Effects of Nonthermal Processes on the Inactivation of Microorganisms and Antioxidants in Minimally Processed Vegetables

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Received August 21, 2008; Accepted December 3, 2008

Effects of several nonthermal disinfection processes, including high pressure carbon dioxide treatment (HCT), gaseous chlorine dioxide treatment (GCT) and pulsed xenon treatment (PXT), on the inactivation of microorganisms and antioxidants in ten cut vegetables (red paprika, green pepper, cucumber, cabbage, lettuce, mizuna, celery, onion, carrot, and bean sprout) were investigated. HCT (6 or 10 MPa, 10 min, 35°C) showed similar inactivation effects on microorganisms using conventional NaOCl treatment (100 ppm, 10 min) by controlling pressure. However, HCT caused a loss of more antioxidants such as vitamin C and phenolic compounds in cut vegetables under severe conditions. In addition, HCT softened the leaf vegetables, cabbage, lettuce, and mizuna. All samples after PXT (500 J, 10 times) had the same antioxidant contents and DPPH radical scavenging activities as the untreated intact samples, suggesting PXT could be a disinfection method that does not influence the antioxidants in cut vegetables. For inactivation of microorganism, PXT decreased the viable cell count by about 10-fold, but not significantly. On the other hand, GCT (10 ppm, 30 min) showed almost the same microorganism inactivation as NaOCl treatment. The loss of vitamin C and phenolic compounds due to GCT were negligible. These results suggest that GCT might be a promising nonthermal disinfection treatment for cut vegetables.

Keywords: cut vegetable, nonthermal treatment, carbon dioxide, chlorine dioxide, pulsed xenon

Introduction

Recent accumulating evidence indicates that several vegetables can provide beneficial effects for health maintenance and prevention of various diseases such as cancer and cardiovascular diseases (Ness & Powles, 1997; Kris-Etherton *et al.*, 2002). The beneficial effects have been attributed to antioxidants including vitamins C and E, flavonoids and carotenoids in vegetables, which are believed to protect living organisms from oxidative damage, and thus resulting in disease prevention (Kaur & Kapoor, 2001; Gossiau & Chen, 2004). Therefore, there is increasing interest in the antioxidant properties of vegetables.

Cut vegetables, also known as minimally processed veg-

etables (MPV), have become popular among consumers in the past few decades, because they need no special preparations to cook. However, MPV are easily contaminated with various microorganisms (Garcia-Gimeno & Zurera-Cosano, 1997; Tournas, 2005) and degraded naturally (Lopez-Galvez *et al.*, 1997; Jacxsens *et al.*, 2003). Contamination is caused by many species of microorganisms, such as bacteria *Pseudomonas*, *Erwinia*, *Listeria*, and *Leuconostoc* (Willcox *et al.*, 1993; Rocourt, 1994), and molds *Penicillium*, *Rhizopus*, and *Aspergillus* (Brackett, 1987; Konuma, 2000). Therefore, nonthermal disinfection treatments are performed to prevent contamination and to supply fresh MPV in cut vegetable industry. In general, washing with sodium hypochlorite (NaOCl) solution is a method used for MPV decontamination (Nascimento *et al.*, 2003; Lee *et al.*, 2004). However, some organic compounds, particularly phenolic

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compounds, react easily with available chlorine to form toxic chlorinated products (Rook 1974; Moriyama *et al.*, 2004a, 2004b). Thus, other disinfection methods to replace NaOCl treatment are being explored.

