Removal of cyfluthrin by fine bubble technology in oranges
(*Citrus reticulata* Blanco)

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Abstract. Cyfluthrin, a synthetic pyrethroid insecticide, is used widely for orange cultivation. Prolong exposure to cyfluthrin causes several human health issues such as kidney inflammation, weight loss and diarrhoea. This study aimed to investigate the fate of cyfluthrin residue in oranges after treatment with fine bubble produced with air or ozone. Micro-bubbles produced from different types of gas (air, oxygen and ozone) were used to treat oranges contaminated with cyfluthrin. Cyfluthrin residue was tested using GPO-TM kit. The pesticide residue was not detected after micro-bubble treatments. An air micro-bubble technology was chosen for further analysis by HPLC. HPLC analysis confirmed that more than 90% of contaminated cyfluthrin was removed by air micro-bubble treatment. Moreover, the insecticide residue was not detected in washing water.

1. Introduction

Oranges (*Citrus reticulata* Blanco) are widely consumed as fresh fruits or ready-to-drink juice. The total area under orange cultivation in Thailand is around 35,600 acres with production of 135,898 tons and productivity of 4.32 tons per acre [1]. To maintain quantity and quality of the produce, a lot of pesticide, mainly cyfluthrin, a synthesis pyrethroid pesticide is used. Cyfluthrin, a synthesis pyrethroid pesticide, has a similar chemical structure as dichlorodiphenyltrichloroethane (DDT) (figure 1).

![Figure 1. Cyfluthrin (Cyano(4-fluoro-3-phenoxy-phenyl)methyl3-(2,2-dichlooroethenyl)-2,2-dimethylcyclopropanecarboxylate).](image)

The mode of action of cyfluthrin is an inhibition of calcium transporting enzyme which causes accumulation of acetylcholine at synapse and continuous muscle contraction [2]. The use of cyfluthrin in orange cultivation said to cause several illness [3] and also toxicity of this pesticide has been investigated on several laboratory animals. Prolong exposure to the pesticide in a very low level (150...
ppb) caused weight loss, vomiting, diarrhoea and kidney inflammation [2]. According to EU pesticide database, Maximum Residue Levels (MRLs) of cyfluthrin in oranges is 0.02 mg/kg [4].

Fine bubble technology is a novel technology in Thailand. Fine bubbles can be generated in liquid medium using different types of generator. Sizes of fine bubble range from one micrometer to one hundred millimeters. Fine bubbles produced from different type of gas show different properties however, shrinking of fine bubbles generate free radicals [5]. Micro-bubble technology exhibited pesticide removal efficacy in several studies [6,7].

GPO TM-Kit (Petty patent no. 7554), is a test kit produced by Government Pharmaceutical Organization (GPO), Thailand. It is used for a rapid detection of insecticide using Thin Layer Chromatography (TLC) coupled with UV (254 nm) reaction. This kit was used for screening the optimal condition in insecticide reduction. The most suitable condition was further confirmed using High Performance Liquid Chromatography (HPLC).

Keeping in view of the above facts, an effort has been made to reduce cyfluthrin residue in oranges by using micro-bubble technology.

2. Materials and methods

2.1. Materials

Oranges were purchased from a local market, Chiang Mai. The fruits were kept at 4°C and used for the experiment within 24 hours after purchase. Cyfluthrin (2.5% w/v) was purchase from Global-crops, Thailand. GPO TM-kits was purchased from the Northern Government Pharmaceutical Organization, Thailand.

2.2. Preparation of cyfluthrin-contaminated oranges

Insecticide was prepared according to the commercial label. Orange fruits were immersed in an insecticide solution for 15 min and then dried at room temperature for 30 min.

2.3. Treatment of cyfluthrin-contaminated oranges using fine bubble technology

After pesticide treatment, the fruits were separated to make five groups viz., control group (C: no further treatment), a positive control group (ROW: treated with Reverse Osmosis-water), an AMB group (treated with air micro-bubble), an OMB group (treated with oxygen micro-bubble) and an OzMB group (treated with ozone micro-bubble). Micro-bubble was generated by an in-house decompression-type micro-bubble generator (Model: RMUTL-KVM-01) with water flow rate of 1.7 L/min, pressure of 0.25 - 0.4 mPa, gas flow rate of 0.1 L/min. AMB was produced by adding five liters of Reverse Osmosis-water (RO-water) in a cylindrical vessel of the generator. OMB and OzMB was conjured in the same manner with addition of oxygen or ozone, respectively. Orange fruits (200 g) were immersed in the vessel containing bubbling micro-bubble for 15 minutes.

2.4. Determination of cyfluthrin contamination using GPO-TM kits and HPLC

Five groups of experimental products were tested for cyfluthrin contamination using GPO-TM kit according to the commercial label.

Briefly, the sample (5 g) was finely chopped and place in 25 mL container then the extracting solution (5 mL) was added. No charcoal powder used in the extraction process. The container was closed, shook for one minute and left at an ambient temperature for five minutes. The clear solution was taken out and dried at 48°C. 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 with GPO-TM4 solution. When the sheet dried, it was put under UV light (254 nm) for 3 minutes. The sheet presented with brown spot reveals that the tested sample is contaminated with organochlorine or pyrethroid insecticides.

The most suitable condition was selected and further analysed using HPLC. Orange skin was
included for the experiment.

