Insecticide Residue Removal by Microbubble Treatments in Fresh Consumed Agricultural Products: A Preliminary Study

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***Abstract*—Four major groups of insecticide, organophosphate, carbamate, organochlorine, pyrethroid, are used in agricultural cultivation to achieve high and constant product quality. However, insecticide residue contamination is one of the major problems that effect human health widely. In this study, two fresh consumed agricultural products, orange and banana, were treated with three types of microbubble, air microbubble (AMB) or oxygen microbubble (OMB) for 15 or 30 min. Then the products were tested for insecticide residue contamination using GPO-TM kit, a test kit for detection of all four insecticide groups. After 15 minutes of treatments with microbubble both AMB and OMB, insecticide residues was still found. Interestingly, both AMB and OMB effectively removed all four-insecticide residue from the products after 30 minutes of the treatments.**

***Index Terms*—Microbubble technology, Insecticide removal, Insecticide contamination, Advance oxidative process.**

I. Introduction

Thailand is an agricultural country. Fresh consumed agricultural products such as banana and orange have been exported and have a great impact on Thai economy. Even though guidelines and appropriate harvesting time of pesticides are available, the products are immediately rushing for sale after harvest which result in large amount of pesticide residue contamination [1]. Recently, the agricultural products from Thailand had been rejected from several countries due to insecticide contamination problems [2].

Four groups of insecticide, organophosphate, carbamate, organochlorine and pyrethroid are widely used for mass plant cultivation. Organophosphates are a class of organophosphorus compounds with the general structure $O=P(OR)_3$ (Fig. 1A).

Organophosphate insecticide such as parathion and malathion are the most common used. Carbamate insecticides contain a carbamate ester functional group (Figure 1B). The common insecticides in this group includes aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb, oxamyl, and methomyl. Organochlorine insecticides is chlorinated hydrocarbon derivatives (Fig. 1C). They are highly toxic, slow degradation and bioaccumulation. Pyrethroids, synthetic insecticides, have similar molecular structure with pyrethrins, natural insecticides found in *Chrysanthemum cinerariifolium* (Fig. 1D).

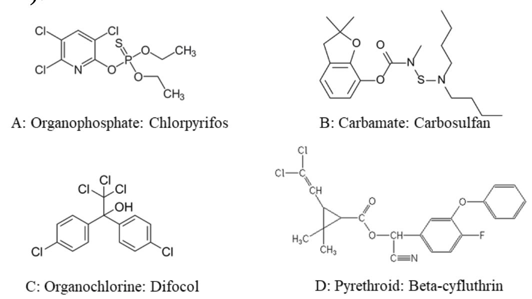

Figure 1. Chemical structure of insecticides from four insecticide groups

Insecticides are designed to intervene various metabolisms of the target organism and subsequently lead to termination of the organism. Therefore, utilization of the insecticide is very important for mass agricultural production. However, insecticide residues leave prejudicial effect on environment and human health [3]. Therefore, a safe method for effective removing of insecticide residue is of interest.

Microbubbles means bubbles with diameter between one micrometer to one hundred millimeters. Microbubbles in water can be produced using different types of generators. Even though characters of microbubbles may vary depend on types of gas used, the bubbles have basic properties which are generation of free radicals, self-pressurization and negative surface charge [4].

Microbubble technique has been reported for insecticide removing effect. Ozone microbubble was able to remove fenitrothion, an organophosphate insecticide, in some agriculture products [5]. Another study showed that bubbling 2 ppm dissolved ozone microbubble can removed both fenitrothion and benomyl, a carbamate insecticide, from red and green persimmon leaves [6].

GPO TM-Kit (Petty patent no. 7554), a special made test kit for detection of four insecticide groups, is produced by the Government Pharmaceutical

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Organization Thailand (Fig. 2). The kit uses Thin Layer Chromatography (TLC) technique couple with UV (254 nm) reaction. White spots will occur if organophosphate or carbamate insecticides present in tested samples while the grey spots will present if the tested samples are contaminated with organochlorine and pyrethroid insecticides.

Figure 2. GPO-TM kit.

Even though several researches have revealed an effect of microbubble treatment on removing some insecticide groups, a study carried out to investigate the effect of microbubble on four widely used insecticide groups has never been occurred. Moreover, ozone is a potent oxidant that may damage mucous and respiratory tissues in animals. Therefore, this study majorly aimed at investigating an insecticide removing effect of microbubble treatment and uncovering the safest technique in removing or reducing insecticide contamination in agricultural products.

