International Journal of GEOMATE, Oct., 2019 Vol.17, Issue 62, pp. 47 - 52
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.62.4738
Special Issue on Science, Engineering & Environment

EFFECT OF OZONE MICROBUBBLES WITH VARIOUS TEMPERATURES ON THE CHLORPYRIFOS INSECTICIDE REMOVAL IN TANGERINE CV. SAI NAM PHUENG

* Wirin Singtoraj [1,4], Jamnong Uthaibutra [1,3,4] and Kanda Whangchai [1,2,3,4*]

[1] Department of Biology, Faculty of Science, Chiang Mai University, Thailand; [2] Center of Excellence in Bioresources for Agriculture, Industry and Medicine Chiang Mai University, Thailand; [3] Postharvest Technology Research Institute, Chiang Mai University, Thailand; [4] Postharvest Technology Innovation Center, Office of the Higher Education Commission, Bangkok, Thailand.

*Corresponding Author, Received: 29 Nov. 2018, Revised: 07 Feb. 2019, Accepted: 25 Feb. 2019

ABSTRACT: Chlorpyrifos, a type of organophosphate insecticide, is widely used to destroy pests in the tangerine orchard to reduce the loss and maintain the quality of fruit. Consequently, the application of insecticides during production leads to the insecticide residues problem in tangerine that can be dangerous to human health. This study investigated the effect of ozone microbubbles (OMBs) on the removal of chlorpyrifos residues in the fruit. Tangerines were sprayed with chlorpyrifos solution at a concentration of 10 minutes. Then, they were treated with OMBs at different temperatures (15, 20 and 25 ºC) for various exposing times (0, 10, 20, 30, 40, 50 and 60 minutes). Removal percentage of chlorpyrifos residues in tangerines was determined by gas chromatography-flame photometric detector (GC-FPD). The results showed that chlorpyrifos residues in tangerines were decreased at lower water temperature, especially at 15 ºC resulting in the removal up to 81% after treating with OMBs for 30 minutes when compared to control (distilled water). After that, the fruits were stored at 25 ºC for 7 days to determine quality changes. The percentage of weight loss, total soluble solids (TSS), titratable acidity (TA), disease incidence and ascorbic acid content in all treatments were not affected by OMBs. These results demonstrate that OMBs treatment at 15 ºC is the most effective treatment for removing chlorpyrifos residues in tangerine and have no effects on the fruit quality.

Keywords: Tangerine, Chlorpyrifos, Insecticide residues, Ozone microbubbles

1. INTRODUCTION

Chlorpyrifos, a type of organophosphate insecticide, is widely used to destroy pests in the tangerine orchard to reduce the loss and maintain the quality of fruit. Consequently, the application of insecticides during production leads to the insecticide residues problem in tangerine that can be dangerous to human health such as neurotoxicity, immunotoxicity and reproductive toxicity [1]. The report from the Regional Environment Office, Chiang Mai, Thailand showed that tangerine fruit in northern Thailand had chlorpyrifos residue exceeded the Maximums Residue Limits (MRLs). The residue problem is rather serious and concern both growers and consumers[2]. Moreover, Thai Pesticide Alert Network reported that tangerine in northern Thailand had chlorpyrifos insecticide residues more than 1.0 mg/L and this exceeded the Maximums Residue Limits (MRLs) [3]. This leads to possible chronic or acute health risk for consumers. Therefore, there is a requirement for effective oxidation technology such as ozone microbubbles that has been applied for removing insecticide residues in tangerine.