2.5. Determination of cyfluthrin contamination using HPLC
Orange fruit samples (peel included) were cut into small pieces and homogenized by means of a kitchen blender. Pesticide extraction method was carried out according to a previous study with slight modification [8]. The blended fruit (100 g) sample was mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate (200 ml) in conical flask. The content was allowed to settle down for half an hour and the ethyl acetate extract was then filtered through a Buchner-funnel fitted with a filter paper covered by (20 g) of anhydrous sodium sulfate. After filtration, the extract was evaporated to dryness and re-dissolved in acetonitrile (5 mL). Then, the volume was reduced to 0.5 mL using stream of liquid nitrogen. The extract was then transferred to a graduated test tube and the final volume was adjusted at exactly 1 mL using acetonitrile. Solutions were then centrifuged and filtered.

The extracts from all experimental groups were analyzed by a HPLC with UV/Visible detector (Agilent Technologies) for identification and quantification of pesticides. Separation was performed on reversed phase C-18 column. The mobile phase: linear gradient of solvent A deionized water: solvent B was acetonitrile (70:30, v/v), flow rate was 0.3 mL min⁻¹. Injection volume was 25 µL. The wavelength of the UV/visible detector was fixed at 254 nm for the pesticides. The wavelength was set at 230 nm.

The pesticide residue was identified by comparing its retention time with respect to technical grade reference standard. The concentration of pesticide residues in fruits washed with micro-bubbles were compared to the unwashed (control) to determine the percentage of pesticide residue reduction.

2.6. Data analysis
All data were analyzed by analysis of variance (ANOVA) and the differences between fine bubble treatments were analyzed by a least significant difference (LSD) comparison. The level of significant difference was indicated with the following: *P ≤ 0.05 for all comparisons.

3. Results and discussion
After cyfluthrin and micro-bubble treatments, oranges from five experimental groups had normal appearance. Determination of cyfluthrin contamination using GPO-TM kits revealed that oranges from C and ROW groups were contaminated with pesticide residue while oranges treated with micro-bubble regardless of gas type showed no pesticide contamination. Therefore, air micro-bubble treatment was selected for further study because it has less complication during preparation process and did not required additional equipment. Determination of cyfluthrin residue using HPLC showed that cyfluthrin and its derivatives were detected. The retention time of cyfluthrin I, II, III and IV were 18.60, 18.86, 19.06 and 19.18, respectively (figure 2). The cyfluthrin level in the control group was 0.52 mg/kg. Treatment with ROW reduced the level of cyfluthrin to 0.48 mg/kg. Oranges treated with air micro-bubble was found with 0.02 mg/kg of cyfluthrin. Interestingly, no cyfluthrin residue was detected in air micro-bubble water used for treatment.

In the present study, approximately 90% of cyfluthrin residue was successfully removed by micro-bubbles. The level of cyfluthrin residue in the sample treated with micro-bubbles met EU MRLs. Like this study, micro-bubbles have been studied for their insecticide removal effects. Organophosphate pesticide, fenitrothion, was reduced to 32% and 52% after treated with ozone micro-bubble in lettuce and cherry tomato, respectively [9]. Also carbamate insecticide contamination in green persimmon leaves was removed after treated with ozone micro-bubbles [7]. Moreover, micro-bubbles conjugated with ultrasonic radiation, an advance oxidative process, reduced more than 70% of pesticide contamination in tangerine [10]. According to the research reports, studies on the efficacy of cyfluthrin removal of micro-bubble technology have not yet occurred.

Cyfluthrin is a persistent pesticide. In California orange production, its abolition rate was almost 100% after 19 days of application [11]. Therefore, it is important to discover a rapid and simple method to eliminate the pesticide residue from agricultural products. Tap water was effective for
removing significant amount of cyfluthrin in olive however, extension of washing time was not positively correlated with cyfluthrin removal ability [12]. Cyfluthrin degradation through oxidation results in 4-fluro-3-phenoxybenzoic acid (FPB acid) as a major by-product [13].

![HPLC chromatograms](image)

**Figure 2.** HPLC chromatograms of cyfluthrin and its derivatives in A: Cyfluthrin-contaminated oranges without further treatment, B: Cyfluthrin-contaminated oranges treated with reverse-osmosis water, C: Cyfluthrin-contaminated oranges treated with air micro-bubble water and D: Air micro-bubble water.

Even though, the pesticide-removal mechanism of micro-bubble technology is still unclear and required further investigation, a possible mechanism has been proposed. Free radical generation is one of the most discussed properties of micro-bubbles. Free radicals are generated after the bubbles disintegrated under non-thermal condition by an augmentation of the ion concentration around the shrinking gas–water interface. Under acidic condition, the generated radical species could break down several organic compounds [14]. Also, Hydroxyl radicals were also hypothesized for the efficacy to
reduce pyrethroid contamination [15].

4. Conclusion
Micro-bubble reduced the level of cyfluthrin contamination by 90% and met the EU MRLs. Cyfluthrin decontamination effect could attributed to hydroxyl radical generated during micro-bubble collapsing. This is the first study reported cyfluthrin reduction effect of micro-bubble technology. Howsoever, oxidative property of micro-bubbles, and insecticide degradation mechanism of free radical species required further investigation.

Acknowledgments
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