|---|---|---|---|
| Fresh | - | $3.30^{a}$ | $3.53^{a}$ | $3.21^{a}$ | $3.43^{a}$ | $3.15^{a}$ | $3.11^{a}$ |
| Tap-water | - | $3.08^{b}$ | $3.34^{b}$ | $2.71^{b}$ | $2.18^{b}$ | $2.60^{b}$ | $2.48^{b}$ |
| Chlorine dioxide | 50 | $2.61^{d}$ | $ND^{d}$ | $ND^{c}$ | $1.60^{d}$ | $1.00^{c}$ | $2.00^{d}$ |
| | 100 | $1.00^{g}$ | $ND^{d}$ | $ND^{c}$ | $ND^{e}$ | $ND^{c}$ | $ND^{f}$ |
| | 200 | $ND^{h}$ | $ND^{d}$ | $ND^{c}$ | $ND^{e}$ | $ND^{c}$ | $ND^{f}$ |
| Sodium hypochlorite | 50 | $2.70^{c}$ | $1.70^{c}$ | $ND^{c}$ | $1.78^{c}$ | $1.85^{c}$ | $2.11^{c}$ |
| | 100 | $2.48^{e}$ | $ND^{d}$ | $ND^{c}$ | $ND^{e}$ | $ND^{c}$ | $1.85^{e}$ |
| | 200 | $1.90^{f}$ | $ND^{d}$ | $ND^{c}$ | $ND^{e}$ | $ND^{c}$ | $ND^{f}$ |

a, b, c, e, f, g, h : Means in column followed by different letters are significantly different ( $p < 0.05$).

## 3.2. Color assessment

The study chose easy browning of apples to assess different washing solution treatments on fresh-cut fruits and vegetables, and the results appear in table 4. The apples showed serious browning reactions after fresh-cutting treatment, and the color of apple was transformed to a darker brown with tap-water washing. Here, the chlorine dioxide showed that it can inhibit enzyme activity and keep the original color, and L value showed no significant difference from fresh apple color. Sodium hypochlorite has some bleaching effect in fresh-cut apples, and L value rose significantly.

Table 4. Lab value of fresh-cut apples by different disinfectants

| Treatment | L | a | b | Hue | Chroma |
|---|---|---|---|---|---|
| Fresh | $80.09^{b}$ | $-4.52^{a}$ | $21.43^{b}$ | $-1.36^{a}$ | $21.90^{c}$ |
| Tap-water | $79.50^{c}$ | $-4.94^{ab}$ | $21.88^{b}$ | $-1.35^{a}$ | $22.43^{b}$ |
| Chlorine dioxide | $80.40^{b}$ | $-4.87^{ab}$ | $22.85^{a}$ | $-1.36^{a}$ | $23.36^{a}$ |
| Sodium hypochlorite | $81.55^{a}$ | $-4.99^{ab}$ | $22.73^{a}$ | $-1.35^{a}$ | $23.27^{a}$ |

a, b, c Means in column followed by different letters are significantly different ($p < 0.05$).

### 3.3. GC-MS analysis

The GC-MS analysis shown in Fig.2, used 200 ppm concentrations of different bactericidal solutions treated for 20 minutes. The treatment of chlorine dioxide solution had 12.85 ppb residuary THMs which is lower than sodium hypochlorite treatment (142 ppb residuary THMs). The treatment of chlorine dioxide solution for fresh-cut vegetables and fruits also conforms to the Taiwan environmental protection regulations, which require counts of less than 80 ppb for THMs. This means that chlorine dioxide solution is a safer disinfectant than sodium hypochlorite.

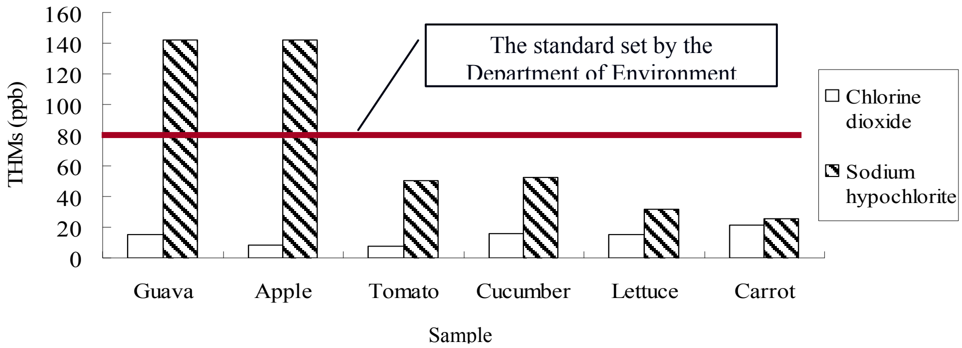


Fig. 2. The residues of THMs of samples with GC-MS analysis.

## 4. Discussion

The results indicated that 100 ppm $ClO_2$ solution can reduce total bacterial and *coliform* counts to 3.5-4.0 log CFU/g ($p < 0.05$) on lettuce, carrots and tomatos, which is better than sodium hypochlorite solution. The flowing washing treatment is better than the dipping method. For the browning test, the apple slices treated with 50 ppm $ClO_2$ solution displayed anti-browning effects, but carrot slices treated with 200 ppm $ClO_2$ solution displayed a fading effect. Gas chromatography-mass spectrometry (GC-MS) analysis also showed that using a 200 ppm bactericide solution treatment for 20 minutes, the treatment of $ClO_2$ solution is 12.85 ppb residuary THMs which is lower than NaOCl treatment (142 ppb residuary THMs). The treatment of $ClO_2$ solution for fresh-cut vegetables and fruits also conforms to the Taiwan environmental protection regulations, which require counts of less than 80 ppb for THMs.

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