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# Removal of Residual Pesticides in Vegetables Using Ozone Microbubbles

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/48744>

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## 1. Introduction

The present agriculture has enabled mass and stable production by using of agricultural pesticides. However, agricultural pesticides can have an adverse effect on the environment in addition to being harmful to humans, animals and fishes. The health hazard to the farmer as well as the residue in crops is also a global problem. Recently, the safety of crops including contamination with agricultural pesticides is a major concern to both the producer and consumer, and the development of a method to remove the pesticides before marketing has been eagerly awaited. In Japan, about 600 agricultural pesticides are included in the Positive List established in 2006. Since agricultural crops cannot be marketed when they contain pesticides exceeding the residual limit, the development of a measure for eliminating residual pesticides in crops is now an important issue (Yamaguchi, 2006).

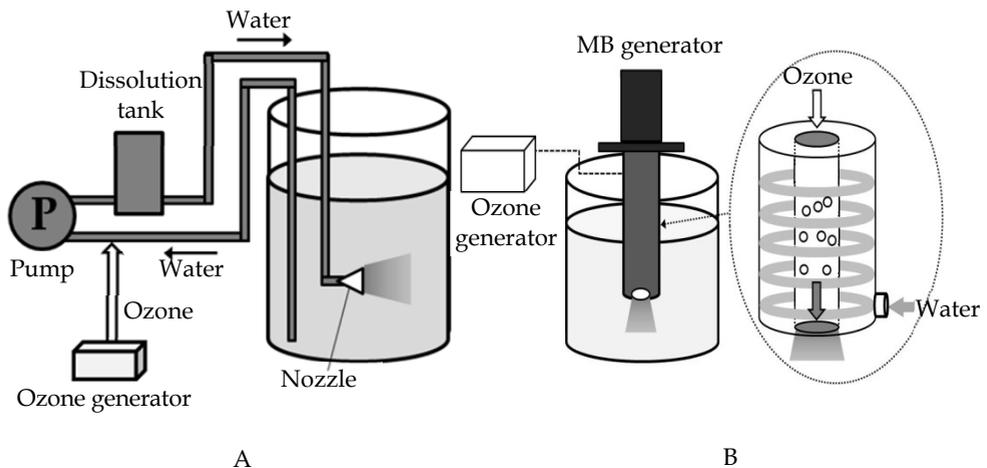
Ozone (O<sub>3</sub>) is the natural substance in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadre et al., 2001). O<sub>3</sub> is generated by the passage of air or oxygen gas through a high voltage electrical discharge or by ultraviolet light irradiation (Mahapatra et al., 2005), then has a strong oxidative power, and is used for sterilization, virus inactivation, deodorization, bleaching (decoloration), decomposition of organic matter, mycotoxin degradation and others (Cataldo, 2008; Karaca and Velioglu, 2009; Karaca et al., 2010; Takahashi et al., 2007a). In addition, O<sub>3</sub> is changed to oxygen by autolysis and does not harm the flavor of vegetables and fruits (Li and Tsuge, 2006). Therefore, O<sub>3</sub> is considered to be most suitable for removing residual pesticides from vegetables and fruits and controlling microbes of food safety concern (Selma et al., 2008; Gabler et al., 2010). The threshold concentration of O<sub>3</sub> for continuous human exposure is 0.075 µL/L (US Environmental Protection Agency, 2008). Although there are many studies on the removal of pesticides using O<sub>3</sub> for water purifications in waste water, there are

several reports on the use of O<sub>3</sub> to remove residual pesticide in vegetables and fruits (Daidai et al., 2007; Hwang et al., 2001a; Hwang et al., 2001b; Hwang et al., 2002; Karaca and Velioglu, 2007; Ong et al., 1995; Wu et al., 2007a; Gabler et al., 2010).

Microbubbles (MB) are less than 50 µm in diameter and have special properties such as generation of free radicals, self-pressurization and negative charge, and their use in the field of food science and agriculture is attracting attention (Sumikura et al., 2007; Takahashi et al., 2007b). Millibubbles generated by using an air pump are 2-3 mm in diameter, rapidly rise in water and burst at the water surface. Therefore, the solubility of the gas in water is very low. On the other hand, MB rise in water slowly and the interior gas is completely dissolved in water (Takahashi et al., 2007a).

Up to now, growth promotion of lettuce in hydroponic cultures with air MB (Park and Kurata, 2009) and inactivation of *Escherichia coli* using CO<sub>2</sub> MB (Kobayashi et al., 2009) have been reported. In addition, reports on disinfecting wastewater using ozone (O<sub>3</sub>) MB have discussed their strong disinfectant activities and relative long-term durability in water (Sumikura et al., 2007; Chu et al., 2007; Chu et al., 2008a; Chu et al., 2008b).

