International Food Research Journal 24(3): 1135-1139 (June 2017)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of ozone microbubbles and ultrasonic irradiation on pesticide detoxification in tangerine cv. Sai Nam Pung

[1,2*]Whangchai, K., [1,2]Uthaibutra, J., [2]Nuanaon, N. and [3]Aoyagi, H.

[1]*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200*
[2]*Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai 50200/ Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400*
[3]*Life Science and Bioengineering, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan*

Article history

Received: 2 June 2016
Received in revised form: 28 June 2016
Accepted: 30 June 2016

Keywords

Ozone microbubbles
Ultrasonic
Ethion
Residue

Abstract

Ethion is an organophosphate pesticide which is widely used in tangerine cultivation. This research was conducted as an investigation on the efficiency of ozone microbubbles and ultrasonic irradiation in combination (OMB/US) on ethion residue reduction in tangerine cv. Sai Nam Pung. Ethion standard (10 ppm) treated with OMB/US was compared with individual ozone microbubbles (OMB) and ultrasonic frequency1000 kHz (US) at various time intervals (15, 30, 45, and 60 min). It was observed that the ethion concentration of treated samples had significantly decreased in direct proportion to the reaction time, as compared with the control. The analysis was carried out to find out which OMB/US treatment showed lower case heading/subheading the most effectiveness on ethion degradation over OMB or US alone. The rate constant (k) of OMB/US was revealed as 0.79×10^{-2} min^{-1}, which is the maximum value obtained of all the treatments. The detoxification levels of OMB/US, OMB, and US were determined using the brine shrimp lethality test method. OMB/US showed the maximum level of LC_{50}, at 281.43 mgL^{-1}, when compared the control which have LC_{50} at 5.41 mgL^{-1}, the ethion residue on tangerine fruit was treated by OMB/US, the same as the previous method. The results showed that the percentage of ethion degradation had increased, which was in addition to the rise in the reaction time. OMB/US showed the highest rate of reduction as occurring within 15 min. It was observed that ethion had reduced by 73% after OMB/US treatment for 60 min reaction time. Thereafter, tangerine fruits were stored at 5°C for 35 days to determine quality changes. The peel color change, percentage of weight loss, TSS, TA, and ascorbic acid content after all the treatments were found to be not affected by OMB/US.

Introduction

Ethion is a well-known insecticide that belongs to the organophosphate group. Its toxic content inhibits cholinesterase enzyme in the insect nervous system. Ethion is widely used to control pest invasion on tangerine cultivation, especially tangerine *(Citrus reticulata)* cv. Sai Nam Pung, one of the most popular tangerine cultivars in northern Thailand. Although ethion is effective in the controlling of pest invasion and diseases, it also has the effect of leaving pesticide residue in the tangerine fruit and in the environment. Krangkrai *et al*. (2009) reported that ethion residue was found to be more than 1.0 $mgkg^{-1}$ in the tangerine fruit sold on shelf, and this is well over the maximum residue limits (MRLs). This leads to major concerns among consumers regarding the safety of the fruit, and it poses a big problem for tangerine trading. To solve this problem, there were many alternative solutions chosen, including oxidative technology such as ozone microbubbles and ultrasonics. These technological methods have high efficiency as regards chemical degradation.

The procedure using ozone microbubbles is a technology that transforms ozone gas into small-sized bubbles (of sizes less than 10 µm) in water. These small bubbles increase the dissolving potential and expand the oxidizing efficiency of ozone, which help to collapse the pesticide structure. Takahashi *et al.* (2007) reported that the ozone microbubbles technique generated more hydroxyl radicals in water. This led to the degradation of polyvinyl alcohol and a reduction in fenitrothion residue in lettuce, cherry tomato, and strawberry (Ikeura *et al*., 2011). Ultrasonic irradiation is a technology that helps to decrease organic and inorganic residues (Weavers *et al*., 1998). Sonolysis of ultrasonic irradiation leads to the production of radical species such as •H, •OH, and •OOH, which directly destroy the chemical structure.

The combination of ozone microbubble

*Corresponding author.
Email: *kanda.w@cmu.ac.th*
Tel: 66-5394-3346-60 Ext. 1204.

technology and ultrasonic irradiation is more effective on chemical pollutant degradation. Zhang *et al.* (2006b) reported that ozone in combination with ultrasonic irradiation increased methyl orange destruction. Additionally, ozone and ultrasonic in combination has been treated to reduce biological toxicity and increase biodegradability in heterocyclic pesticide wastewater (Xiong *et al.*, 2011). The toxicity of the by products from pesticide degradation using OMB/US should be evaluated. Bioassay can be used to determine toxicity by the estimation of LC_{50} which have been reported for toxins, pesticides and other contaminants (Kanwar, 2007). Therefore, the objective of this research is to study the effect of ozone microbubbles and ultrasonic irradiation on the degradation of ethion standard and the reduction of ethion residue in tangerine cv. Sai Nam Pung.