II. Materials and Methods

A. Materials

Bananas and oranges were purchased from a local market, Chiang Mai. The products were kept at 4 ℃ and used in the experiment within 24 hours after purchased. A common organophosphate insecticide, Chlorpyrifos (40% w/v), a common carbamate insecticide, Carbosulfan (20% w/v), a common pyrethroid insecticide, Beta-cyfluthrin (2.5% w/v) and a common organochlorine insecticide, Dicofol (20% w/v)

All insecticides were purchase from Global-crops, Thailand. GPO TM-kits was purchased from the Northern Government Pharmaceutical Organization, Thailand

B. Treatment with Insecticides

Each insecticide was prepared according to the commercial label. Orange fruits or banana fruits were immersed in each insecticide solution for 15 min then dried for 30 min at room temperature. After pesticide treatment, the fruits were separated to five groups including a control group (C: no further treatment), a positive control group (ROW: treated with Reverse Osmosis-water), an AMB group (treated with air microbubble) and an OMB group (treated with oxygen microbubble).

C. Treatment with Microbubble

Five liters of Reverse Osmosis-water (RO-water) was added in a cylindrical vessel. AMB was generated in the water using an in-house decompression-type microbubble generator (Model: RMUTL-KVM-01) with water flow rate 1.7 L/min, pressure 0.25 - 0.4 mPa, gas flow rate 0.1 L/min. OMB was produced in the same manner with addition of oxygen. Orange fruits or banana fruits (200 g) were immersed to the vessel containing bubbling microbubble for 15 or 30 min.

D. Pesticide Residue Analysis Using GPO TM-kit

The treated fruits were tested for pesticide residue contamination as described in the kit manual.

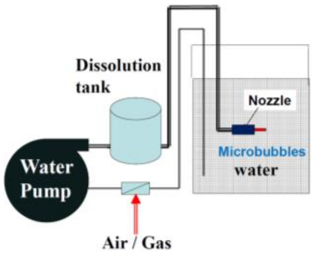

Figure 3. RMUTL-KVM-01 microbubble generator diagram.

For organophosphate and carbamate detection, the peel sample (5 g) was finely cut and place in 25 mL container. Extracting solution (5 mL) was added to the container followed by 0.25 g activated charcoal powder and mixed thoroughly. The container was left at room temperature for 5 min then the clear part was taken out and dried at 48C. The extracting solution (20 uL) was added to the dried sample and used for TLC analysis. The TLC sheet was until the solvent reached a solvent front line (8.5 cm). The sheet was taken out from the mobile phase then dried at room temperature and sprayed with a testing solution 1. The sheet was then incubated at 37 ℃ for 10 min. Finally, the sheet was sprayed again with a color testing solution then left at room temperature for three min before reading the result. A present of a white spot means the sample is contaminated with organophosphate or carbamate insecticides (Fig. 4A).

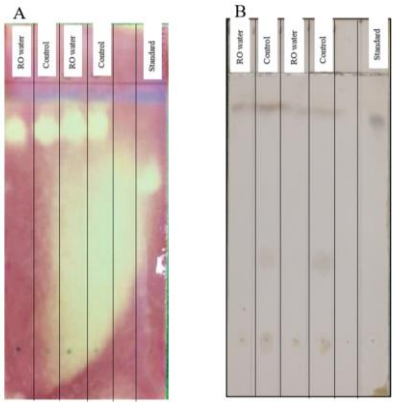

Figure 4. GPO-TM TLC sheets. A. Samples contaminated with organophosphate or carbamate insecticides. B. Samples contaminated with organochlorine or pyrethroid insecticides.

In order to examine organochlorine and pyrethroid insecticide contamination, the method was slightly different. Five grams of the peel sample was finely chopped and place in 25 mL container then the extracting solution (5 mL) was added. The container was then tightly closed and vigorously shook for one min. The container was left at the room temperature for five min. The clear solution was taken out and dried at 48 ℃. The extracting solution (20 uL) was added to the dried sample and used for TLC analysis. The TLC sheet was put in a mobile phase until the mobile phase reach a solvent front line (8.5 cm). The sheet was dried at room temperature and sprayed GPO-TM4 solution then dried before putting under UV light (254 nm) for three min. A present of a dark brown spot means the sample is contaminated with organochlorine or pyrethroid insecticides (Fig. 4B).

III. Result and Discussion

An average size of microbubbles produced from an in-house decompression-type microbubble generator was 37 µm and the concentration of bubble was 6241 bubble/mL.

Similar results were found in both agricultural products. The control sample (C), contaminated sample without further treatment were tested positive for all four insecticide groups. When the contaminated products were treated with RO-water (ROW), the result showed that ROW was not effective in reducing or removing the insecticides in the products regardless of the treatment time. Insecticide residues from all four insecticide groups were found in the products washed with AMB and OMB for 15 min. Interestingly, when the treatment time with AMB or OMB increased, insecticide contamination vanished. The results are shown in Table I.