There are two types of O<sub>3</sub> MB (OMB) generators, a decompression type and a gas-water circulation type. In the former, a sufficient amount of gas is dissolved in water under a 3-4 atmospheric pressure to cause a supersaturated condition (Figure 1-A). Under such a condition, supersaturated gas is unstable and escapes from the water generating a large amount of air bubbles, which are MB. In the latter, gas is introduced into the water vortex, and the formed gas bubbles are broken into MB by breaking the vortex (Figure 1-B) (Takahashi, 2009).



**Figure 1.** Schematic diagrams used for MB generation. (A) The decompression-type MB generator, (B) the gas-water circulating-type MB generator.

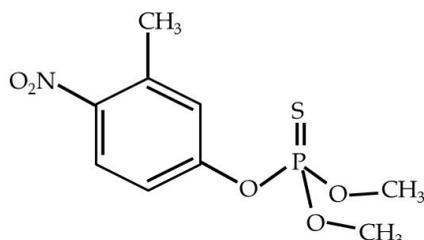
By the way, although there have been several studies on the use of O<sub>3</sub> millibubble for removing residual pesticides from vegetables and fruits, few studies have reported on the use of OMB to remove them. Therefore, since microbubbled gas is highly soluble in water and O<sub>3</sub> is a powerful decomposer of organic matter, OMB were expected to remove residual pesticides efficiently from vegetables and fruits.

No comparative studies exist on the effects of OMB generated by different methods on the removal of residual pesticides in vegetables. In this study, we examined 1) the effects of OMB generated by different methods and 2) the effects of OMB dissolved by different concentrations on the removal of pesticide (fenitrothion, FT) infiltrated into vegetables with different shapes. Since FT has been utilized widely as pesticide and acaricide, or mixture with organophosphorus agent, carbamate, pyrethroid and antibiotic in Japan, we used it in this study.

## 2. Materials and methods

### 2.1. Materials

Lettuce, cherry tomatoes and strawberries used in this study were purchased from a supermarket in Kawasaki city. A common organic phosphorous pesticide, fenitrothion, (Sumithion emulsion, 50% of MEP) and d<sub>6</sub>-fenitrothion were obtained from Sumitomo Chemical Co. Ltd. (Osaka, Japan) and Kanto Chemical Co. Ltd. (Tokyo, Japan), respectively. The structure of FT (C<sub>9</sub>H<sub>12</sub>NO<sub>5</sub>PS, MW 277.25) is shown in Figure 2. FT agent contains 50 % of *O,O*-Dimethyl-*O*-(3-methyl-4-nitrophenyl) thiophosphate, 50% of organic solvent and surfactant agent, and generally it is used by 1000-fold dilution with tap water.



**Figure 2.** Chemical structure of fenitrothion

### 2.2. Treatment with agricultural pesticide

FT was 1000-fold diluted in tap water, and three drops of a spreading agent (Haiten power, Hokko Sangyo, Co., Ltd. Tokyo, Japan) were added. The concentration of FT solution was 500 ppm. Lettuce leaves and fruits of cherry tomatoes and strawberries were immersed in this solution (60 L) for 1 min and left in a cool dark room for 24 hr to infiltrate FT into vegetables. Thereafter, they were washed in tap water for 1 min and treated with O<sub>3</sub> as follows.

## 2.3. O<sub>3</sub> treatment

### 2.3.1. Experiment 1

Forty liters of tap water was pooled in a cylindrical vessel (55 cm×32 cm i.d.) and kept at 20 °C in a room to remove chlorine in tap water for 24 hr. We confirmed that all the chlorine had been removed 24 hr later by a chlorine comparator (Photometer CL, OYWT-31, OYALOX Co., Ltd., Tokyo, Japan). OMB was generated in dechlorinated water by using a MB generator of a gas-water circulation type (FS101-L1, Fuki Co. Ltd., Saitama, Japan) or a decompression type (20NEDO4S, Shigen-Kaihatsu Co. Ltd., Kanagawa, Japan) combined with an O<sub>3</sub> generator (ED-OG-A10, Ecodesign Co. Ltd., Saitama, Japan) at a flow rate of 2.5 L/min. Under this condition, no more than 2.0 ppm of O<sub>3</sub> could be dissolved. Therefore, O<sub>3</sub> generation was stopped when the concentration of dissolved O<sub>3</sub> reached 2.0 ppm and three kinds of vegetables were immersed in the solutions for 0, 5, or 10 min. Solution temperature was kept at 20 °C in all treatments. In addition, the concentration of dissolved O<sub>3</sub> was measured with a dissolved O<sub>3</sub> meter (OZ-21P, DKK-TOA Co. Ltd., Tokyo, Japan), by shaking the electrode with 10 cm/s in solutions. Analyses were run in triplicate.

### 2.3.2. Experiment 2

O<sub>3</sub> millibubbles (OMLB) were generated in the dechlorinated water with an ED-OG-A10 O<sub>3</sub> generator at a flow rate of 2.5 L/min. The maximum amount of O<sub>3</sub> that could be dissolved under these conditions was 0.2 ppm, so O<sub>3</sub> generation was stopped when the concentration of dissolved O<sub>3</sub> reached 0.2 ppm. Then the vegetables were immersed in this solution for 0, 5, or 10 min.