Ozone is environmentally friendly and confirmed as a Generally Recognized As Safe (GRAS) for food contact application. Ozone is a powerful oxidant with a wide range of sanitizing application. Due to its instability with a half-life of 20-30 minutes in distilled water at 20 ºC [4], it degrades quickly into oxygen and leaves no residues after application [5]. Ozone has been used as a sanitizer in food surface hygiene and to extend the shelf life of many postharvest agricultural products [6]. Moreover, it was found that oxidation by ozone is an effective treatment to degrade the insecticide residues on agricultural products [7]. For example, Whangchai et al. [8] reported that using ozone gas for 60 minutes can reduce 45% chlorpyrifos insecticide on lychee fruits without changes in fruit quality. Kusvuran et al. [9] found that fumigation with 10 minutes of ozone gas for 5 minutes can reduce the tetradifon and chlorpyrifos insecticides on the surface of citrus fruits in the ratio of 100% and 98.6% respectively and Wu et al. [10]. stated that low level dissolved ozone (1.4 mg/L) was able to reduce diazinon, parathion and methyl parathion insecticide residues in Pak Choi (Brassica rapa) in 30 minutes. Heleno et al. [11] suggested that washing table grapes that contaminated with chlorothalonil insecticide using the ozonated water for 60 min reduced residues about 60%. Nevertheless, the low water solubility and rapid degradation of ozone are problems for an attack on insecticide molecules.

Ozone microbubbles (OMBs) is a good alternative technique that can transform ozone gas into micro-size bubbles (less than 10 µm) in the water. These micro-bubbles can keep the ozone inside for a longer period and they will be slowly floated up to the water surface that increased the dissolving potential of ozone. In this process, the surface of microbubbles will be surrounded by anionic molecules, therefore they will not be combined together to form big- bubbles. Likewise, the microbubbles enhance the oxidizing efficiency of the ozone which helps to destroy the insecticide structures [12]-[14]. It has been reported that OMBs removed water pollutants (ammonia and diethyl phthalate) from wastewater in a pilot plant [15], [16]. Furthermore, Ikeura et al. [17] found that sanitizing with OMBs (2 mg/L) for 20 minutes could reduce the fenitrothion insecticide residues in lettuce, cherry tomatoes and strawberries with no effect on product quality. Therefore, the objective of this research is to study the effect of OMBs with various temperatures on the chlorpyrifos insecticide removal in tangerine cv. Sai Nam Phueng.

2. MATERIALS AND METHODS

Chemicals Preparation

Standard chlorpyrifos insecticide was purchased from Sigma-Aldrich Laborchemikalien GmbH (Stienheim, Germany) with 99% purity. Standard chlorpyrifos insecticide stock solution (1000 mg/L) for the insecticide residues analysis was prepared in acetone. The chlorpyrifos insecticide solution was diluted with distilled water to appropriate working concentrations.

Ozone Microbubbles Preparation

The OMBs used ozone generator (Ozonizer, Model SO5AE) and microbubbles water generator (Model 15KED02S, Nikuni Co., Ltd., Japan) at a flow rate of 7L/minute with an internal pressure of 0.25 MPa for distilled water. OMBs water came out from the microbubbles nozzle and circulated in the microbubbles bath.

2.1 Effect of Ozone Microbubbles on the Removal of Standard Chlorpyrifos Insecticide at Different Temperatures and Exposing Times

The optimum condition for the removal of chlorpyrifos insecticide using OMBs water was determined at various conditions at different temperatures (15, 20 and 25 ºC) and various exposing times (0, 10, 20, 30, 40, 50 and 60 minutes). Standard chlorpyrifos insecticide solution was prepared in the flask. Each standard chlorpyrifos insecticide solution sample was added with 20 ml of OMBs water treatment previously mentioned and distilled water (control). The concentration of dissolved ozone was determined by an ozone solution detector (Prominent Dulcotest DT1B photometer) and the removal percentages of each standard chlorpyrifos insecticide sample were determined by gas chromatography-flame photometric detector (GC-FPD).

2.2 Ozone Microbubbles Treatment on the Removal of Chlorpyrifos Insecticides and Postharvest Quality Changes in Tangerine

Organic tangerines (*Citrus reticulata* Blanco cv. Sai Nam Pueng) were purchased from the orchards in Mae Tang district, Chiang Mai Province, Thailand. The fruits were sorted for free of the wound from harvesting and insect bites. Each fruit sample was sprayed with 10 mg/L chlorpyrifos insecticide solution and then air dried at room temperature. Then, they were sanitized in OMBs with the same conditions as the best result from the above Experiment. After that, they were divided into 2 groups.

