Fine-bubble technology application for pesticide-removal effect in ‘Gros Michel’ banana (Musa acuminata ‘Gros Michel’)

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Abstract. ‘Gros Michel’ bananas are among highly exported agricultural product in Thailand. Their cultivation involves several pesticide applications to meet consumer satisfaction. In several countries, pesticide residues found in the bananas may result in rejection and further banned the imported products which may affect export income of Thailand. Therefore, it is important to reduce the level of the pesticide residue to meet Maximum Residue Levels (MRLs). The aim of this study was to investigate the impact of fine-bubble technology on commonly used pesticides, chlorpyrifos and cypermethrin, in banana fruits. The contact times, 15, 30, 60, and 90 minutes, and types of gas, air and ozone, used for microbubble generation were also studied. Pesticide removal effect of fine-bubble technology which was first screened using GPO-TM kits, a test kit for pesticide detection. The findings revealed that both organophosphate and pyrethroid pesticides were not detected after 30 minutes contact time when treated with fine-bubbles regardless of the gas type. Air microbubble treatment was chosen for further quantitative analysis using gas chromatography (GC). In comparison to the unwashed products (Control), air microbubble application lessened chlorpyrifos and cypermethrin by 80% and 72%, respectively. Even though fine-bubble technology successfully reduced pesticide residue in banana fruits, modes of action require further investigation.

1. 1 Introduction

‘Gros Michel’ bananas (Musa acuminata ‘Gros Michel’) are a popular fruit due to their delicious taste and high nutritional value. ‘Gros Michel’ banana cultivation area in Thailand was approximately 13,500 hectares. A total banana production in 2016 was 171,199 tons with 126 tons per hectare productivity [1]. An export value of ‘Gros Michel’ banana was 685 USD per ton.

According to National Bureau of Agricultural Commodity and Food Standard 0006-2548 banana, the banana fruit appearance was a focus [2]. The skin of the fruit should be evenly green without any obvious visible defects. In order to maintain quality and quantity of the product, various pesticides have been used widely in banana cultivation process.

Chlorpyrifos is a potent neurotoxicant in organophosphate pesticide family. Maximum Residue Limits (MRLs), the highest level of a pesticide residue that is legally tolerated in or on the agricultural product, of chlorpyrifos in banana is 3 mg/kg according to EU pesticide database [3]. In banana cultivation, the pesticide is used to control bunch pests, including flower thrips, rust thrips, and scab moth [4]. Briefly, when insects expose to chlorpyrifos, the insecticide binds to the active site of the
cholinesterase (ChE) enzyme, causing an accumulation of acetylcholine, a neurotransmitter, in the synaptic cleft and its accumulation induces continuous muscle contraction [5].

Cypermethrin is also a neurotoxic pesticide in Class II pyrethroid group [6]. The pesticide targets sodium channel by extending the channel opening resulted in hyper-excitation of the central nervous system. Moreover, voltage-gated calcium and potassium channels are also influenced by the pesticide. DNA damage and oxidative stress in the neuronal cells may occur after the pesticide exposure [6]. Cypermethrin is used in agricultural and domestic applications [7].

However, pesticide contamination problems have emerged, and the use of pesticide may affect in agricultural product exportation. Therefore, a technology for pesticide residue reduction is needed.

Fine-bubble sizes are between one micrometer to one hundred millimeters. The bubbles are generated using different types of gas in liquid media [8]. The bubbles from different gas types shows unique characteristic. Interestingly, generation of free radicals, self-pressurization and negative surface charge are common characters in microbubbles regardless of gas types [8]. Fine-bubble technology applications in agriculture vary from plant and animal growth stimulation to food safety. Several studies have shown pesticide removal effect of micro-bubble. Lettuces and cherry tomatoes contaminated with fenitrothion, an organophosphate pesticide, was treated with ozone micro-bubble. Following the treatment, the pesticide residue was reduced to 32% and 52% in lettuce and cherry tomato, respectively [9]. Ozone micro-bubble coupled with ultrasonic radiation reduce more than 70% of ethion residue in tangerine [10].

