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# Analytical performance for determination of synthetic pyrethroids residue in Indonesian fermented cocoa using GC-ECD

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# Analytical performance for determination of synthetic pyrethroids residue in Indonesian fermented cocoa using GC-ECD

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**Abstract**. Pyrethroid insecticides are causing problems for the environment because they are toxic at such small concentrations and very difficult to determine. Characteristics of pyrethroids insecticides include high efficiency, wide spectrum, low biodegradability, and low mammalian and avian toxicity. They also demonstrate strong selectivity for insects and invertebrates. This study presents the results of the analysis conducted on four synthetic pyrethroids in fermented cocoa samples from Indonesia. The pesticides were extracted using acetonitrile and the analysis was carried out by Gas Chromatography with Electron Capture Detector (GC-ECD). Column programming temperature was conducted to get a complete separation. The total operation took less than 10 minutes to separate four standards of synthetic pyrethroids. The synthetic pyrethroids were identified by their retention times and quantified using an external calibration method. The linearity test was carried out for four standard synthetic pyrethroids, namely: lambda-cyhalothrin, permethrin, cypermethrin, and fenvalerate, from 1 µg/kg to 10 µg/kg. The coefficient of correlation (r) was found to be 0.9999; 0.9998; 0.9944; and 0.9999 for lambda-cyhalothrin, permethrin, cypermethrin, and fenvalerate, respectively. The detection limit for synthetic pyrethroids residue was found to be below the EU and Japan regulations, and the method can be applied for the determination of synthetic pyrethroids residues in fermented cocoa.

## 1. Introduction

The natural insecticide pyrethrum has seldom been used for agricultural purposes because of its cost and instability in sunlight. However, recently, several synthetic pyrethrin-like materials have become available and are referred to as synthetic pyrethroids. These materials are very stable in sunlight and are generally effective against most agricultural pests when used at a low rate of 0.11 to 0.23 kg/ha. There are three groups of synthetic pyrethroids; the first group includes allethrin, bioallethrin, bioresmethrin, phenothrin, resmethrin, and tetramethrin, the second group includes deltamethrin, fenvalerate, and permethrin [1], and the latest group includes bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, fenpropathrin, flucythrinate, fluvalinate, and tralomethrin.

Cacao beans are very vulnerable to pests and disease [2–4], so pesticides are applied on fermented cocoa to prevent, control, monitor, or kill the pest. In Indonesia, lambda-cyhalothrin is used as insecticide both in cultivation stage with frequencies of 3-5 times a year and in fermented cocoa beans storage [4]. Currently, lambda-cyhalothrin has been classified as a third class of pesticide or moderately hazardous [1]. However, the latest generation of pyrethroids such as permethrin, cypermethrin,

deltamethrin, fenvalerate, lambda-cyhalothrin, and beta-cyfluthrin are quite resistant and have a higher neurotoxic characteristic to mammals than previous generation [4,5].

From previous research, analysis of pyrethroid was applied for honey [6], vegetables [7,8], cacao seed [9,10], and fermented cacao [5,11,12]. The analysis method for pyrethroid was divided into four steps: preparation, extraction, clean up, and quantification using instrument [5,11,12]. The previous methods of pyrethroid extraction were liquid-liquid and liquid-solid extraction using acetonitrile and acetone [13]. Other researchers used vortex, ultrasonic, and centrifuge for extraction [7–9,11,12]. The solvent used from previous studies using multiresidue systems was high in volume [11,14]. In order to meet the green analytical chemistry concept, another extraction method should be developed. Quecher (The Quick, Easy, Cheap, Effective, Rugged and Safe) method was applied for the clean up step of pyrethroid in cacao seed [9,15]. Identification and quantification of pyrethroid was performed using GC-ECD [5,12], GC-MS [10,14], and HPLC [8,11].

**Table 1.** Pesticide used in Indonesia[a].

| Active substances | Pesticide type | % User |
|---|---|---|
| Cypermethrin 50EC | Insecticide | 42 |
| Lambda-cyhalothrin + Thiamethoxam | Insecticide | 18 |
| Lambda-cyhalothrin 25EC | Insecticide | 12 |
| Chlorpyrifos | Insecticide | 12 |
| Deltamethrin 2.5 EC | Insecticide | 5 |
| Isopropylamine glyphosate 480 g/L | Herbicide | 43 |
| Paraquat dichloride 276 g/L | Herbicide | 36 |
| Azoxystrobin + difenoconazole | Fungicide | 6 |

[a] From Hafid *et al* in Khaerati and Indriati [16]

Table 1 shows that two types of pyrethroids, cypermethrin and lambda-cyhalothrin, were mostly applied in Indonesia [16]. Since these pesticides are toxic and can cause severe adverse health effects when contaminated foods are consumed [4,5], their residue levels in fermented cocoa produced in Indonesia should be ascertained.

## 2. Methodology

### 2.1. Sampling
A total of five fermented cocoa samples were collected from the largest cocoa producing area in Indonesia, East Java and South Sulawesi, within the period of June to August 2018.