High pressure carbon dioxide treatment (HCT) is one of the promising nonthermal disinfection methods. Many studies have shown that HCT can inactivate microorganisms using gaseous and supercritical states and that kills bacteria including spores (Dillow *et al.*, 1999; Furukawa *et al.*, 2004; Nakamura *et al.*, 1994; Watanabe *et al.*, 2003). Pulsed xenon treatment (PXT) is a pulsed UV light treatment using a xenon lamp. Some PXT studies on the inactivation of microorganisms have suggested that PXT is also a promising nonthermal surface disinfection method (Takeshita *et al.*, 2003; Wang *et al.*, 2005). Chlorine dioxide is considered an effective disinfectant and is now used in purification and disinfection in the United States (Knapp & Battisti, 2001). Chlorine dioxide treatment can be divided into a gaseous treatment (GCT) and an aqueous treatment (ACT). Many reports have shown microorganism inactivation effects of GCT and ACT (Puente *et al.*, 1992; Radziminski *et al.*, 2002), with some showing that GCT is more effective than ACT (Sapers *et al.*, 2003; Izumi, 2005). Moreover, the effect of chlorine dioxide treatment on decontamination of vegetables including MPV has been reported (Lee *et al.*, 2004; Rodgers *et al.*, 2004; Gomez-Lopez *et al.*, 2005a). Thus, the chlorine dioxide treatment appears to be a promising method for microorganism decontamination of vegetables.

While there are many reports on the inactivation of microorganisms in vegetables, there is little information about the functional components in MPV after disinfection. In this study, we investigated the effects of several nonthermal processes, including HCT, PXT, and GCT, on microorganism inactivation and antioxidants in ten cut vegetables.

Materials and Methods

Vegetables Red paprika, green pepper, cucumber, cabbage, lettuce, mizuna (*Brassica*), celery, onion, carrot and bean sprout were all commercially available and purchased at a local store in Fujisawa City, Japan. Leaf-vegetables (cabbage, lettuce, and mizuna) were cut into about 0.5×2.0 cm pieces and the remaining vegetables were cut into about $0.5 \times 0.5 \times 2.0$ cm sticks using a ceramic knife or scissors to avoid oxidation of organic compounds in vegetables. The vegetables before cutting were stored at 4°C and used within 2 days of purchase.

Sodium hypochlorite treatment (NaOCl-treatment) Cut vegetable samples (about 50 g) were immersed in 100 ppm sodium hypochlorite solution (500 mL) for 10 min at room temperature, and then washed for 1 min in tap water (500

mL) three times to remove residual NaOCl. The samples were centrifuged (1,000 rpm) for 1 min for surface liquid removal (Koseki & Itoh, 2001; Izumi, 2005).

High pressure carbon dioxide treatment (HCT) Cut vegetable samples were placed in the stainless steel vessel (104×17 mm) of a prototype pressurization apparatus (AKI-CO Co., Tokyo, Japan) (Watanabe *et al.*, 2003), and pressurized with CO₂. Carbon dioxide changes its state at 7.38 MPa and 31.1°C; it is in the supercritical state above 7.38 MPa and 31.1°C and in the gaseous state below this condition. Therefore, the samples were treated as follows:

- 1) Samples were placed in the vessel without solution, and treated at 6 MPa for 10 min at 35°C (G-dry).
- 2) Samples were placed in the vessel containing 0.85% physiological saline solution (10 mL), and treated at 6 MPa for 10 min at 35°C (G-wet).
- 3) Samples were placed in the vessel without solution, and treated at 10 MPa for 10 min at 35°C (S-dry).
- 4) Samples were placed in the vessel containing 0.85% physiological saline solution (10 mL), and treated at 10 MPa for 10 min at 35°C (S-wet).

Gaseous chlorine dioxide (ClO₂) treatment (GCT) Cut vegetable samples were placed on a turntable in a non-commercial box ($30 \times 38 \times 50$ cm³, Nishikawa Sangyo Co., Ltd., Tokyo, Japan) and exposed with gaseous chlorine dioxide (10 ppm), produced by a mixture of 3% HCl and 3.3% NaClO₂ solutions, for 30 min. Gas was circulated in the box, and the gaseous chlorine dioxide concentration around the samples were measured by a gas detector (Kitagawashiki-Gastec, Komyo Rikagaku Kogyo Co., Ltd., Kanagawa, Japan) from outside of the box.

Pulsed xenon treatment (PXT) Cut vegetable samples were placed on a table in a prototype apparatus (Iwasaki Electric Co., Ltd., Tokyo, Japan) and irradiated 10 times with xenon lamp pulses at 1 Hz produced by 500 J electric discharges.

