INTRODUCTION

Pesticides are used to eliminate pests so that agricultural harvests can be optimal and are also used to preserve woods and forestry products. Hence, human pesticide use has grown and developed considerably, and formerly used natural pesticides have been replaced with artificial agents [1].

Due to toxic effects in humans, animals, and the environment, bans have been placed on the use of several pesticides, including organochlorine agents such as aldrin, dieldrin, DDT, and BHC [2]. Organochlorines are known persistent organic pollutants [3] that bioaccumulate and disperse into food chains. The fat solubility of these organochlorine pesticides is an important contributor to chronic toxicity. Moreover, DDT affects axonal transmission by decreasing sodium intake and inhibiting egress of potassium. Cyclodiene and hexachlorocyclohexane also inhibit chloride uptake in response to gamma-aminobutyric acid, which is a central nervous system mediator. As a consequence, these agents cause neuron hyperactivity, and death can occur within 24–72 h due to consequent breathing disturbances [4].

Despite their chronic toxic effects in humans and proven persistence in the environment, many organochlorines, such as chlorothalonil, have been used in Indonesia [5].

In addition to organochlorine, organophosphates, carbamates, and pyrethroids are used in Indonesia. Pyrethroid is a derivative of a molecule from chrysanthemum flowers [6] and is classified into two types on the basis of the presence or absence of cyano (C≡N). Addition of cyan to the pesticide increases its insecticidal activity [7]. Cyan groups, including in fenpropatrin and lambda-cyhalothrin, can cause reversible paretetic fever, numbness, tickling sensations of the skin, and nervous system perturbations [6]. Hence, evaluations of pesticide concentrations are crucial for food products. Several studies show health effects of pyrethroid residues in contaminated fruit and vegetables, and chronic consumption has a high potential to adversely affect humans [7].

Organic vegetables have become increasingly popular in recent years, on the basis of the assumptions of higher nutrition and the absence of toxic chemicals [8]. On the basis of a study by Yu and Yang in Singapore, 1 in 10 organic vegetables was not free of pesticide [9-11]. Hence, since fenpropathrin, lambda-cyhalothrin, and chlorothalonil are used in tomato and potato commodities [5], we tested samples of these vegetables.

Concentrations of these three pesticides were analyzed using a high activity liquid chromatography method with gas chromatography (GC) and a mass spectrometer (MS) detector and electron capture detector (ECD). The data herein show high sensitivity and selectivity of the ECD.

MATERIALS AND METHODS

Materials

Non-organic and organic potatoes and tomatoes of various brands were purchased in Depok, Jakarta, and Tangerang. The standard pesticides fenpropatrin, lambda-cyhalothrin, and chlorothalonil were purchased as 200-ppm solutions in acetone from National Plant Quality Assay Laboratory in Pasar Minggu, Jakarta, and were stored at 20°C in a refrigerator. Acetone pure absolute (p.a.; Merck, US), sodium sulfate anhydride p.a. (Merck, US), petroleum ether p.a. (Merck, US Canada), dichloromethane p.a. (Merck, US), iso-octane p.a. (Merck, US), and toluene p.a. (Merck, US) were purchased from respective suppliers.

Analytical instrumentation

Analyses were performed using a Shimadzu model GC-17A GC with an ECD, a 30-m capillary column of 0.25 mm in diameter and 0.25 µm in
Procedures
Preparation of solutions
Standard primary solutions were prepared by evaporating standard pesticide (200 ppm) solutions of up to 1 mL under a flow of nitrogen gas in a 60°C evaporator over 10 min. Pesticide residues were then dissolved in 10-mL aliquots of isooctane-toluene (9:1) to produce solutions of 20 ppm as described by Lozano et al. [12,13].

The calibration curve of fenpropathrin standard liquid was prepared by placing 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, and 4.5-mL aliquots of 100-ppm solutions into six 10-mL glass measuring flasks and filling to volume with isooctane-toluene (9:1). Standard fenpropathrin concentrations were of 20, 25, 30, 35, 40, and 45 ppb. Using 100-ppb cyhalothrin standard solution, we prepared standard solutions of 15, 20, 25, 30, 40, and 45 ppb. Similarly, from 100-ppb cyhalothrin solution, we prepared solutions at 5, 10, 15, 20, 25, and 30 ppb.

Analysis conditions
Analyses were performed with an injection volume of 1 µL and an injector and detector temperature of 300°C using nitrogen as the carrier gas at 1 mL/min. The oven temperature program was 80°C for 1 min, followed by increases to 180°C at 25°C/min and then to 280°C at 8°C/min and finally to 300°C at 30°C/min. The temperature was then maintained at 300°C for 3.17 min. The total time of analysis was about 22 min (Lozano et al., 2016).