### 2.2. Chemicals and reagents
Pesticide standards (lambda-cyhalothrin, permethrin, cypermethrin, and fenvalerate) were obtained from Sigma Aldrich, high-grade acetonitrile was obtained from Merck, and 15 mL SPE kit (4000 mg PSA, 400 mg C18EC, 1200 mg $MgSO_4$) was obtained from Agilent.

### 2.3. Sample extraction
The sample extraction was developed from Zaenudin *et al* [11]. Fermented cocoa sample (1.5 g) was weighed in a centrifuge tube and labelled accordingly. Then, 4.5 mL of acetonitrile was added to each tube, stirred to form a homogeneous mixture and allowed to stand for 1 minute. After that, the samples were centrifuged for 15 minutes at 4000 rpm. Two types of experiment were conducted. The first experiment involved no clean up step. One mL of supernatant was filtered through 0.45 μm PTFE filter into an autosampler vial before analysis. The second experiment consisted of SPE clean-up using 400 g $MgSO_4$, 150 g C18CE, and 150 g PSA as sorbents. Two mL of the supernatant was transferred to SPE

tube. The tube was mixed by vortex for 60 s. After centrifugation at 4000 rpm for 15 min, an aliquot of 1 mL extract was filtered through 0.45 μm PTFE filter into an autosampler vial before analysis.

*2.4. Instrumentation*

The final extracts were analysed by Gas Chromatography (GC) Agilent 9680 series equipped with autosampler and Electron Capture Detector (ECD). The GC column used for the analysis was capillary column HP-5 (30 m length, 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperature were set at 250 °C and 350 °C, respectively, split injection ratio was 1:1. The oven temperature was programmed as follows: initial temperature 200 °C, held for 1 minute, ramp for 20 °C/minute to 280 °C, then held for 8 minutes. The auto-injection system was applied.

## 3. Results and discussions

Using the above condition of GC-ECD instrument, four standards of synthetic pyrethroids were separated well. Each retention time of pesticide standard is shown in Table 2, and the chromatogram of the mixture of synthetic pyrethroid standards is shown in Figure 1.

**Table 2.** Retention time for synthetic pyrethroids standards.

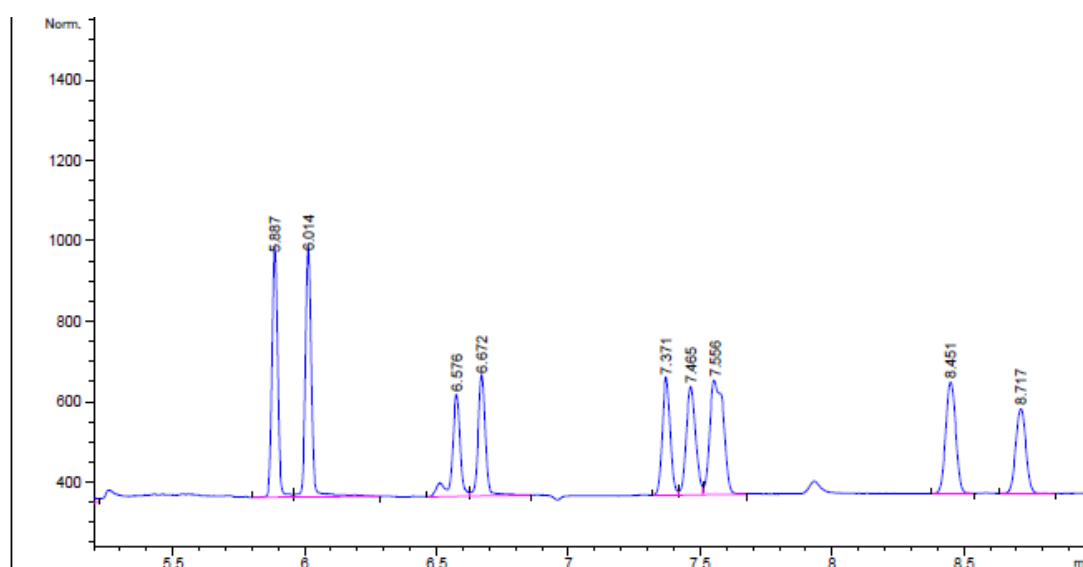| Pesticide | Retention time (minute) |
|---|---|
| Lambda-cyhalothrin | 5.882 ; 6.01 |
| Permethrin | 6.562 ; 6.67 |
| Cypermethrin | 7.367 ; 7.456 ; 7.573 |
| Fenvalerate | 8.442 ; 8.707 |



**Figure 1.** Chromatogram of synthetic pyrethroid standards.

Each synthetic pyrethroid standard (lambda-cyhalothrin, permethrin, cypermethrin and fenvalerate) was prepared in the concentration range of 1 to 10 μg/kg. The coefficient of correlation obtained for each pesticide standard ranged from 0.9937 to 0.9999 (Table 3). This range shows that the linearity and regression model obtained by standards testing were accountable. Lambda-cyhalothrin dan fenvalerate had higher correlation coefficients, each consisted of two peaks of 0.9998 and 0.9999. On the other hand, cypermethrin had a lower correlation coefficient, ranging from 0.9937 to 0.9952, and consisted of three peaks. The equations and correlation coefficients in Table 3 were then used as standards for estimating the concentration of pyrethroid compounds in each cacao sample.