In the view of fact that pesticide contamination in agriculture products is an important concern, Government Pharmaceutical Organization of Thailand has created a rapid, affordable, and user-friendly test kit (GPO TM-Kit; Petty patent no. 7554). The kit applies thin layer chromatography (TLC) technique and UV (254 nm) reaction to identify pesticide contamination in agricultural products [11]. Even though the kit produces prompt results, quantitative information was unobtainable. Therefore, in this study, the kit was purposely used for preliminary examination to find the most suitable condition for pesticide reduction. Then, gas chromatography (GC) was used for quantitative analysis.

By taking all the above facts into consideration, an effort was undertaken to diminish pesticide contamination in ‘Gros Michele’ banana fruits using fine-bubble technology. Moreover, this study also aimed to identify the most appropriate condition for pesticide-removal effect of fine-bubble technology. Quantitative analysis of pesticide residues after fine-bubble treatment was studied.

2. Materials and methods

2.1. Materials
Bananas were purchased from a market in Chiang Mai. The fruits were kept at 4°C with 95% relative humidity. After purchase, the fruit were used within 24 hours. An organophosphate insecticide, chlorpyrifos (40% w/v) and a pyrethroid pesticide, cypermethrin (35% w/v) were obtained from Global-crops, Thailand and Extra Agrochemical, Thailand, respectively. GPO TM-kits was purchased from the Northern Government Pharmaceutical Organization, Thailand. All chemicals used were analytical grade and purchased from Sigma-Aldrich, Singapore. Helium gas of MS-grade was used and supplied by Sigma.

2.2. Preparation of pesticide-contaminated bananas
The pesticide solution was prepared by adding 15 mL chlorpyrifos (40% w/v) and 2.5 mL cypermethrin (35% w/v) in RO-water (5 L). Banana fruits were submerged in an insecticide solution for 15 minutes and then dried at 25°C with 50% relative humidity at room temperature for 30 minutes.

2.3. Treatment of pesticide-contaminated bananas by fine-bubble technology
After pesticide submerging process, the fruits were separated into four experimental groups. The experimental groups and their conditions were presented in table 1.
Table 1 All experimental groups and their conditions.

| Experimental group         | Condition                             | Abbreviation |
|----------------------------|---------------------------------------|--------------|
| Control group              | No treatment                          | C            |
| Reverse Osmosis-water group| Treated with Reverse Osmosis-water    | ROW          |
| Air micro-bubble           | Treated with air micro-bubble          | AMB          |
| Ozone micro-bubble         | Treated with ozone micro-bubble        | OzMB         |

Micro-bubble generator used in this study was an in-house decompression-type micro-bubble generator (Model: RMUTL-KVM-01) and the condition used in this study and the production of AMB and OzMB were adopted from the previous work [12]. The contaminated fruits (200 g) were submerged in the vessel of bubbling micro-bubble for 15, 30, 60 and 90 minutes.

2.4. Pesticide residue determination using GPO-TM kits

After fine-bubble treatment, the fruit samples were examined for pesticide residue using GPO-TM kit according to the commercial label [11].

The fruit sample was extracted with 5 mL extracting solution provided with the kit. The clear solution was removed and dried at 48°C for 30 minutes. The dried sample was re-dissolved in the extracting solution 20 µL and used for TLC analysis. The TLC sheet was dried at room temperature and sprayed with a testing solution 1 and then incubated at 37°C for 10 min. Finally, a color testing solution was applied and left at room temperature for three minutes. White spot appeared on TLC sheet suggests that the sample was contaminated with organophosphate pesticide. For pyrethroid pesticides, after TLC analysis the sheet was dried and sprayed with GPO-TM4 solution and placed under UV light 254 nm. A dark brown spot presented on the TLC sheet showed pyrethroid contamination.

The most appropriate washing condition was selected and further analysis using GC.