Measurement of survivors in vegetable samples The treated cut vegetable samples were cut by sterile scissors and allowed to stand for 10 min before the colony count analysis. The number of surviving cells of microorganisms on the cut vegetable samples was determined by the plated viable count method using nutrient agar (Becton Dickinson, USA). Plates were incubated at 37°C for 48 h and the colonies were enumerated.

Measurement of surviving cells of *E. coli* To determine the appropriate disinfection conditions for cut vegetables by HCT, a preliminary investigation was carried out using agar block cultivated *Escherichia coli* K-12 (MG1655) obtained from the Department of Cell Genetics, The National Institute of Genetics (Mishima, Japan). *E. coli* were cultivated in

Trypticase Soy Broth (Becton Dickinson, USA) overnight at 37°C and the stationary growth phase cells were washed two times followed by centrifugation at $8,000 \times g$ for 10 min at 4°C. Following resuspension in 0.85% physiological saline solution, 10-mm cubic agar blocks were immersed into these *E. coli* solutions for 30 min at 4°C, and then subjected to NaOCl treatment or HCT. The treated agar blocks were cut by sterile scissors and allowed to stand for 10 min. The number of surviving cells of *E. coli* was determined by the plated viable count method using nutrient agar. Plates were incubated at 37°C for 48 h and the colonies were quantified.

Measurement of vitamin C Vitamin C in MPV (5 g of fresh weight; 1.25 g for red paprika; 2.5 g for green pepper and mizuna; and 10 g of carrot) was extracted with 2% metaphosphoric acid (50 mL) using a Polytron PT2100 homogenizer (30,000 rpm, 20 s, Kinematic AG, Switzerland). The level of vitamin C was determined by the colorimetric method using 2,4-dinitrophenylhydrazine (Pharmaceutical Society of Japan, 1980).

Measurement of total phenolic content The total phenolic content in MPV was analyzed photometrically using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The phenolic compounds in MPV (5 g of fresh weight; 2.5 g for red paprika) were extracted with 100 mL of 50% acidified methanol solution (pH 2.2) using a Polytron PT2100 homogenizer. Each sample solution (1 mL) was mixed with 1.5 mL of 0.4 M Na_2CO_3 and 1.5 mL Folin reagent. The solution was allowed to stand for 30 min at 37°C, and the absorbance was measured at 760 nm. Catechin was used as the standard, and the total phenolic content is expressed as (+)-catechin equivalents.

Measurement of antioxidant activity The antioxidant activity was evaluated by the DPPH radical scavenging assay

(Blois, 1958; Shimamura *et al.*, 2007). About 5 g MPV (1.25 g for red paprika, 2.5 g for green pepper and mizuna, and 10 g for carrot) was extracted with 100 mL of 50% methanol. An aliquot of extract (1 mL) was mixed with 4 mL of 100 mM Tris-HCl buffer (pH 7.4) and then added to 5 mL of 0.15 mM DPPH in ethanol. The mixture was shaken vigorously (10 sec) and left for 20 min at room temperature in the dark. The absorbance at 517 nm was measured by a Shimadzu UV-1700 spectrophotometer. DPPH radical scavenging activity was evaluated from the following equation:

$$\text{Radical scavenging activity (\%)} = (BL - S) / BL \times 100 \quad \text{Eq. (1)}$$

where *BL* is the absorbance of DPPH in the absence of the sample and *S* is the absorbance of DPPH in the presence of the sample.

Statistical analysis All experiments were done in at least triplicate. The data presented are the means of at least three replicate experiments. Statistical significance of the results was evaluated using analysis of variance (ANOVA) and Tukey multiple-range test at $p < 0.05$. All statistical analyses were performed in GraphPad Prism for Windows, GraphPad software.