OMB were generated in the dechlorinated water using a gas-water circulation type MB generator together with the ED-OG-A10 O<sub>3</sub> generator described above. Ozonated water solutions were produced containing 0.5, 1.0, or 2.0 ppm dissolved O<sub>3</sub>. Then, the vegetables were immersed in these solutions for 0, 5, or 10 min. Treatment with OMB solution containing 0.2 ppm dissolved O<sub>3</sub> was not fully tested because a preliminary experiment showed that its pesticide-removing activity was not significantly different from that of the OMLB solution.

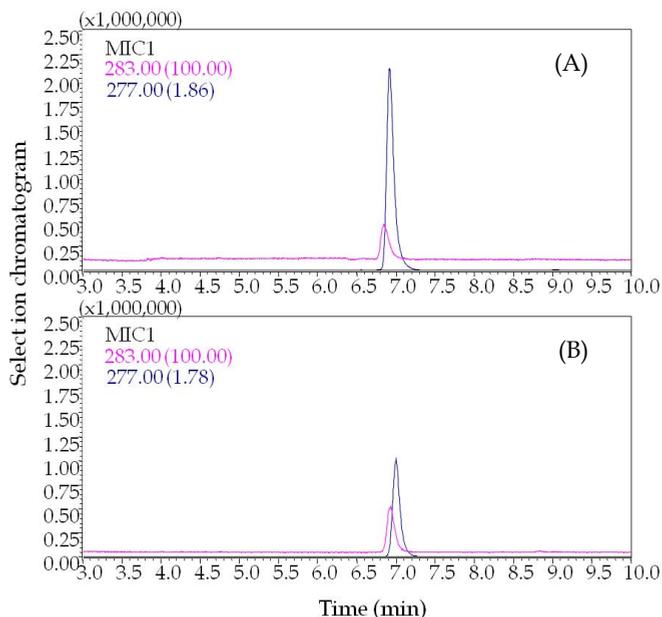
A further treatment was set up where MB were continuously generated during vegetable immersion in the ozonated solutions (bubbling OMB). In these treatments O<sub>3</sub> microbubbling was continued to maintain the concentration of dissolved O<sub>3</sub> at 2.0 ppm for 0, 5, or 10 min vegetable immersion.

A control treatment was also conducted where the vegetables were immersed in dechlorinated water. The solution temperature was maintained at 20 °C during all treatments and the concentrations of dissolved O<sub>3</sub> were measured using an OZ-21P O<sub>3</sub> analyzer with a DO<sub>3</sub> electrode. All analyses were performed in triplicate.

## 2.4. Residual pesticides analysis

Lettuce leaves or fruits of cherry tomato or strawberry (20 g) and 100 µL of 20 ppm d<sub>6</sub>-FT as internal standard were added with liquid nitrogen, homogenized for 3 min by a blender

(18000 rpm, Nissei Co. Ltd., Aichi, Japan), and then extracted by shaking in 100 mL of acetone for 30 min. After the extraction was filtered with a glass filter under reduced pressure and was evaporated until about 5 ml by a rotary evaporator, the extraction was added 5 mL of distilled water, and then was poured into a diatomite column (CHEM ELUT-20mL, UNBUFFERED, 100/PK, VARIAN Technologies Japan, Co. Ltd., Tokyo, Japan). The column was washed with 10 ml of hexane at twice, the pesticide followed by 120 ml of hexane to elute. The elution was evaporated to dryness by a rotary evaporator and refused in 2 ml of acetone, and this solution (10  $\mu$ L) was injected into Gas Chromatograph-Mass Spectrometry.

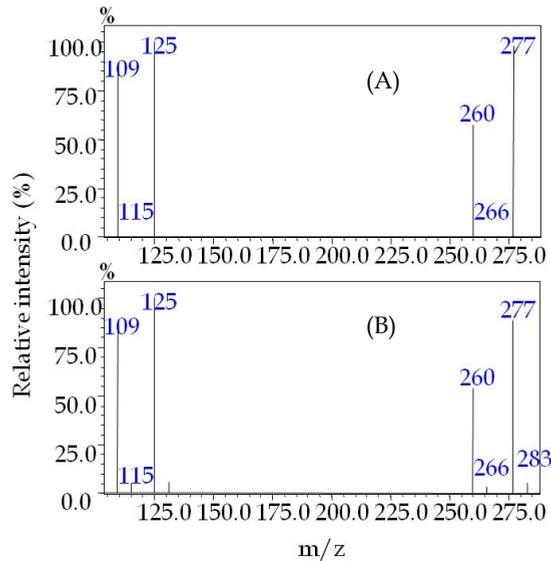


**Figure 3.** GC-chromatogram of fenitrothion and  $d_6$ -fenitrothion before and after OMB treatments. (A) before  $O_3$  treatment. (B) after  $O_3$  treatment.