2.5. Determination of pesticide contamination using GC

Chlorpyrifos and cypermethrin residues were extracted from the fruit samples as reported by Steinwander [13] with slight modification. Banana fruit sample with peel (100 g) was mixed with acetone (200 mL), NaCl (30 g) and hexane (150 mL). The mixture was blended using high speed kitchen blender for 5 minutes. The aqueous phase was discarded and the organic phase (200 mL) was reduced to 5 mL. Hexane (5 mL) was added to the organic phase then further evaporated to complete acetone removal. Lastly, the extract was dried using anhydrous sodium sulphate and resuspended in hexane (5 mL) prior GC analysis.

For chlorpyrifos analysis, the compounds were detected using GC-MS/MS TQ system (Agilent 7890GC/7000C/MS Triple Quad) according to previous published method [14] with some modification. The column used for analysis was DB-5 MS, 30m x 0.250µm, film thickness 0.25 µm. The extract 1 µL was injected. An initial column temperature was 70 °C. Helium (He) gas was used as a mobile phase at flow rate 1.2 ml/minutes. Oven temperature gradient started from 70°C and held for 2 minutes then increased to150°C for 25°C/minute, and to 200°C for3°C/minute. Finally, the oven temperature elevated to 280°C and maintained for 10 minutes. Mass spectrometric analysis was operated using Electron Ionization (EI) source in both positive and negative modes using split/split less injection with Mass Hunter Workstation software. The condition was adjusted as follow; ion spray voltage, 70 kV for EI (+) and 70 Kv for EI (-); collision gas flow was nitrogen with flow rate 1.5 ml/minute. Quenching gas flow was 2.5 ml/minute. Ion source temperature was 230°C. The pesticide detection was confirmed with NIST library.
Cypermethrin was analyzed by a Gas Chromatography/micro-Electron Capture Detector (µ ECD-CG, Agilent 6820) with Agilent 7683B auto-sampler and a RTX-CLPesticides column. Injection volume was 2 µL with nitrogen as a carrier (6.0 psi, 1.2 mL/minutes (60°C). Instrument control, signal acquisition and data processing were achieved by Cerity Networked Data System (NDS) software. Oven gradient temperature started from 60°C (1 minute), 30°C/minute to 180°C, 5°C/minute to 250°C (10 minutes) and 3°C/minute to 280°C and maintained for 10 minutes. The detector temperature was 330°C with nitrogen (60 mL/minute).

2.6. Data analysis
Data are presented as mean ± standard deviation (SD). Differences between groups were assessed by a one-way analysis of variance (ANOVA), followed by multiple comparisons utilizing Tukey's test. Statistically significant was considered when probability values were less than 0.05.

3. Results and discussion
An average size of microbubbles produced from RMUTL-KVM-01 microbubble generator was 37 µm and the concentration of bubble was 6241 bubbles/mL.

3.1. Determination of pesticide contamination using GPO-TM kits
No visual abnormality was observed in all experimental groups after pesticide and micro-bubble treatments. The findings from GPO-TM kits revealed that samples in C were contaminated with both pesticides as expected. Pyrethroid pesticide was not detected after 15 minutes contact time with either AMB or OzMB treatments while the pyrethroid removal effect of ROW accomplished after 90 minutes contact time. AMB or OzMB successfully removed organophosphate pesticide after 30 minutes contact time while ROW required longer contact time to attain the same effect. The determination of pesticide contamination using GPO-TM kits was summarized in table 2.

| Contact Time | C | ROW | AMB | OzMB |
|--------------|---|-----|-----|------|
|              | OP | PY  | OP  | PY  | OP  | PY  | OP  | PY  |
| 15           | +  | +   | +   | +   | -   | +   | -   | -   |
| 30           | +  | +   | -   | -   | -   | -   | -   | -   |
| 60           | +  | +   | -   | -   | -   | -   | -   | -   |
| 90           | -  | -   | -   | -   | -   | -   | -   | -   |

* OP: Organophosphate (chlorpyrifos), PY: Pyrethroid (cypermethrin)

Normal tap water was generally used for cleaning dirt and contaminants in fresh agricultural products. In this study RO water was effective in pesticide residues removal however, it required longer contact time (90 minutes) than micro-bubble water. Several studies have investigated pesticide removal effect of tap water and revealed contrary findings. Various insecticides, chlorpyrifos, cypermethrin, fenithrothion, dimethoate and trochlorfon, were reduced after treated with tap water [15]. While another study showed that bifenthrin, a pyrethroid insecticide and chlorpyrifos, an organophosphate insecticide samples cannot be cleaned using sole tap water and did not related to pesticide rinsability [16].