Results

Effects of high pressure CO_2 treatment on the inactivation of microorganisms and antioxidants in MPV The effects of four types of HCT (S-dry, S-wet, G-dry and G-wet) on the inactivation of *E. coli* were first examined as a preliminary investigation to determine the HCT treatment for MPV (Fig. 1). The wet treatments (S-wet and G-wet) showed more effective inactivation than the dry treatments (S-dry and G-dry), with S-wet (10 MPa, 10 min, 35°C) being the most

Fig. 1. Effects of the four types HCT, S-dry, S-wet, G-dry and G-wet on the inactivation of the *Escherichia coli* in agar blocks. S-dry and wet, 35°C, 10 MPa, 10 min; G-dry and wet: 35°C, 6 MPa, 10 min; NaOCl, 100 ppm, 10 min.

effective. In fact, S-wet was as effective as NaOCl-treatment (100 ppm, 10 min) for inactivation of microorganisms. However, S-wet caused the vegetable samples to freeze in the decompression procedure when S-wet was applied to MPV (data not shown). Therefore, G-wet was applied to MPV, as this treatment was also effective for inactivation.

Figure 2 shows the effects of G-wet on the inactivation of microorganisms in the ten cut vegetables. G-wet (6 MPa, 10 min, 35°C) showed the same or less inactivation of

NaOCl treatment, with variations in the effectiveness among the vegetables. The vitamin C and total phenolic contents in samples after G-wet treatment were significantly decreased ($p < 0.05$), and these losses due to G-wet treatment were greater than that due to NaOCl treatment (Table 1), as well as greater than that due to the G-dry treatment. Thus, even though the wet treatment most likely caused the losses in the antioxidants, particularly water-soluble components such as vitamin C from cut section of vegetables, it showed effective

Fig. 2. Effects of G-wet on the inactivation of microorganisms in ten cut vegetables. □, intact; ▨, NaOCl; ▩, G-wet. Vertical bars indicate standard deviation of means. Means within each vegetables with different letter differ significantly ($p < 0.05$).

Table 1. Effects of high pressure carbon dioxide treatments on antioxidant components in cut vegetables.

	Red paprika	Green pepper	Cucumber	Cabbage	Lettuce	Mizuna	Celery	Onion	Carrot	Bean sprout
Vitamin C (mg / 100 g of fresh weight)										
Intact	132.9 a	70.8 a	9.8 a	24.0 a	4.4 a	32.5	5.9 a	3.6 a	2.2	2.9
NaOCl	107.1 bc	66.0 ab	7.2 ab	19.2 a	3.8 a	29.9	4.6 ab	2.4 ab	2.0	1.9
G-wet	81.0 c	45.6 b	3.0 b	9.2 b	1.5 b	18.2	2.3 b	1.8 b	0.8	1.2
G-dry	115.4 ab	56.5 ab	8.0 ab	22.4 a	4.2 a	21.3	5.0 ab	2.8 ab	1.9	2.4
Total phenolic contents (mg catechin equivalent / 100 g of fresh weight)										
Intact	152.4	109.9 d	35.4	71.8 d	93.4 d	105.2 d	55.1	82.5 d	86.8	114.1 d
NaOCl	142.5	102.2 de	33.0	62.2 de	93.6 d	103.4 d	50.4	72.5 d	84.6	111.7 d
G-wet	139.3	86.6 e	28.7	45.1 e	42.2 e	49.3 e	41.8	67.8 e	85.9	76.6 f
G-dry	141.4	103.4 de	34.5	66.0 de	94.4 d	77.4 d	50.7	79.6 d	87.9	100.9 e

Values within a column with the different letters (a-c and d-f) are significantly different ($p < 0.05$).

microorganism inactivation.

Effects of gaseous chlorine dioxide and pulsed xenon treatments on the inactivation of microorganisms and antioxidants in MPV Treatments of cut vegetables under dry conditions were also examined, as wet treatments caused losses in antioxidants. The effects of GCT and PXT on microorganism inactivation in the ten cut vegetables are shown in Fig. 3. GCT (10 ppm, 30 min) showed almost the same inactivation as NaOCl treatment (100 ppm, 10 min), while

PXT (500 J, 10 times) decreased the number of viable cells by about 10-fold; there was no significant difference in the number of viable cells between untreated intact and PXT samples, except for bean sprouts. Thus, the effectiveness to inactivate microorganisms in cut vegetables was as follows: PXT $\approx$ NaOCl < GCT; the results suggested that GCT has almost similar potential as NaOCl treatment.