A Shimadzu GC-MS QP2010 (Shimadzu Co., Ltd., Kyoto, Japan) was used for the analysis, with ionization achieved by electron impact at 70eV. The capillary column used was an Inertcap 1MS capillary column (30 m  $\times$  0.25 mm i.d.; J&W Scientific, Folsom, CA). The operating conditions were: injection port temperature, 250°C; interface temperature, 280°C; column oven temperature, 200 °C for 5 min, ramped at 1 °C /min to 215°C, followed by 20°C/min to 280°C; helium carrier gas (flow rate of 30 cm/s); 10  $\mu$ L injection volume. The split/splitless injector was operated in the splitless mode for 0.5 min after injection of the sample. The selected ion of the labeled standard ( $d_6$ -FT) was analyzed in the single ion monitoring (SIM) mode and the intensity calculated by a Shimadzu GC-MS solution. SIM of  $d_6$ -FT used the ion: for  $d_6$ - FT,  $m/z=283$ ; for FT,  $m/z=277$  were quantification. Analyses were run in triplicate. Chromatogram and mass spectra of FT and  $d_6$ -FT were shown in Figure 3 and 4, respectively.

## 2.5. Statistical analysis

Mean separation of O<sub>3</sub> concentration, residual percentage of FT and concentration of FT in vegetables between treatments were determined by Turkey-Kramer test at P < 0.05 and standard division of the mean (SD).



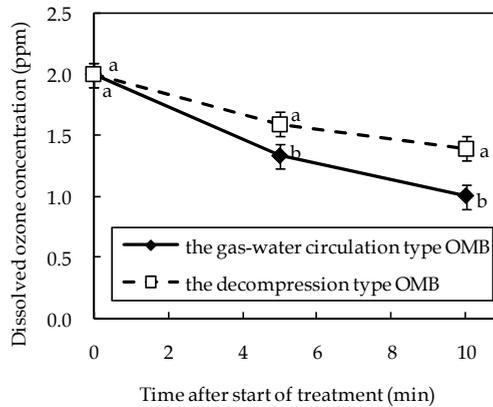
**Figure 4.** Mass spectrum of fenitrothion and d<sub>6</sub>-fenitrothion (A) fenitrothion, (B) d<sub>6</sub>-fenitrothion

## 3. Results and discussion

### 3.1. Removal of residual pesticide, fenitrothion, in vegetables by using OMB generated by different methods (Experiment 1)

Figure 5 shows the change in the concentration of dissolved O<sub>3</sub> in solutions after the start of OMB treatments with the gas-water circulation type and the decompression type. In both solutions, measured in the absence of vegetables, the concentration of dissolved O<sub>3</sub> decreased gradually with time, and the concentration at 5 and 10 min after the start of OMB treatments was 1.3 and 1.0 ppm in the gas-water circulation type solution, and 1.6 and 1.4 ppm in the decompression type solution, respectively. Thus, the concentration of dissolved O<sub>3</sub> was kept higher in the decompression type solution than in the gas-water circulation type solution. The half-life of dissolved O<sub>3</sub> by using an air pump is reported to be 2.27 min in tap water at 25°C (Dhillon et al., 2009). That by using the gas-water circulation type was about 10 min and that by using the decompression type was much longer, though the solution temperature was 20°C in this study.

Figure 6 shows the residual percentage of FT in lettuce (A), cherry tomatoes (B) and strawberries (C) at 5 and 10 min after the immersion into the solutions of OMB treatments



**Figure 5.** Change in the  $\text{DO}_3$  concentrations after OMB treatments by using the gas-water circulating-type and the decompression-type.

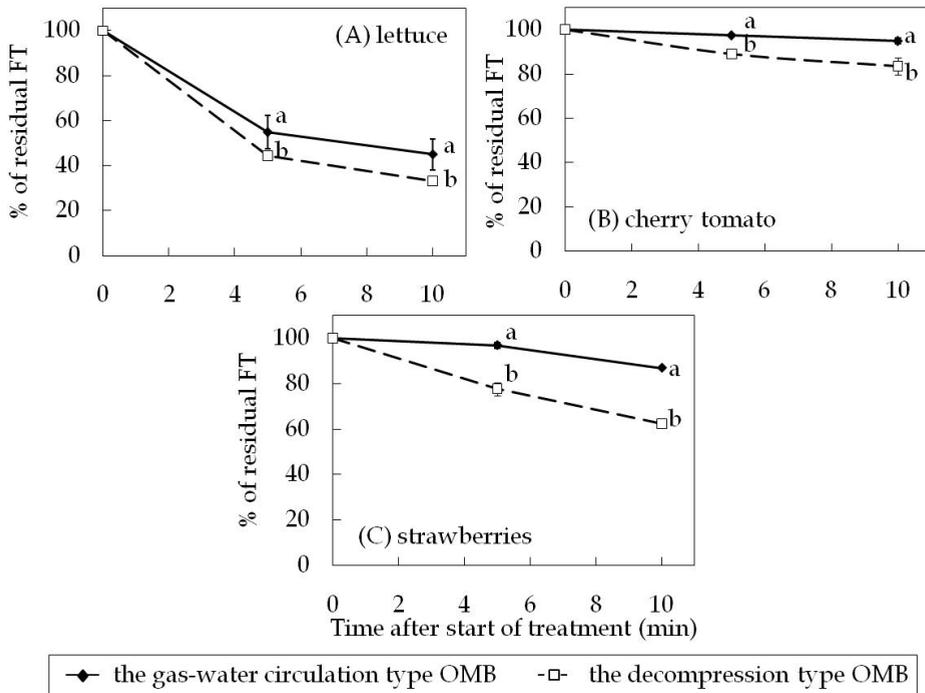
Vertical bars represent the standard division of the mean ( $n=3$ ).