Materials and Methods

Reduction and detoxification of ethion standard by ozone microbubbles and ultrasonic reduction of ethion standard

Reference standard ethion was purchased from Sigma-Aldrich Laborchemikalien GmbH (Stienheim, Germany) with 99.9% purity. Ethion stock solution (1000 mgL^{-1}) was prepared by dissolving standard ethion in 99.8% acetone. The solution was diluted with distilled water to the appropriate concentrations.

The systematic diagram of ozone microbubbles in combination with ultrasonic irradiation was prepared. Ozone microbubbles (OMB) were generated using an ozone generator (OZONIZER: 200 mgL^{-1}) connected to the circulatory water pump. OMB water came out from the microbubble nozzle and circulated in the ultrasonic bath. The ultrasonic device size 44 x 51 x 35 cm. equipped with eight transducers and ultrasonic frequency at 1 MHz 24 W.

The ethion solution (10 mgL^{-1}) was placed in the bath while the combination of ozone microbubbles with ultrasonic irradiation (OMB/US) was being generated. Sample solutions were taken every 15 min of reaction time for 60 min to determine the reduction percentage of the pesticide residue by gas chromatography. In addition, ethion solutions were treated using the same method but with only the OMB system (OMB), only the ultrasonic system (US), and the control (that is, using neither OMB nor US). The values of $\ln(C_t/C_o)$ and the rate constant *(k)* were calculated to report the efficiency of the OMB/US combination, in comparison with the OMB and the US alone.

Toxicity test of degraded ethion after OMB and US treatment by bioassay method.

The levels of detoxification of the ethion standard in all the treatments (OMB, US, and OMB/US) were determined by brine shrimp lethality test method. Diluted solutions with 0 ppm, 0.1 ppm, 10 ppm, and 100 ppm ethion were prepared in laboratory marine water for the OMB, US, and OMB/US treatments. Ten brine shrimps nauplii were placed in each vial containing 5 ml of treated ethion solution. The mortality of the brine shrimps nauplii in each treatment was checked every 2 hours for 24 hours of incubation. The percent mortality was determined 18 hours after application. Probit analysis in SPSS version 17 was used for the calculation of the LC_{50} value.

Ozone microbubbles and ultrasonic irradiation treatment on ethion residue reduction in tangerine cv. Sai Nam Pung and its quality changes during storage ehion residue reduction in tangerine cv. Sai Nam Pung

Tangerine (cv. Sai Nam Pung) fruits were purchased from an organic agriculture farm in Fang district, Chiang Mai, Thailand. The fruits were cleaned and the ones that are uniformly shaped and lesion free were selected. Each fruit sample was dipped into 10 ppm ethion solution for 30 min, and then left to dry at room temperature. Thereafter, the fruits were washed in the sample bath that was connected to ozone microbubbles in combination with ultrasonic irradiation. The fruit samples were collected at washing times of 15 min, 30 min, 45 min, and 60 min to determine the reduction percentage of pesticide residue by gas chromatography.

Quality changes during storage

Tangerine fruits were washed in the bathtub that was connected to ozone microbubbles in combination with ultrasonic, in the same way as the previous experiment. Fruit samples were collected at 15 min, 30 min, 45 min, and 60 min washing time, and then were kept in sealed PE bags. All the treatment samples were stored at 5°C for 21 days. The fruits were selected every 7 days to measure the quality changes during storage. The percentage of weight loss, peel color change, total soluble solids (TSS) content, titratable acidity (TA), and ascorbic acid content (AOAC) were measured.

Statistic analysis

All the experiments were replicated three times and evaluated with regression procedure, using SPSS version 17 for analysis of variances (ANOVA). The differences between the treatments were analyzed for significance, using Duncan Multiple Range Test ($p^{*}<0.05$).