The effects of GCT and PXT on the ascorbic acid and phenolic contents in ten samples are summarized in Table

Fig. 3. Effects of GCT and PXT on the inactivation of microorganisms in ten cut vegetables. □, intact; ▨, NaOCl; ▩, GCT; ▤, PXT. Vertical bars indicate standard deviation of means. Means within each vegetables with different letter differ significantly ($p < 0.05$).

Table 2. Effects of chloride dioxide and pulsed xenon treatments on antioxidant components in cut vegetables.

	Red paprika	Green pepper	Cucumber	Cabbage	Lettuce	Mizuna	Celery	Onion	Carrot	Bean sprout
Vitamin C (mg / 100 g of fresh weight)										
Intact	131.5 a	53.9 a	9.0	30.2	4.7	27.1	3.8	5.0 a	2.8	3.5
NaOCl	111.4 b	41.7 b	6.6	27.5	4.1	33.3	2.3	3.5 b	2.2	2.5
GCT	122.8 ab	53.0 ab	7.6	28.9	4.8	26.5	3.1	3.6 b	2.2	2.8
PXT	132.0 a	52.9 a	8.2	29.4	4.3	28.3	3.0	3.9 b	3.0	3.3
Total phenolic contents (mg catechin equivalent / 100 g of fresh weight)										
Intact	157.7 d	120.6	46.3	68.6	79.4	125.1 d	43.7 d	84.0 d	85.5	111.1
NaOCl	126.5 e	117.4	41.3	63.2	70.7	112.8 de	42.5 de	67.5 e	81.6	109.8
GCT	163.5 d	114.1	40.8	66.6	66.7	103.4 e	38.4 e	77.8 de	79.7	106.4
PXT	156.4 d	117.6	45.0	64.7	83.3	114.6 d	44.1 d	82.5 d	84.9	109.4

Values within a column with the different letters (a and b and d and e) are significantly different ($p < 0.05$).

Fig. 4. DPPH radical scavenging activity of cut vegetables after disinfection treatment. □, intact; ▨, NaOCl; ▩, G-wet; ▤, GCT; ▧, PXT. Vertical bars indicate standard deviation of means. Means within each vegetables with different letter differ significantly ($p < 0.05$).

Fig. 5. Effects of GCT on the ascorbic acids in red paprika and green pepper. □, ascorbic acid; ■, dehydroascorbic acid.

2. As expected, the losses of vitamin C and phenolic compounds due to these treatments were negligible. The DPPH radical scavenging activities of samples after these two treatments are shown in Fig. 4. The activities decreased relative to loss of antioxidants. However, the activities of red paprika

and green pepper after GCT were slightly, but not significantly, decreased compared with those of untreated intact samples, despite no change in the content of the antioxidants. Figure 5 shows the changes in content of ascorbic and dehydroascorbic acids in red paprika and green pepper by several

Fig. 6. Cut vegetables after disinfection treatment.

treatments. The dehydroascorbic acid content in both red paprika and green pepper was increased after GCT, indicating that some ascorbic acids were oxidized to dehydroascorbic acid due to chlorine dioxide. When DPPH radical scavenging activities of ascorbic and dehydroascorbic acids were measured using commercial reagents, dehydroascorbic acid showed no activity (ascorbic acid, $69.8 \pm 1.3\%$; dehydroascorbic acid, $1.4 \pm 0.6\%$). Thus, the decrease in the antioxidant activities of red paprika and green pepper following GCT could be due to oxidation of ascorbic acid to dehydroascorbic acid.

Images of the treated vegetables are shown in Fig. 6. Based on visual observation alone, G-wet treatment appeared to soften the leaf-vegetables such as cabbage, lettuce, and mizuna; physical analysis was not performed in this study.

On the other hand, no differences in color and texture were observed between GCT or PXT samples and untreated intact samples.