Different letters indicate a difference significant at the 5% level by Turkey-Kramer test between treatments.

by using the gas-water circulation type and the decompression type. In lettuce, the concentration of residual FT after washing in water was 212.21 ppm (Table 1), and the concentration rapidly decreased after the start of both the treatments of the decompression type and the gas-water circulation type, reaching 44 and 55% at 5 min, and 33 and 45% at 10 min, respectively. Thus, in lettuce, both the treatments of the decompression type and the gas-water circulation type removed residual FT effectively, and the decompression type was more effective than the gas-water circulation type. The dissolved  $\text{O}_3$  in the solutions of OMB treatments generates hydroxyl radicals that are highly effective at decomposing organic molecules like the residual FT (Sumikura et al, 2007; Takahashi et al., 2007b), and hydroxyl radicals are generated by the collapse of OMB in solutions (Chu et al., 2008a). The decompression type would have generated a high enough concentration of dissolved  $\text{O}_3$  to produce a large amount of hydroxyl radicals. However, the gas-water circulation type was lower effective than the decomposing type, because the concentration of dissolved  $\text{O}_3$  was lower and fewer hydroxyl radicals would have been generated.

In cherry tomatoes, the concentration of FT after washing with water was 3.02 ppm (Table 1), and the residual FT percentage at 5 min after the start of OMB treatments of the decompression type and the gas-water circulation type was 89 and 97%, respectively, showing a low pesticide-removing effect. At 10 min after the start of OMB treatments, it was 84 and 95%, respectively. Thus, the decompression type was slightly more effective than the gas-water circulation type. The most likely explanation for the lower reduction of residual FT in cherry tomatoes is that the dissolved  $\text{O}_3$  and hydroxyl radicals could not penetrate through the thick pericarp of the cherry tomatoes and not reach the sarcocarp, and were inactivated by contact with the pericarp.

In strawberries, the concentration of FT after washing with water was 37.80 ppm (Table 1), and the residual percentage of FT at 5min after the start of OMB treatments of the



**Figure 6.** Residual fenitrothion percentages for lettuce (A), cherry tomatoes (B), and strawberries (C) after immersion in solutions containing OMB generated by using the gas-water circulating-type and the decompression-type.

Vertical bars represent the standard division of the mean (n=3).

Different letters indicate a difference significant at the 5% level by Turkey-Kramer test between treatments.

decompression type and the gas-water circulation type was 78 and 97%, respectively. That at 10 min after the start of OMB treatments of the decompression type and the gas-water circulation type was 62 and 87%, respectively, showing that the pesticide could be removed effectively by using the decompression type. The amount of FT removed in strawberries was higher than that in cherry tomatoes at both types of OMB generators. We think that strawberries have a rougher surface and larger surface area than cherry tomatoes and then can contact with O<sub>3</sub> efficiently, removing FT easily in the sarcocarp.

The decompression type had a high FT-removing effect on all vegetables examined even though the initial concentration of dissolved O<sub>3</sub> was 2.0 ppm. The difference in the pesticide-removing effect between the decompression type and the gas-water circulation type may be caused by the difference in the size and the number of the bubbles (Takahashi, 2009). The diameter of the MB generated using the decompression type shows about 10 μm, and the number of bubbles smaller than 50 μm in diameter amounted to several thousand per ml (Takahashi et al., 2007a). On the other hand, the diameter of the MB generated using the gas-water circulation type shows about 40 μm, and the number of MB smaller than 50 μm in

	Time	Concentration (ppm)				Coefficient of variation (%)	
		The gas-water circulation type OMB	± SD	The decompression type OMB	± SD	The gas-water circulation type OMB	The decompression type OMB
lettuce	before <sup>z</sup>	346.64	± 5.75	346.64	± 5.75	1.66	1.66
	0	212.21	± 31.24	212.21	± 31.24	14.72	14.72
	5	117.77	± 4.30	95.11	± 1.96	3.65	2.06
	10	96.50	± 0.79	92.75	± 3.93	0.82	4.24
tomatoes	before	3.41	± 0.14	3.41	± 0.14	4.05	4.05
	0	3.02	± 0.17	3.02	± 0.17	5.70	5.70
	5	2.75	± 0.04	2.70	± 0.11	1.50	4.08
	10	2.75	± 0.12	2.53	± 0.23	4.25	9.00
Straw-berries	before	43.50	± 2.12	43.50	± 2.12	4.88	4.88
	0	37.80	± 4.58	37.80	± 4.58	12.11	12.11
	5	34.80	± 4.12	29.35	± 2.18	11.84	7.44
	10	32.25	± 3.07	23.59	± 0.15	9.53	0.62

<sup>z</sup> before washing in tap water.