2.1.1 The removal of chlorpyrifos insecticides on tangerine

Insecticide residues of tangerine were extracted with the Agilent Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction kit. Then the samples were analyzed insecticide residues by GC-FPD to determine the removal percentage of chlorpyrifos insecticide.

2.1.2 Postharvest quality changes in tangerine

Tangerines were stored at 25 ºC and taken every day for 7 days to determine the fruit quality. The percentage of weight loss, total soluble solids (TSS), titratable acidity (TA), disease incidence and ascorbic acid content were measured.

Statistic Analysis

All the experiments were replicated 3 times and evaluated with regression procedure using the SPSS version 17, while the differences among various treatments by Duncan's New Multiple Range test ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Effect of Ozone Microbubbles on the Removal of Standard Chlorpyrifos Insecticide at Different Temperatures and Exposing Times

The concentration of dissolved ozone in water was determined using an ozone solution detector (Prominent Dulcotest DT1B photometer). It was found that OMBs treatments at 25 ºC obtained the concentration of 0.22, 0.24, 0.29, 0.24, 0.26 and 0.29 mgO3/L, at 20 ºC obtained the concentration of 0.25, 0.30, 0.35, 0.30, 0.30 and 0.35 mgO_3/L and at 15 ºC obtained the concentration of 0.49, 0.53, 0.61, 0.52, 0.48 and 0.48 mgO_3/L at 10, 20, 30, 40, 50 and 60 minute, respectively (Fig. 1). The dissolved ozone concentration of OMBs treatment was increased when exposure time 10-30 minutes and then they were stabled during experiment times. All OMBs treatment at 15 ºC obtained the concentration of dissolved ozone more than at 20 and 25 ºC. Due to the low temperature may increase the dissolved ozone in water, while the high temperature increases the chemical reaction between substrate and oxidant, but decrease the dissolved ozone in water [18].

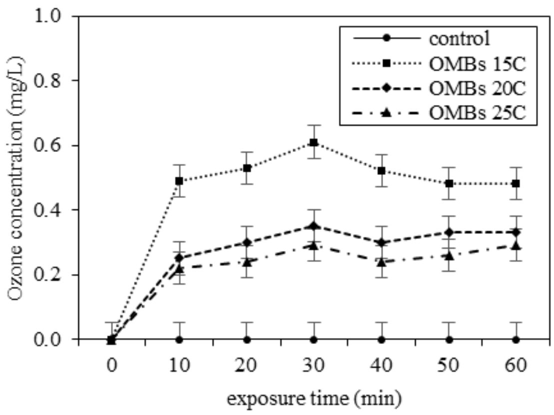

Fig.1 Concentration of dissolved ozone after OMBs at different temperatures and exposing times

The effect of OMBs to remove the standard chlorpyrifos insecticide solution was also examined. It was found that the removal percentage of standard chlorpyrifos insecticide was significantly induced with low temperature and prolonged OMBs exposure time for 30 minutes. Furthermore, OMBs at 15 ºC for 30 minutes was the most effective treatment for removing standard chlorpyrifos insecticide residues, insecticide removal was 42%. But, the trend of standard chlorpyrifos insecticide residues removal percentage after exposure time for 40-60 minutes in all OMBs treatment was decreased. There were significant ($P<0.05$) difference in each OMBs treatment (Fig. 2).

Ozone is a powerful oxidant, probably modified the chemical structure of chlorpyrifos insecticide by oxidizing sulfate ion in thiophosphorile bonds (P=S) of chlorpyrifos insecticide into phosphorile bonds (P=O) [19], [20]. And, decreasing of ozonated water temperature cause a positive effect on the removal percentage of organophosphate insecticides [21]. Moreover, microbubbles have a high surface area, which can maintain the ozone for a long time. In addition, the usefulness effect of low OMBs water temperature may be due to the increasing dissolved ozone in water, which enhance the oxidizing efficiency of the OMBs to collapse with insecticide structure [22].