Results and Discussion

Reduction and detoxification of ethion standard by ozone microbubbles and ultrasonic irradiation

The concentration of ethion was found to significantly decrease in all treatments, which was in direct proportion to the increasing of the reaction times (Figure 1). Moreover the OMB/US treatment for 60 min was the most effective reducing ehtion concentration. The plot of $\ln(C_t/C_o)$ versus contact time (t) is shown in Figure 2. The values of the first order rate constant, k, of ethion under different treatments are summarized. The rate constants varied from 0.23×10^{-2} to 0.79×10^{-2} min^{-1}. The degradation kinetics of ethion (k) was related with ethion degradation, the highest k value was obtained 0.79 x10^{-1}m^{-1} when using OMB/US combination. These values demonstrate the synergistic efficiency of the OMB/US combination on the degradation of the ethion concentration over US or OMB alone. The synergistic effect of OMB and US increased the water temperature during the reaction period, which resulted in the increase in the degradation of the methyl orange dye pollutant (Zhang *et al*., 2006b). According to Matouq *et al*., (2008), kinetic modeling applied for the obtained results should that the derivative of diazinon by US were followed a pseudo-first model with rate constant around of 0.01s^{-1}. Also, the combination process of the two technologies certainly helped to enhance the oxidizing power of ozone during the reaction. Moreover, ultrasonic irradiation individually increased the potential of ozone in water dissolution (Zhang *et al*., 2006a). This was the cause of the degradation of malachite green, which was as a result of the ultrasonic irradiation increasing the dissolving potential of ozone in water (Zhou *et al*., 2013).

The toxicity values of the ethion in all the treatments were revealed by LC_{50}. While the OMB and the US treatments increased the LC_{50} value to 9.53 mgL^{-1} and 38.18 mgL^{-1}, respectively. Toxicity was slightly reduced after OMB US and the combination treatments as revealed by LC_{50} value especially, the combination treatment which increased from 5.41 to 281.43 mgL^{-1} and significantly different from other treatments (Figure 3). The toxicity evaluation indicated that the toxicity of ethion decreased after the OMB/US treatment. This showed the high efficiency of the OMB/US combination on ethion standard degradation and detoxification. Similarly,

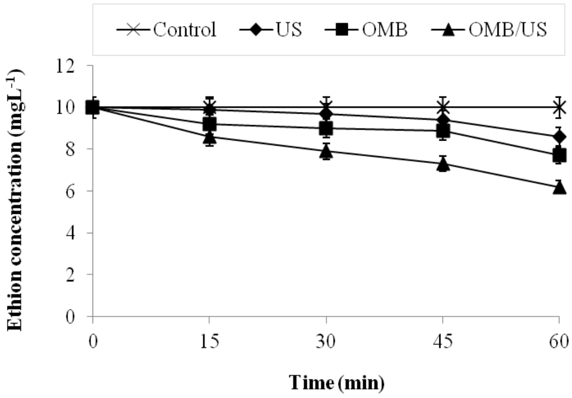

Figure 1. The ethion concentrations after treatment with US and OMB, showing statistically significant degradation ($p<0.005$). The values are expressed as mean ± S.E. of measurements made on three replicates of the treatment

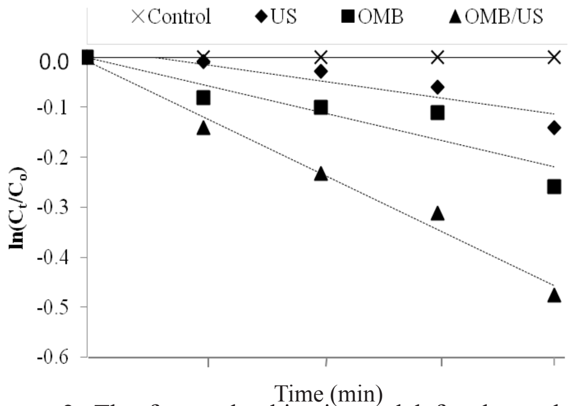

Figure 2. The first order kinetic model fitted to ethion degradation by ozone microbubbles combined with ultrasonic irradiation treatments

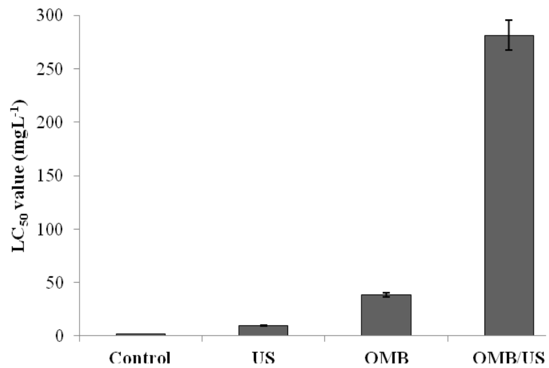

Figure 3. The LC_{50} value to the brine shrimp (*Artemia salina* L.) toxicity of ethion after treatment with US and OMB

Pengphol *et al.* (2012a) reported that the US and ozone in combination had a synergistic effect in reducing chlorpyrifos concentration as well as in lowering the toxicity of chlopyrifos, as determined using the shrimps nauplii bioassay.