**Table 1.** Concentration of residual FT for lettuce, cherry tomatoes, and strawberries after immersion in solutions containing OMB generated by using the gas-water circulating-type and the decompression-type.

diameter amounted to several hundred per ml, which is less than that of the decompression type (Takahashi et al., 2003). These findings show that the decompression type had a strong pesticide-removing effect, which could be attributed to the larger number of small OMB that could more easily infiltrate into the vegetables than the gas-water circulation type. There have been no reports on the effects of OMB generated by different methods on the removal of residual pesticides in vegetables. This is the first report showing the pesticide-removing effect of OMB with the different methods of generation. In this study, we tested that whether vegetable containing in high concentration of pesticide was removed or not, and so we confirmed that vegetables were removed efficiently by treatment with the OMB. In near future, we should be attempted to confirm safety of vegetable treated by OMB.

### 3.2. Removal of residual pesticides in vegetables using OMB dissolved by different concentration (Experiment 2)

Experiment 1 was conducted, in the absence of any vegetables, to determine how dissolved O<sub>3</sub> concentrations changed in the ozonated water solutions over time at 20°C. Figure 7

shows how the concentration of dissolved  $O_3$  changed over a 10 minute period (the maximum length of subsequent vegetable treatments), once the ozonated water solutions had been prepared. Over the 10 min period the concentration of dissolved  $O_3$  in the MB solution with a starting concentration of 2.0 ppm (2.0 ppm OMB solution) decreased steadily to 1.0 ppm. Similarly, the concentrations of dissolved  $O_3$  in the 0.5 and 1.0 ppm OMB solutions, and the 0.2 ppm OMLB solution also decreased steadily, with all dissolved  $O_3$  lost from the 0.5 ppm OMB and 0.2 ppm OMLB solutions within 10 minutes.

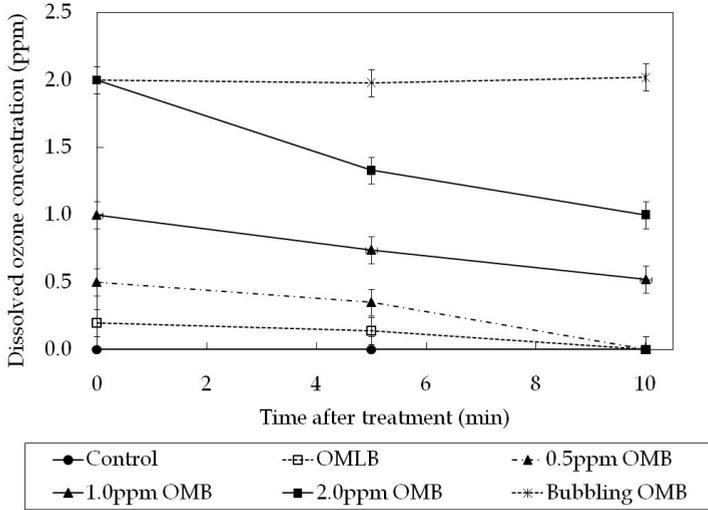
Figure 8 shows the reduction in residual FT in lettuce treated with the OMB and OMLB solutions. Before treatment with OMLB or OMB solutions, but after washing with tap water, the concentration of residual FT in lettuce was 212.2 ppm (data not shown). The amount of residual FT decreased with increasing treatment time and dissolved  $O_3$  concentration. The residual FT in lettuce was reduced to 67%, 55% and 45% after 5 minutes treatment with the 1.0 ppm OMB, 2.0 ppm OMB, and 2.0 ppm bubbling OMB solutions, respectively. After 10 minutes treatment the respective amounts of residual FT had been further reduced to 49%, 45% and 42%. The similarly high reductions in residual FT achieved with the 1.0 ppm OMB, 2.0 ppm OMB, and 2.0 ppm bubbling OMB, indicates that immersion of lettuce in an OMB solution containing 1.0 ppm or more dissolved  $O_3$  may be sufficient to effectively remove residual FT from the lettuce, possibly because lettuce has thin leaves. In contrast, after 10 minutes treatment with the OMLB and 0.5 ppm OMB solutions the residual FT had only been reduced to 87% and 78%, respectively.