Method validation
Linearity tests were performed by injecting six standard solutions of various concentrations. Data were then analyzed using linear regression of concentration versus area, and corresponding r values were generated. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the calibration curve [14].

Precision tests and extractions were conducted as described by Lozano et al. at three concentrations [12,13]. Simulations were performed with organic vegetables as matrix, and pesticide-free status was confirmed. The requirements of this study were 70%–120% and percentage VC <20% [11].

Standard solutions of 200 ppm in acetone were diluted to 100 ppb and were added to the matrix and then extracted as described previously [12,13]. Fenpropathrin was diluted in acetone to 80, 140, and 180 ppb. Subsequently, 6.0-g samples were weighed into 18 different centrifuge tubes and 5-mL aliquots of 20-ppb fenpropathrin solution were added to the first six tubes, 5-mL aliquots of 35-ppb fenpropathrin were added to glass tubes 7–12, 5-mL aliquots of 45-ppb fenpropathrin in acetone were added to tubes 13–18, and all tubes were shaken and then left standing for 5 min. 6 g of anhydride Na2SO4 and 3 mL of acetone were then added, and all tubes were centrifuged for 30 s at 1500 rpm (extraction stage). Thereafter, 8-mL aliquots of petroleum ether and 4-mL aliquots of dichloromethane were added, and the tubes were centrifuged again for 30 s at 1500 rpm (partition stage). After centrifuging at 3300 rpm for 3 min, up to 3 mL of the upper layers were taken and evaporated in a water boiler with increasing temperature from 45°C until extracts were dry. Residues were diluted in 0.9 mL of isooctane-toluene (9:1) and were analyzed under the described conditions. The resulting fenpropathrin solutions had concentrations of 20, 25, 35, 45 ppb, and 1-, 10-, and 40-ppb lambda-cyhalothrin, and 5-, 20-, and 30-ppb chlorothalonil solutions were processed as described above.

Sample preparation
1 km samples of conventional and organic potatoes and tomatoes of differing brands were purchased in Depok, Jakarta, and Tangerang. Extraction procedures were performed according to the study by Lozano et al. but with a volume weight ratio of 2.5 [12,13]. Vegetables were cut and blended, and 0.1-g samples were placed in centrifuge tubes with 8 mL of acetone and 6 g of anhydride Na2SO4. After centrifuging at 1500 rpm for 30 s (extraction stage), 8-mL aliquots of petroleum ether and 4-mL aliquots of dichloromethane were added and the tubes were centrifuged again for 30 s at 1500 rpm (partition stage) and then at 3300 rpm for 3 min. Up to 3 mL upper layers were then taken and evaporated in a water bath with increasing temperature from 45°C to 63°C until residues were dry. Residues were finally dissolved in 0.9-mL aliquots of isooctane-toluene (9:1) and were analyzed as described above.

Establishment of samples
Qualitative and quantitative analyses were performed with 1-µL samples in triplicate using the described analysis conditions.

RESULTS AND DISCUSSION
Condition analysis
Analyses were performed as described by Lozano et al. with slight modifications [13]. Briefly, the oven temperature program was 80°C for 1 min, followed by increasing temperature to 180°C at 25°C/min, to 280°C at 8°C/min, and then to 300°C at 30°C/min, which was maintained for 3.17 min. The total analysis time was about 22 min. Comparisons with the analysis conditions described by Lozano et al. are listed in Table 1 [12,13].

Establishment of retention times
After injecting fenpropathrin, lambda-cyhalothrin, and chlorothalonil at 20 ppm, retention times were 18.363, 19.278, and 10.851 min, respectively.

Validation of analysis methods
Linearity tests
Linearity was tested using fenpropathrin at 20, 25, 30, 35, 40, and 45 ppb. Regression analysis of concentration versus area gave the linear equation \( y = 1061.7x - 177.8 \) with an \( r \) value of 0.9993. Linearity tests for lambda-cyhalothrin were performed at 10, 15, 20, 25, 30, and 40 ppb and gave the equation \( y = 1879.4x + 12787 \) with an \( r \) value of 0.9992. For chlorothalonil at 5, 10, 15, 20, 25, and 30 ppb, the linear equation \( y = 1061.7x - 177.8 \) was generated with an \( r \) value of 0.9993.