Table 1. Changes in Peel Color, Weight Loss, TSS, TA, and Ascorbic Content of Tangerine Fruits after Washing with Microbubble Ozone in Combination with Ultrasonic Irradiation (OMB/US) at 15, 30, 45, and 60 min, and Storage at 5°C for 21 Days

Treatment	Peel Color Change			Weight loss (%)	TSS (%)	TA (%)	Ascorbic Content (mg/ml)
	L*	Chroma	Hue				
Control	27.68a	10.87	178.81a	12.50a	11.00a	0.09ab	1.10a
OMB/US 15 min.	27.94a	10.85a	178.80a	12.30a	10.76a	0.07a	1.18a
OMB/US 30 min.	28.20a	11.44a	178.78a	11.89a	11.38a	0.09ab	1.20a
OMB/US 45 min.	27.87a	11.13a	187.80a	12.03a	10.87a	0.09ab	1.10a
OMB/US 60 min.	27.86a	11.34a	178.84a	11.38a	11.63a	0.07a	1.11a

Note: The data followed by the same letter within the column are not significantly different ($p^* < 0.05$)

Ozone microbubbles and ultrasonic irradiation on ethion residue reduction in tangerine cv. Sai Nam Pung and its quality changes during storage

The ethion residue on tangerine fruit is related to the treatment period with the OMB/US combination at different times. The results showed that the percentage of ethion degradation increased in proportion to the rise in the reaction time. The OMB/US combination showed a high rate of ethion reduction occurring within 15 min, at 42%. ethion was reduced by 73% after the OMB/US combination treatment for 60 min. (Figure 4). The combination between ozone microbubbles and ultrasonic irradiation showed the synergistic effect of both the technologies. Ultrasonic irradiation would help ozone to get better dissolved in water and expand the oxidizing power by increasing hydroxyl radical production. Zhang *et al.* (2009) reported that the increasing of chemical degradation during ozone and ultrasonic reaction was effected by their synergistic power. The reaction of ozone in combination with continuous ultrasonic irradiation resulted not just in the increasing of ozone dissolution but also in the enhancing of hydroxyl radical production (Yue *et al.*, 2008). This is important as far as ethion residue reduction in tangerine fruit as well as chemical degradation on water treatment are concerned (Xiong *et al.*, 2011). Gong *et al.* (2011) found that ozone in combination with ultrasonic irradiation had higher effectiveness on malathion, diphenylamine, carbendazim, and chlorothalonil residue reduction in apple fruits rather than individual ozone treatment or ultrasonic irradiation. Similary, Pengphol *et al.* (2012b) reported that ultrasonic 1000 kHz together with ozone for 60 min could reduce 73.05% of chlorpyrifos residue in bird chili.

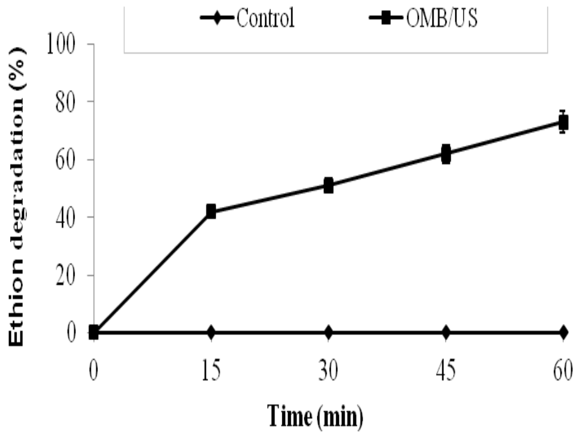

Figure 4. The percentage of ethion degradation in tangerine fruit after washing with ozone microbubbles in combination with ultrasonic irradiation (OMB/US)

The quality changes in the tangerine fruits after washing with the OMB/US treatment, and then storing at 5°C after 21 days are summarized and shown in Table 1. None of the treatments had significant differences as regards fruit quality changes during storage at 5°C for 21 days. The peel color changes in all the treatments were measured by L*, Chroma and Hue values. All the treatments demonstrated slight decreases in the L* and Chroma values. However, there were no significant changes observed in the peel color, weight loss, TSS, TA and ascorbic acid content in all the treatments during the storage. The low temperate obviously delayed the weight loss during storage in all the treatments. Tangerine is a non-climacteric fruit, so most of the chemical changes as regards TSS, TA, and ascorbic acid content occur during the preharvest period.