The dissolved  $O_3$  in the OMB treatment solutions generates hydroxyl radicals that are highly effective at decomposing organic molecules like the residual FT (Sumikura et al., 2007; Takahashi et al., 2007b). Hydroxyl radicals are generated by the collapse of OMB in solution, and so the 1.0 ppm OMB, 2.0 ppm OMB and bubbling OMB solutions would have had a high enough concentration of dissolved  $O_3$  to produce a large amount of hydroxyl radicals. However, the OMLB treatment was not nearly so effective because the concentration of dissolved  $O_3$  was much lower and so far fewer hydroxyl radicals would have been generated.

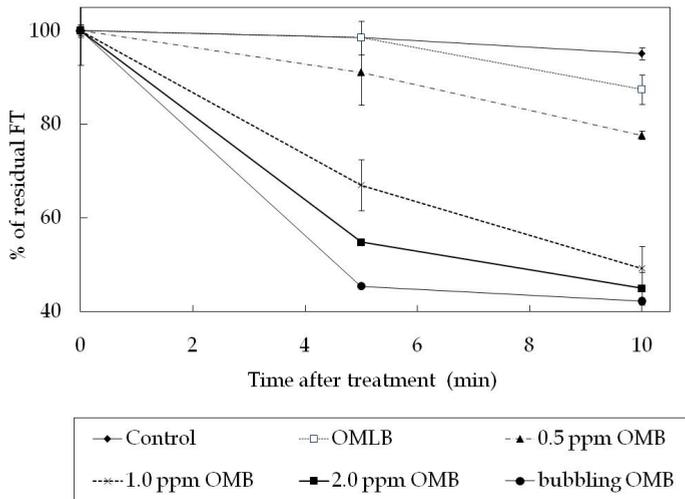
Figure 9 shows the reduction of residual FT in cherry tomatoes for each treatment. The starting concentration of residual FT in the cherry tomatoes was 3.0 ppm (data not shown), prior to  $O_3$  solution treatments. Removal of residual FT by the various treatment solutions was much less in the cherry tomatoes than in the lettuce. After 10 minutes treatment residual FT had been reduced to 65% in 2.0 ppm bubbling OMB solution, but remained at >90% for all other treatments. The most likely explanation for the lower reduction of residual FT in the cherry tomatoes is that the dissolved  $O_3$  and hydroxyl radicals could not penetrate through the thick pericarp of the tomatoes and to reach the sarcocarp, and were inactivated by contact with the pericarp. The greater effectiveness of the bubbling OMB solution was probably because the concentration of dissolved  $O_3$  remained high and so hydroxyl radicals continued to be generated throughout the treatment.

Figure 10 shows the reductions in residual FT in strawberries for each treatment. The starting concentration of residual FT in strawberries was 37.8 ppm (data not shown). After 10 min of

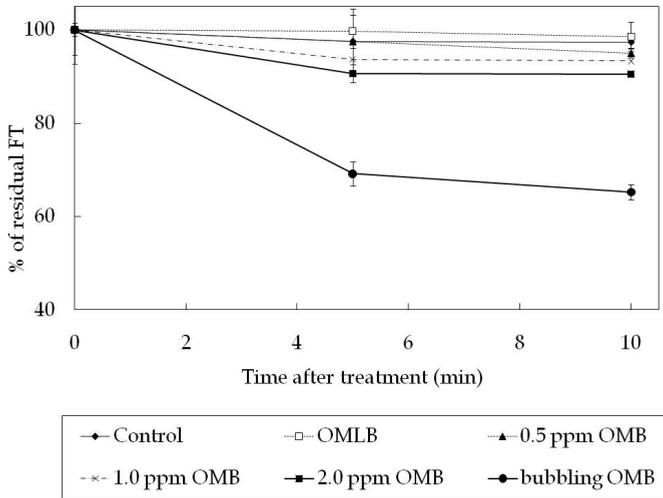
treatment, the greatest reduction in residual FT was in the 2.0 ppm bubbling OMB treatment where 75% residual FT remained. The other treatments ranged from 85% residual FT remaining in the 2.0 OMB treatment to 91% in the OMLB treatment. The amount of FT that



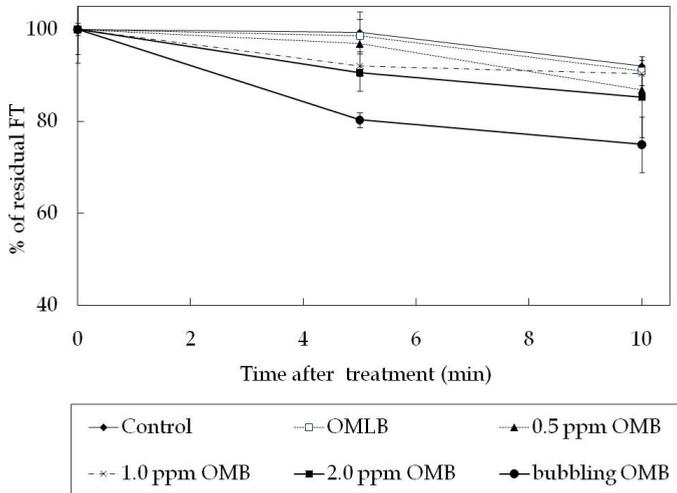
**Figure 7.** Change in the concentration of dissolved O<sub>3</sub> after the start of the O<sub>3</sub> treatments, in the absence of vegetables. Vertical bars represent one standard deviation of the mean.