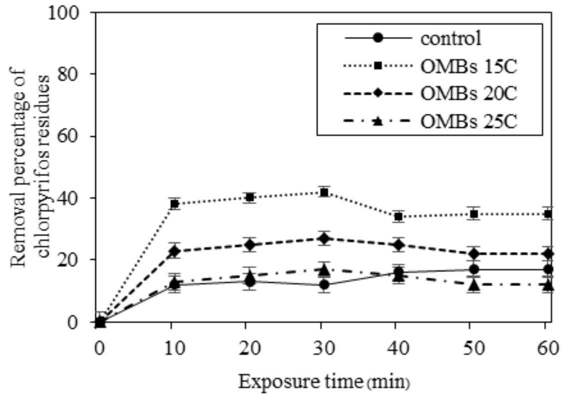

Fig.2 Removal percentage of standard chlorpyrifos solution after OMBs treatment at different temperatures and exposing times

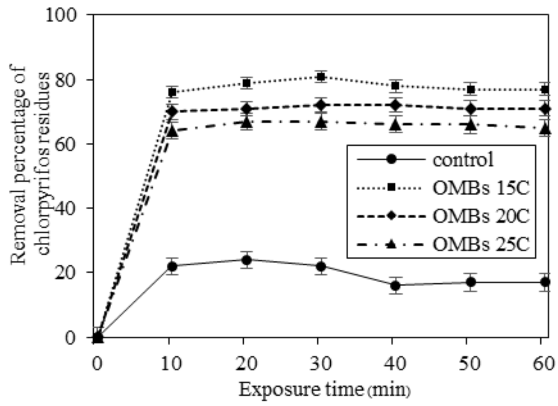

Fig.3 Removal percentage of chlorpyrifos in fruit after OMBs at different temperatures and exposing times.

Table 1 Changes in the percentage of weight loss, TSS, TA, disease incidence and ascorbic acid content of tangerine after sanitizing with ozone microbubbles at 15°C for 60 minutes, and storage at 25 ºC for 7 days

treatment	Weight loss (%)	TSS (%)	TA (%)	Disease incidence (%)	Ascorbic content (mg/ml)
Control	0.92a	10.76a	0.23a	3.43ab	0.91ab
OMBs 10 minutes	0.80a	10.42a	0.28ab	3.33a	1.01a
OMBs 20 minutes	0.83a	9.60a	0.32ab	3.33a	1.09a
OMBs 30 minutes	0.82a	9.80a	0.28ab	3.33a	1.04a
OMBs 40 minutes	0.81a	9.82a	0.30ab	3.43ab	1.06a
OMBs 50 minutes	0.81a	9.75a	0.29ab	3.43ab	1.05a
OMBs 60 minutes	0.83a	9.75a	0.29ab	3.43ab	1.06a

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

3.2 Ozone Microbubbles Treatment on the Removal of Chlorpyrifos Residues and Postharvest Quality Changes in Tangerine

Tangerines were sanitized in OMBs treatment in order to remove residual chlorpyrifos insecticide. The results showed that OMBs at 15 ºC for 30 minutes was the most effective to remove chlorpyrifos insecticide from tangerine that related to a concentration of dissolved ozone. It was found that the chlorpyrifos residues on fresh tangerine showed the highest removal percentage of chlorpyrifos residues (81%) with significant ($P<0.05$) different when compared with individual treatment and the control, which was sanitized in distilled water (Fig. 3).

This trend could be explained by the reaction of OMBs with low temperature increasing dissolved ozone in the water leading to high hydroxyl radical producing, which effectively distinguished chlorpyrifos residues on the tangerine surface. Similarly, Wu et al. [10] reported that application of the ozonated water at 14 ºC for insecticide removal from Pak Choi (Brassica rapa) was more effective than at 24 ºC.

Thus, this research showed that the efficiency of OMBs on chlorpyrifos insecticide removal depended on the decreasing of the temperature of OMBs water and exposure times. The temperature of OMBs water related to the efficacy of dissolved ozone in water. Therefore, the low temperature could increase the ability of dissolved ozone in water.