Conclusion

Ozone microbubbles and ultrasonic irradiation in combination (OMB/US) had more effectiveness on the ethion standard degradation than individual ozone treatment or ultrasonic irradiation. OMB/US combination treatment for the 60 min reaction time showed the highest rate of ethion degradation. The LC_{50} value of the OMB/US combination showed a similar relation between ethion detoxification and ethion degradation. The efficiency of the OMB/US combination as regards the reduction of ethion residue in tangerine cv. Sai Nam Pung is related directly to the increase in the reaction time. The best result in ethion residue reduction in tangerine was obtained in the combined treatment with ozone microbubbles and ultrasonic irradiation for 60 min. This was not observed to have any effect on the quality changes of the tangerine fruit during storage at 5°C for 35 days.

Acknowledgments

This work has been supported by Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok, and Postharvest Technology Research Institute and Department of Biology, Faculty of Science, Chiang Mai University.

References

Gong, J., Li, Z., Zhong, H., Tang, J. and Li, Y. 2011.Ozone/ultrasound degradation effect on residual pesticides in commercially available apples. 2011 5th International conference on Bioinformatics and Biomedical Engineering (ICBBE 2011), Wuhan, China, 10-12 May, 2011, IEEE. pp. 5179-5182.

Ikeura, H., Kobayashi, F. and Tamaki, M. 2011. Removal of residual pesticide, fenitrothion, in vegetables by using ozone microbubbles generated by different methods. Journal of Food Engineering 103: 345-349.

Kanwar, A.S. 2007. Brine shrimp *(Artemia salina)* a marine animal for simple and rapid bioassay. Journal of Chinese Clinical Medicine 2: 236-240.

Krangkrai, J., Sarut, S., Srijamnam, S., Srikacha, S., Butsabong, M., Manus, M., Wipada, P. and Wanapon, W. 2009. Pest and Pesticide on Tangerine In Proceeding of the Production Tangerine for Safety the Environment Conference,Chiangmai, Thailand.

Matouq, M.A., Al-Arber, Z.A., Tagawa, T., Alibour, S. and Al-Shannag, M. 2008. Degradation of dissolved diazinon pesticide in water using high frequency of ultrasound wave. Ultrasonic Sonochemistry 15: 869-874.

Pengphol, S., Uthaibutra, J., Arquero, O.A., Nomura, N. and Whangchai, K. 2012a. Oxidative degradation and detoxification of chlorpyrifos by ultrasonic and ozone treatments. Journal of Agricultural Science 4(4): 164-172.

Pengphol, S., Uthaibutra, J., Arquero, O.A., Nomura, N. and Whangchai, K. 2012b. Reduction of residual chlopyrifos on harvested bird chilies (*Capsicum frutescenes* Linn.) using ultrasonic and ozonation. Thai Journal of Agricultural Science 44(5): 182-187.

Takahashi, M., Chiba, K. and Li, P. 2007. Formation of hydroxyl radicals by collapsing ozone microbubbles under strong acid conditions. Journal of Physical Chemistry 111: 11443-11446.

Weavers, L. K., Ling, F. H. and Hoffmann, M. R. 1998. Aromatic compound degradation in water using a combination of sonolysis and ozonolysis. Environmental Science and Technology 32: 2727-2733.

Xiong, Z., Cheng, X. and Sun, D. 2011. Pretreatment of heterocyclic pesticide wastewater using ultrasonic/ozone combined process. Journal of Environmental Science 23: 725-730.

Yue, W., Yao, P., Wei, Y. and Mo, H. 2008. Synergetic effect of ozone and ultrasonic radiation on degradation of chitosan. Polymer Degradation Stability 93:1814-1821.

Zhang, H., Duan, L. and Zhang, D. 2006a. Absorbtion kinetics of ozone in water with ultrasonic radiation. Ultrasonics Sonochemistry 14: 552-556.

Zhang, H., Duan, L. and Zhang, D. 2006b. Decolorization of methyl orange by ozonation in combination with ultrasonic irradiation. Journal of Hazardous Materials B138: 53-59.

Zhang, Y., Xiao, Z., Chen, F., Ge, Y., Wu, J. and Hu, X. 2009. Degradation behavior and products of malathion and chlorpyrifos spiked in apple juice by ultrasonic treatment. Ultrasonics Sonochemistry 17: 72–77.

Zhou, X. J., Guo, W.Q., Yang, S.S., Zheng, H.S. and Ren, N.Q. 2013. Ultrasonic-assisted ozone oxidation process of triphenylmethane dye degradation: evidence for the promotion effects of ultrasonic on malachite green decolorization and degradation mechanism. Bioresource Technology 128: 827-830.