**Figure 8.** Change in the residual FT in lettuce treated with the OMB and OMLB solutions. Vertical bars represent the standard deviation of the mean.



**Figure 9.** Change in the residual FT in cherry tomatoes treated with the OMB and OMLB solutions. Vertical bars represent the standard deviation of the mean.



**Figure 10.** Change in the residual FT in strawberries treated with the OMB and OMLB solutions. Vertical bars represent the standard deviation of the mean.

was removed with the 2.0 ppm bubbling OMB solution was lower than that in the cherry tomatoes. Strawberries have a rougher surface and larger surface area than cherry tomatoes and this may cause O<sub>3</sub> and hydroxyl radicals to lose their specific activity upon contact with the surface of strawberries, preventing them from removing FT in the sarcocarp.

There have been several studies on the decomposition of pesticides by O<sub>3</sub> treatment (Daidai et al., 2007; Hwang et al., 2001a; Hwang et al., 2001b; Hwang et al., 2002; Karaca and Velioglu, 2007; Ong et al., 1995; Wu et al., 2007a). For example, it was reported that 140 ppm FT was completely decomposed within 40 min in 13% ozonated solution produced by millibubbling (Tanaka et al., 1992). Another study reported that 53% of diazinon, 55% of parathion, 47% of methyl parathion, and 61% of cypermethrin were removed from the brassicaceous vegetable "Pakchoi" (*Brassica campestris* L. ssp. *chinensis* Makino) treated with 0.1 ppm of these pesticides and then immersed in ozonated solution containing 2.0 ppm dissolved O<sub>3</sub> for 30 min (Wu et al., 2007b). A further study demonstrated that 2.0 ppm residual captan, azinphos-methyl, and formetanate HCl on the surface of apples after harvest were reduced effectively by immersing them in 0.25 ppm O<sub>3</sub>-millibubbled solution for 30 min (Ong et al., 2007a). Although these studies show that residual pesticides can be removed from vegetables and fruits by immersion in ozonated solution, prior to our study there had not been any reports on using these techniques to remove residual pesticides from fruity vegetables such as tomatoes and strawberries. Interestingly, one report showed that 11 kinds of pesticides (alachlor, atrazine, bentazon, butylate, carbofuran, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion, metolachlor, metribuzin, and trifluralin) could be removed by O<sub>3</sub> generation (454 g O<sub>3</sub>/day) and UV irradiation, but several hours of treatment were necessary (Philip et al., 1987).

Clearly, residual pesticides in leafy vegetables can be removed by immersion in ozonated solution, but the concentrations of residual pesticide in the earlier studies were low. In the present study, high concentration of residual FT founded in lettuce (>200 ppm) could be reduced to less than 100 ppm in 5–10 min by treatment with 1.0–2.0 ppm OMB solution. Such a large reduction may be possible because the chemical structure of FT is similar to diazinon, which can be easily decomposed by hydroxyl radicals (Kouloumbos et al., 2003), and so the oxidative powers of O<sub>3</sub> and hydroxyl radicals may act in concert to effectively degrade FT. This effective joint action was only possible in the MB generated solutions because the millibubble generated solutions could not achieve high enough dissolved O<sub>3</sub> concentrations and not generate hydroxyl radicals.

This study showed that OMB can remove high concentrations of residual pesticides within a short time from not only leafy vegetables but also fruity vegetables. Thus, OMB could be useful for removing residual pesticides from a wide range of vegetables. In near future, we should be attempted to confirm the quality and safety of vegetable and fruits treated by OMB.

#### 4. Conclusion

The effectiveness of OMB for removal of residual pesticides varies with the methods of the OMB generation. The decompression type was more effective than the gas-water circulation type on removing the residual pesticide in vegetables, which could be attributed to the larger number of small OMB that could more easily infiltrate into vegetables than the gas-water circulation type.

OMB quickly and effectively removed high concentrations of residual FT from lettuce. In addition, continuously bubbled OMB effectively removed residual FT from fruity vegetables with a thick pericarp and sarcocarp, such as cherry tomatoes and strawberries. Unlike millibubbles, MB allow O<sub>3</sub>, which is highly insoluble in water, to be easily dissolved in water at high concentrations. As a result, OMB solutions are more effective than OMLB solutions at removing residual pesticides from vegetables because the OMB solutions combine the oxidative power of O<sub>3</sub> with the generation of hydroxyl radicals from the collapsing OMB.

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## Acknowledgement

This work was supported by grant-aid for scientific research (C) (21580407) from Japan Society for the Promotion of Science.

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