TABLE I. Insecticide Removal Effect of Microbubble

Insecticide group		Oragnophosphate & Carbamate			Oragnochlorine& Pyrethroid		
Treatment time (minute)		15	30	60	15	30	60
Banana	C	+	+	+	+	+	+
	ROW	+	+	+	+	+	+
	AMB	+	-	-	+	-	-
	OMB	+	-	-	+	-	-
Orange	C	+	+	+	+	+	+
	ROW	+	+	+	+	+	+
	AMB	+	-	-	+	-	-
	OMB	+	-	-	+	-	-

+: Contaminated -: Not contaminated

Mass agriculture required insecticide to stabilize production yield. Elimination of insecticide residue in agricultural products is a challenge in food safety around the globe. Moreover, insecticide possesses genotoxic effect and carcinogenicity. Their relation to human chronic diseases has been reviewed [7]. Water is a main use in the household to remove dirt and other contaminated substance including insecticides from agricultural products. It has been a controversy on the efficiency of tap water on insecticide removal. An extensive review explained that rinsing vegetables with tap water significantly decreased level of several insecticides, chlopyrifos, cypermethrin, fenithrotion, dimethoate and trochlorfon in the products [8]. However, A study of insecticide removal effect of tap water showed that water was unable to remove residues of bifenthrin, a pyrethroid insecticide and chlorpyrifos, an organophosphate insecticide in agricultural product samples. Moreover, the study also revealed that water solubility of each insecticide did not link to its rinsability [9].

Microbubble is a technique of induction of fine bubble (<50 µm) in solution. One of the most interested properties of microbubbles is free radical generation after collapsing under non-thermal condition. The free radical generation might result from an augmentation of the ion concentration around the shrinking gas–water interface. The free radical species generated by the bubble collapsing was detected by an electron-spin-resonance test using 5,5-dimethyl-1-pyroroline-N-oxide as a spin-trap reagent. The study also showed that free radicals generated from collapsed microbubbles was confirmed to decompose several chemicals under acidic condition [10].

Several studies have reported insecticide removal properties of microbubble technique in fresh consumed agricultural products. Ikeura and coworker investigated two different types of microbubble generator on insecticide-removal efficiency. They revealed that a decompression generator was more effective than a gas-water circulation generator because the decompression generator generated higher concentration of dissolved ozone in the aqueous solution than the gas-water circulation generator which resulted in a higher production of hydroxy radical in the solution [11]. Pesticide-removal effect was difference in each agricultural product. Ozone microbubble treatment was tested against fenitrothion (FT), an organophosphate insecticide, in various products. A research showed that bubbling ozone microbubble at 30 ℃ reduced residual FT to 32% and 52% in lettuce and cherry tomato, respectively [12]. Another study investigated an insecticide removal effect of both organophosphate (fenitrothion) and carbamate (benomyl) in green persimmon leaves. Bubbling ozone microbubble was the most effective treatment in removing both fenitrothion and benomyl with 44% and 50% residual, respectively [6]. A combination of ozone microbubble and ultrasonic radiation decreased Ethion, an organophosphate pesticide residue, in tangerine. After 60 minutes treatment, the pesticide contamination was reduced by 73% [13]. The study also showed that increasing of reaction time has positive effect on insecticide-removal property of an advance oxidative process.

Even though a clear mechanism of insecticide-removal effect has not yet been proved, a possible mechanism has been discussed. Hydroxyl radicals (˙OH) can be generated by the reaction of excited atomic oxygen in water. They are short-lived and highly reactive [14]. The radicals have been purposed to deconstruct several organophosphate pesticide molecules including, malathion, chlorpyrifos [15], diaznion [16] and phorate [17]. Carbamate insecticides was also found to be degraded by hydroxyl radicals. The main reaction was a removal of hydrogen atom from a methyl group presented in carbamate insecticides [18]. Moreover, hydroxyl radicals were able to destruct organochlorine insecticides in water.

Researchers relied on advance oxidative process using ultrasonic combined with hydrogen peroxide for hydroxyl radicals generation. They revealed that the reaction time has obvious effect on reduction of contaminated pesticide [19]. Another study found that a concentration of Lindane, an organochlorine insecticide, in drained water decreased after treated with advance oxidation process using nano photo-Fenton like reagent as a source of hydrogen radicals [20]. As far as the information has been gathered, there is no evidence on using microbubble technology on dissipation of pyrethroid insecticides. However, hydroxyl radicals were hypothesized for their efficacy to reduce pyrethroid contamination [21].

IV. Conclusion

This study exhibited that an in-house microbubble generator effectively produced microbubbles with desirable size and concentration. Both air and oxygen microbubbles can reduce level of insecticide contaminant in orange and banana fruits after 30 min of microbubble treatment. Insecticide-removal effect of microbubble may attribute to generation of free radical by microbubbles. This is the first study showing that normal air microbubble effectively reduced concentration of all four pesticide groups in fresh consumed agriculture products. However, this study is a preliminary research, further investigation on insecticide degradation mechanism of hydroxyl radicals, oxidative property of microbubbles, and parameters affect insecticide removal effect need to be explored.

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