3.2. Determination of pesticide contamination using GC
Following the screening for the most suitable condition using GPO-TM kits. AMB was chosen for further investigation because AMB generation process did not require additional equipment and it was less intricate compared to OzMB preparation. The pesticide removal effect was further confirmed using gas chromatography. The chromatograms showed that both chlorpyrifos and cypermethrin residues were detected. The chromatograms of chlorpyrifos and cypermethrin residues in all experimental sample were shown in figure 1 and 2, respectively.
Chlorpyrifos and cypermethrin levels in untreated samples were 7.82±0.7 mg/kg and 0.09±0.02 mg/kg, respectively. Chlorpyrifos residue level was 2.9±0.3 mg/kg and 1.55±0.1 mg/kg, after ROW and AMB treatment, respectively. Statistical analysis showed that the level of chlorpyrifos after ROW treatment was significantly lower than untreated sample (P<0.05) but the level still exceeds MRLs. Chlorpyrifos level in AMB treated sample was also significantly lower (P<0.05) than untreated and ROW treated samples. Likewise, cypermethrin residues decreased after ROW and AMB treatments to 0.04±0.01 mg/kg and 0.025±0.04, respectively. The cypermethrin residues after ROW and AMB treatments was significantly lower than untreated sample (P<0.05). Moreover, both chlorpyrifos and cypermethrin levels after AMB treatment met EU Pesticide MRLs. Quantitative analysis of the pesticide residues is shown in figure 3.

Figure 1. GC chromatograms of chlorpyrifos in
A: Chlorpyrifos-Control
B: Chlorpyrifos-ROW
C: Chlorpyrifos-AMB

Figure 2. GC chromatograms of cypermethrin and its derivatives in
A: Cypermethrin-Control
B: Cypermethrin-ROW
C: Cypermethrin-AMB
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Figure 3. Quantitative analysis of pesticide residues (A: Chlorpyrifos, B: Cypermethrin) by gas chromatography.

Similar to our findings, several studies have shown pesticide removal effect of micro-bubble. To our knowledge, very few researches has been carried out regarding pyrethroid removal using micro-bubbles. Micro-bubble treatment on *Citrus reticulata* Blanco revealed cyfluthrin-removal effect. Air micro-bubble treatment removed approximately 90% of cyfluthrin after 15-minute contact time [12]. However, most studies used ozone which may contributed to their pesticide-removal effect. A study on chlorpyrifos contamination showed that ozone micro-bubble at 15°C decreased approximately 80% of chlorpyrifos residue in tangerine cv Sai Nam Phueng with 30-minute contact time [17]. The study suggested that pesticide-removal effect of micro-bubble may vary depend on contact time and temperature. Removal percentage when studied on waxy samples was higher than rough surface samples. It is possible that types of plant surface affect pesticide attachment on different surfaces [18].

Although pesticide degradation action of micro-bubble is indefinite, a possible mechanism has been suggested. As mention previously, a unique characteristic of microbubble is free-radicals generation, caused by an augmentation of the ion concentration around the shrinking gas–water interface, may be responsible for pesticide degradation [19]. A study showed that hydroxyl radicals (•OH) exhibited destroying effect on malathion and chlorpyrifos contamination in apple juice [20]. Moreover, it has been showed that hydroxy radicals efficiently destroyed pyrethroid contamination [21].

4. Conclusion
The level of chlorpyrifos and cypermethrin residues was reduced by 72% and 80%, respectively, compared to their non-treated counterparts. The pesticide levels after treated with microbubble met the EU MRLs. Hydroxyl radical may be responsible for both chlorpyrifos and cypermethrin decontamination. Cypermethrin reduction effect of fine-bubble technology was also mentioned. This novel technology was rapid and user friendly. Nonetheless, further studies regarding free-radicals generation from fine-bubble and their effect on pesticide dissipation are required.

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