Table 1: Differences between analysis conditions between Lozano et al. and the present study

| Notes | Lozano et al. | This study |
|-------|---------------|------------|
| Tools | Bruker 436 GC | Shimadzu GC-17A |
| Detector | MS | ECD |
| Columns | Capillary columns 30 m in length and 0.25 mm in diameter, 0.25-µm film thickness with VF-5 idle phase | Capillary columns 30 m in length and 0.25 mm in diameter, 0.25-µm film thickness with DB-5 idle phase |
| Media phase | Helium with argon | Nitrogen (manual) |
| Injector | Autosampler Variant CP-9400 | 1 µL |
| Injection volume | 5 µL | External standard |

GC: Gas chromatography, ECD: Electron capture detector
Validation of analytical methods

**Linearity tests**

Linearity tests were performed with fenpropathrin at 20, 25, 30, 35, 40, and 45 ppb; lambda-cyhalothrin at 10, 15, 20, 25, 30, and 40 ppb; and chlorothalonil at 5, 10, 15, 20, 25, and 30 ppb. Using plots of concentration versus area, the equations $y=583.57x-4418.2$ ($r=0.9994$), $y=187.4x+12787$ ($r=0.9992$), and $y=1061.7x-177.8$ ($r=0.9993$) were generated for the respective pesticides (Fig. 1).

**LOD and LOQ**

LOD and LOQ values for fenpropathrin, lambda-cyhalothrin, and chlorothalonil were 1.36 and 4.58, 1.45 and 4.85, and 1.12 and 3.73 ppb, respectively.

**Accuracy and precision tests**

The result of accuracy and precision test was summarized in Tables 2-7.

**Establishment of sample concentrations**

Extracted samples were analyzed using GC with an ECD. Analyses were performed in triplicate and no traces of pesticides were found in any of the samples (Table 8).

### DISCUSSION

In the present study, we used GC-ECD instead of GC-MS-MS because it has greater efficiency. Although GC-ECD is limited by the potential for the matrix to capture other electrons, the requirements of linearity were met in our analyses of fenpropathrin, lambda-cyhalothrin, and chlorothalonil, with $r>0.9990$ in all cases [14].

LOD and LOQ of these analyses were 0.005 µg/kg and LOD 1.22 ppb, and these were better than in previous pesticide analyses of lettuces and oranges [14]. These LOD and LOQ values of the three pesticides indicate sufficient sensitivity of these analyses [14].

The present percentage RT and percentage VC values for the three pesticides were 70%–120% and ≤20%, respectively, and fulfilled the associated criteria. However, accuracy values did not increase in these analyses, likely reflecting technical limitations such as variations in additions of standards to vegetables, manual shaking and durations of waiting times, or variations in vortexing during extraction and partition stages.

No pesticide traces were found in any of the present samples, although chromatogram peaks with retention times of 10.78–10.79 min were

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**Table 2: Accuracy and precision tests for fenpropathrin in tomatoes**

| Standard Concentration (ppb) | Area | Standard Measurement (ppb) | RT (%) | $\bar{x}$ | SD (%) | (VC) (%) |
|-----------------------------|------|-----------------------------|--------|-----------|--------|----------|
| 20                           | 8231.9 | 21.677                      | 108.386 | 100.861   | 0.953  | 0.945    |
|                             | 6916.6 | 19.423                      | 102.880 |            |        |          |
|                             | 6708.1 | 19.066                      | 98.705  |            |        |          |
|                             | 7589.4 | 20.576                      | 102.750 |            |        |          |
|                             | 7102.1 | 19.741                      | 102.750 |            |        |          |
|                             | 7574.2 | 20.550                      | 98.705  |            |        |          |
|                             | 12 754.8 | 29.427                      | 84.079  | 86.819     | 3.295  | 3.795    |
|                             | 13 255.4 | 30.285                      | 86.529  |            |        |          |
|                             | 13 612.6 | 30.897                      | 88.278  |            |        |          |
|                             | 13 965.3 | 31.502                      | 90.005  |            |        |          |
|                             | 12 318.5 | 28.680                      | 81.942  |            |        |          |
|                             | 13 980.2 | 31.527                      | 90.078  |            |        |          |
|                             | 15 891.5 | 34.803                      | 77.339  | 79.124     | 4.035  | 5.099    |
|                             | 15 498.6 | 34.129                      | 75.843  |            |        |          |
|                             | 15 684.7 | 34.448                      | 76.551  |            |        |          |
|                             | 16 627.1 | 36.063                      | 80.140  |            |        |          |
|                             | 16 087  | 35.138                      | 78.083  |            |        |          |
|                             | 18372.3 | 39.054                      | 86.786  |            |        |          |
Table 3: Accuracy and precision tests for fenpropathrin in potatoes

| Standard Concentration (ppb) | Area       | Standard Measurement (ppb) | RT (%) | ̄x   | SD (%) | (VC) (%) |
|-----------------------------|------------|---------------------------|--------|------|--------|----------|
| 20                          | 6496.8     | 18.704                    | 93.519 | 96.725 | 0.518  | 0.536    |
| 35                          | 13735.3    | 31.108                    | 88.079 | 85.540 | 3.325  | 3.888    |
| 45                          | 17440.2    | 37.456                    | 83.236 | 85.779 | 0.882  | 1.028    |