The postharvest quality changes in the tangerine after sanitizing with OMBs treatment, and then storing at 25 ºC and taken every day for 7 days were concluded and shown in Table 1. All treatments had non-significant differences as fruit quality changes during storage at 25 ºC for 7 days. The percentage of weight loss, TSS, TA, disease incidence and ascorbic acid content in all treatment were not significant changes during storage in all the treatments. Similarly, treatment of vegetables such as arugula, bell pepper, cabbage, carrot, cucumber, leek, parsley and tomatoes with ozonated water at 25 ºC can remove chlorpyrifos insecticide residues higher than at 35 ºC without changing in antioxidant capacity, total phenolic and ascorbic acid [23]. Qiang et al. [24] reported that ozone treatment could reduce the decay rate and respiratory intensity, maintain color, and delay softening of mulberry fruit, thus extending their shelf-life during refrigerated storage. Moreover, Lauana et al. [25] reported that after sanitized carrots with ozone did not change its characteristics (weight loss percentage, firmness and color) but increase in soluble solids during storage, thereby increasing the shelf life of carrots.

4. CONCLUSION

OMBs at different temperatures had effects on the removal of chlorpyrifos insecticide. The exposure times of OMBs at 15 ºC for 30 minutes provide the best result on the removal of chlorpyrifos insecticide (81%) in tangerine and have no effects on the fruit quality.

5. ACKNOWLEDGMENTS

This research has been supported by Postharvest Technology Innovation Center, Office of the Higher Education Commission, Bangkok, Thailand., Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand and the Graduate School Chiang Mai University, Chiang Mai, Thailand.

6. REFERENCES

[1] Kassa J., Krocova Z., Sevelov V., Sheshko V., Kassalova I., and Neubauerova V., Low-level