**Table 3.** Linear equation and coefficient of correlation (r) of synthetic pyrethroid standards at range concentration of 1-10 µg/kg.

| Pesticide Standard | Linear equation | Coefficient of correlation (r) |
|---|---|---|
| Lambda cyhalothrin (1) | y = 90.111x - 14.843 | 0.9999 |
| Lambda cyhalothrin (2) | y = 91.003x - 15.168 | 0.9998 |
| Permethrin (1) | y = 45.488x - 3.5322 | 0.9998 |
| Permethrin (2) | y = 51.949x - 0.9934 | 0.9998 |
| Cypermethrin (1) | y = 64.281x - 14.781 | 0.9941 |
| Cypermethrin (2) | y = 70.706x - 9.7724 | 0.9937 |
| Cypermethrin (3) | y = 107.46x - 19.428 | 0.9952 |
| Fenvalerate (1) | y = 74.63x - 14.676 | 0.9999 |
| Fenvalerate (2) | y = 57.27x - 11.688 | 0.9998 |

Based on Frimpong *et al* [5], nine maximum residue levels (MRL) of pyrethroids pesticide have been appointed by Europe and Japan regulations (see Table 4). This study only involved MRL values from four types of synthetic pyrethroids that were often used in cocoa cultivation in Indonesia, i.e lambda-cyhalothrin, permethrin, cypermethrin and fenvalerate.

The maximum residue levels (MRL) for synthetic pyrethroid in fermented cocoa products appointed by Europe regulation are generally higher than the values set by Japan. For example, the MRL value set by Japan for 4 types of synthetic pyrethroid used in this study ranges from 10 to 50 µg/kg, with the lowest value set for lambda-cyhalothrin (10 µg/kg) and the highest value set for permethrin (50 µg/kg). Whereas the MRL set by Europe for lambda-cyhalothrin and permethrin are 50 and 100 µg/kg, respectively. This value implies that the world's awareness of the presence of lambda-cyhalothrin and fenvalerate is relatively higher compared to permethrin and cypermethrin.

The pesticide residues in fermented cocoa from Indonesia samples (Java and South Sulawesi) were found between 8-24 µg/kg for cypermethrin and 27-33 µg/kg for lambda-cyhalothrin. Only one sample was found to have permethrin residue (12 µg/kg), and the concentration was below EU's and Japan's MRLs. The value of relative standard deviations (%RSD) was calculated (standard deviation/average x 100) and found that the % RSDs were between 3.5%-25.2%. These results were found to be of sufficient precision for concentrations below 30 µg/kg.

**Table 4.** Maximum residue levels (MRL) of synthetic pyrethroids pesticide in fermented cocoa [8].

| Pesticides | EU (µg/kg) | Japan (µg/kg) |
|---|---|---|
| Allethrin | 10 | 10 |
| Bifenthrin | 100 | 100 |
| Fenpropathrin | 20 | 10 |
| **Lambda-cyhalothrin** | **50** | **10** |
| **Permethrin** | **100** | **50** |
| Cyfluthrin | 100 | 20 |
| **Cypermethrin** | **100** | **30** |
| **Fenvalerate** | **50** | **10** |
| Deltamethrin | 50 | 50 |

**Table 5.** Pesticide residue in Indonesian fermented cocoa.

| Sample Code (n=3) | Pesticide residue | Concentration (μg/kg) | % RSD |
|---|---|---|---|
| A | Lambda-cyhalothrin | * | - |
| | Permethrin | 12 | 25.2 |
| | Cypermethrin | 28 | 2.3 |
| | Fenvalerate | * | - |
| B | Lambda-cyhalothrin | 27 | 9.6 |
| | Permethrin | * | - |
| | Cypermethrin | 24 | 8.7 |
| | Fenvalerate | * | - |
| C | Lambda-cyhalothrin | 27 | 11.3 |
| | Permethrin | * | - |
| | Cypermethrin | 22 | 5.6 |
| | Fenvalerate | * | - |
| D | Lambda-cyhalothrin | 33 | 3.8 |
| | Permethrin | * | - |
| | Cypermethrin | 9 | 17.5 |
| | Fenvalerate | * | - |
| E | Lambda-cyhalothrin | 29 | 1.5 |
| | Permethrin | * | - |
| | Cypermethrin | 8 | 11.9 |
| | Fenvalerate | * | - |

*: below the detection limit of 0.5 μg/kg

## 4. Conclusions

The method can be applied for the determination of four types of synthetic pyrethroid pesticides. The range of linearity was found between 1–10 μg/kg, this concentration was below the maximum residue level (MRL) for synthetic pyrethroid pesticide in fermented cocoa required by EU and Japan. Two types of pesticide residue were found in Indonesian fermented cocoa, and the concentrations of pesticide residues were under EU's MRL requirement, but higher than Japan's MRL.

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