Discussion

In this study, the effects of nonthermal processes, HCT (G-wet), GCT, and PXT on microorganism inactivation and antioxidants in MPV were examined for ten cut vegetables. G-wet, PXT and GCT showed similar inactivation effects to NaOCl treatment. For antioxidants such as ascorbic acid and phenolic compounds, the contents were unchanged following either PXT and GCT, while the contents were decreased following G-wet and NaOCl treatments.

Comparing the loss of antioxidants after disinfection treatment, PXT is thought to be the best disinfection method,

as the contents of antioxidants and DPPH radical scavenging activity in all the samples following PXT were unchanged. Antioxidant activity is strongly correlated to the content of antioxidants such as ascorbic acid and phenolic compounds (Kaur & Kapoor, 2002; Suda *et al.*, 2005; Matsufuji *et al.*, 2007). The results suggest that PXT would be a disinfection method that does not influence antioxidant in cut vegetables, but its microorganism inactivation is lower than that of NaOCl treatment. Gomez-Lopez *et al.* (2005b) have reported a reduction (log reductions, 0.24 – 2.04) in the initial microbial load on minimally processed vegetables by intense light pulses. Their pulse intensity was 7 J with lamp flashes at 15 Hz for 45 to 180 sec. In our study, the pulse intensity was 500 J with lamp flashes at 1 Hz for 10 s (about 10-fold the average number of pulses). Although both studies could not be directly compared, we both obtained almost the same results. However, they reported that the intense light pulse treatment alone under the conditions in their study showed no increase in the shelf-life of cut vegetables (cabbage and lettuce), but applying an antibrowning treatment in combination with the intense light pulses increased the shelf-life.

Previous studies (Lee *et al.*, 2004; Rodgers *et al.*, 2004; Gomez-Lopez *et al.*, 2005a) on the application of GCT to vegetables showed that chlorine dioxide gas was effective at inactivating food poisoning bacteria on vegetables without deteriorating sensory qualities. Our results also support these results. On the other hand, ascorbic acid in cut vegetables was oxidized to dehydroascorbic acid by GCT, resulting in lower antioxidant activity, with the total content remaining the same. As dehydroascorbic acid is known to be reduced to ascorbic acid *in vivo*, it is thought to be a vitamin C compound (Dayton *et al.*, 1966). Thus, GCT may be a potential nonthermal disinfectant method for MPV.

As PXT is a clean treatment that requires no chemicals compared to other treatments such as NaOCl and GCT, it is harmless to the environment. In addition, GCT uses ClO₂ gas and is conducted just under atmospheric pressure in a non-liquid environment. Above all, neither PXT nor GCT causes deterioration in the color and texture quality of cut vegetables, based on visual observations. Further studies on the effects of these nonthermal disinfection treatments on vegetables using physiological measurements are needed. In addition, investigation of the effects of disinfection treatments, including PXT, GCT and combined treatments, on vegetable shelf-life will also be important for food safety.

Conclusion

It is necessary to disinfect cut vegetables that are minimally processed, as they are easily contaminated with microorganisms. Although wet HCT (G-wet; 6 MPa, 10 min,

35°C) causes losses of antioxidants, particularly vitamin C in cut vegetables, it shows almost the same microorganism inactivation as NaOCl treatment (100 ppm, 10 min). PXT (500 J, 10 times) does not cause losses in antioxidants and shows the same DPPH radical scavenging activity as untreated intact samples, but its microorganism inactivation is lower than that of NaOCl treatment. GCT (10 ppm, 30 min) shows similar microorganism inactivation to NaOCl treatment with no changes in the vitamin C and phenolic contents. These results suggest that GCT is a potential nonthermal disinfection method for minimally processed vegetables.

Acknowledgments We appreciate Iwasaki Electric and Nishikawa Sangyo Companies for lending us the pulsed xenon and gaseous chlorine dioxide treatment apparatus, respectively. This work was supported by a Food Safety and Functionality Project Grant from The Ministry of Agriculture, Forestry and Fisheries of Japan to H.F. and S.F.

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