Sarin-induced Alteration of Immune System Reaction in Inbred BALB/c Mice, Toxicology Vol. 187, 2003, pp. 195-203.
[2] Regional Environment Office 1 (Chiang Mai), 2017. http://www.reo01.mnre.go.th/th/.
[3] Ousap P., Road Map of Food Safety, Report from Thai Pesticide Alert Network, 2017. http://www.thaipan.org/node/831.
[4] Chelme-Ayala P., El-Din M.G., and Smith D.W., Kinetics and Mechanism of the Degradation of Two Pesticides in Aqueous Solutions by Ozonation, Chemosphere, Vol. 78, Issue 5, 2010, pp. 557–562.
[5] Carletti L., Botondi R., Moscetti R., Stella E., Monarca D., Cecchini M., and Massantini, R., Use of Ozone in Sanitation and Storage of Fresh Fruits and Vegetables, Journal of Food, Agriculture and Environment, Vol. 11, 2013, pp. 585-589.
[6] Coke A.L., For Chapter in a Book, Ozone in Food Processing, Ed. Blackwell Publishing Ltd., 2012, pp. 48-51.
[7] Ormad M.P., Miguel N., and Claver A., Pesticides Removal in the Process of Drinking Water Production, Journal of Antimicrobial Chemotherapy, Vol. 71, Issue 1, 2008, pp. 97-106.
[8] Whangchai K., Uthaibutra J., Phiyanalinmat S., Pengphol S., and Nomura N., Effect of Ozone Treatment on the Reduction of Chlorpyrifos Residues in Fresh Lychee Fruits, Ozone: Science and Engineering, Vol. 33, 2011, pp. 232-235.
[9] Kusvuran E., Yildirim D., Mavruk F., and Ceyhan M., Removal of Chlorpyrifos Ethyl, Tetradifon and Chlorothalonil Pesticide Residues from Citrus by Using Ozone, Journal of Hazardous Materials, Vol. 2, Issue 4, 2012, pp. 287-300.
[10] Wu J.G., Luan T., Lan C.Y., Lo T.W.H., and Chan G.Y.S., Removal of Residual Pesticides on Vegetable Using Ozonated Water, Food Control, Vol. 18, 2007, pp. 466-472.
[11] Heleno F.F., Maria E.L.R., Antonio A.N., Leda R.A.F., Flaviane A.S., and Andre F.O., Ozone Treatment for the Removal of Residual Chlorothalonil and Effects on the Quality of Table Grapes, Journal of the Brazilian Chemical Society, Vol. 26, Issue 4, 2015, pp. 687-694.
[12] Sumikura M., Hidaka M., Murakami H., Nobutomo Y., and Murakami T., Ozone Microbubble Disinfection Method for Wastewater Reuse System, Water Science and Technology, Vol. 56, Issue 5, 2007, pp. 53-61.
[13] Takahashi M., Chiba K., and Li P., Formation of Hydroxyl Radicals by Collapsing Ozone Microbubbles under Strong Acid Conditions, The Journal of Physical Chemistry, Vol. 111, 2007, pp. 11443-11446.
[14] An T., Gao Y., Li G., Kamat P.V., Peller J., and Joyce M.V., Kinetics and Mechanism of OH Mediated Degradation of Dimethyl Phthalate in Aqueous Solution: Experimental and Theoretical Studies, Environmental Science and Technology, Vol. 48, Issue 1, 2014, pp. 641-648.
[15] Khuntia S., Majumder S.K., and Gosh P., Removal of Ammonia from Water by Ozone Microbubbles, Industrial and Engineering Chemistry Research, Vol. 52, Issue 1, 2013, pp. 318-326.
[16] Jabesa A., and Ghosh P., Removal of Diethyl Phthalate from Water by Ozone Microbubbles in a Pilot Plant, Journal of Environmental Management, Vol. 180, 2016, pp. 476-484.
[17] Ikeura H., Hamasaki S., and Tamaki M., Effects of Ozone Microbubble Treatment on Removal of Residual Pesticides and Quality of Persimmon Leaves, Food Chemistry, Vol. 138, Issue 1, 2013, pp. 366-371.
[18] Hart E.J., and Henglein A., Free Radical and Free Atom Reactions in the Sonolysis of Aqueous Iodide and Formate Solutions, Journal of Physic Chemistry, Vol. 89, 1985, pp. 4342- 4347.
[19] Pond A.L., Chambers H.W., and Chambers J.E., Organophosphate Detoxication Potential of Various Rat Tissues via A-esterase and Aliesterase Activities, Toxicology Letters, Vol. 78, Issue 3, 1995, pp. 245–252.
[20] Wu J.G., Lan C.Y., and Chan G.Y.S., Organophosphorus Pesticide Ozonation and Formation of Oxon Intermediates, Chemosphere, Vol. 76, 2009, pp. 1308-1314.
[21] Kusvuran E., Yildirim D., Mavruk F., and Ceyhan M., Removal of Chlorpyrifos Ethyl, Tetradifon and Chlorothalonil Pesticide Residues from Citrus by Using Ozone, Journal of Hazardous Materials, Vol. 2, Issue 4, 2012, pp. 287-300.
[22] Guidance Manual Alternative technique and Oxidant, Information from the United States Environmental Protection Agency, 2001. http://water.epa.gov/lawsregs/rulesregs/sdwa/mdbp/upload_2001_12_mdbp_alter.pdf.
[23] Khaled A.O., Fahad B., and Abdullah A., Ozone as a Safety Post-Harvest Treatment for Chlorpyrifos Removal from Vegetables and Its Effects on Vegetable Quality, International Journal of Food and Nutritional Science, Vol. 3, Issue 2, 2017, pp. 1-11.
[24] Lauana P.S., Leda R.D.A.F., Fernanda F.H., Paulo R.C., Thamiris D.C.G., Priceline J.S., and Lucas H.F.P., Effects of Ozone Treatment on Postharvest Carrot Quality, Food Science

and Technology, Vol. 90, 2018, pp. 53–60.
[25] Qiang H., Haiyan G., Hangjun C., Xiangjun F., and Weijie W., Precooling and Ozone Treatments Affects Postharvest Quality of black mulberry (Morus nigra) fruits, Food Chemistry, Vol. 221, 2017, pp. 1947–1953.