Table 4: Accuracy and precision tests for Lambda-Cyhalothrin in Tomatoes

| Standard Concentration (ppb) | Area       | Standard Measurement (ppb) | RT (%) | ̄x   | SD (%) | (VC) (%) |
|-----------------------------|------------|---------------------------|--------|------|--------|----------|
| 10                          | 29410.4    | 8.845                     | 88.451 | 93.095 | 1.305  | 1.401    |
| 25                          | 63747.8    | 27.115                    | 108462 | 97.605 | 2.361  | 2.419    |
| 40                          | 73764.7    | 32.445                    | 81.113 | 82.810 | 1.740  | 2.101    |

Table 5: Accuracy and precision tests for Lambda-Cyhalothrin in Potatoes

| Standard Concentration (ppb) | Area       | Standard Measurement (ppb) | RT (%) | ̄x   | SD (%) | (VC) (%) |
|-----------------------------|------------|---------------------------|--------|------|--------|----------|
| 10                          | 34865.4    | 11.748                    | 117.46 | 114.526 | 0.383  | 0.335    |
| 25                          | 47795.8    | 18.628                    | 73.294 | 86.710 | 3.247  | 3.745    |
| 40                          | 63613.4    | 27.044                    | 90.147 | 100.448 | 3.599  | 3.583    |
Table 6: Accuracy and precision tests for Cyhalothrin in tomatoes

| Standard Concentration (ppb) | Area       | Standard Measurement (ppb) | RT (%) | x̄     | SD (%) | (VC) (%) |
|-----------------------------|------------|----------------------------|--------|--------|--------|----------|
| 5                           | 4465.9     | 4.374                      | 87.477 | 97.288 | 0.3604 | 0.370    |
|                             | 5300.4     | 5.160                      | 103.197|        |        |          |
|                             | 4622.2     | 4.521                      | 90.421 |        |        |          |
|                             | 4954.4     | 4.834                      | 96.679 |        |        |          |
|                             | 5440.7     | 5.292                      | 105.840|        |        |          |
|                             | 5136.7     | 5.006                      | 100.113|        |        |          |
| 20                          | 15 851.5   | 15.098                     | 75.489 | 78.534 | 1.014  | 1.291    |
|                             | 16 499.5   | 16.561                     | 78.258 |        |        |          |
|                             | 15 134.9   | 14.423                     | 72.114 |        |        |          |
|                             | 18 136.8   | 17.250                     | 86.251 |        |        |          |
|                             | 17 319.6   | 16.481                     | 82.403 |        |        |          |
|                             | 16 106.8   | 15.338                     | 76.691 |        |        |          |
| 30                          | 23 581.2   | 22.378                     | 74.594 | 83.108 | 1.991  | 2.397    |
|                             | 25 829.6   | 24.495                     | 81.650 |        |        |          |
|                             | 27 123.5   | 25.715                     | 85.716 |        |        |          |
|                             | 26 812.5   | 25.422                     | 84.739 |        |        |          |
|                             | 29 671.3   | 28.114                     | 93.715 |        |        |          |
|                             | 24 740     | 23.470                     | 78.232 |        |        |          |

Table 7: Accuracy and precision tests for Cyhalothrin in potatoes

| Standard Concentration (ppb) | Area       | Standard Measurement (ppb) | RT (%) | x̄     | SD (%) | (VC) (%) |
|-----------------------------|------------|----------------------------|--------|--------|--------|----------|
| 5                           | 4439.2     | 4.349                      | 86.974 | 84.884 | 0.354  | 0.417    |
|                             | 4366.3     | 4.280                      | 85.600 |        |        |          |
|                             | 3834.5     | 3.779                      | 75.583 |        |        |          |
|                             | 3938.6     | 3.877                      | 77.546 |        |        |          |
|                             | 4813.2     | 4.701                      | 94.019 |        |        |          |
|                             | 4577.7     | 4.479                      | 89.585 |        |        |          |
| 20                          | 15 699.1   | 14.954                     | 74.771 | 79.473 | 1.181  | 1.486    |
|                             | 16 132.8   | 15.363                     | 76.814 |        |        |          |
|                             | 18 476.2   | 17.570                     | 87.850 |        |        |          |
|                             | 15 191.5   | 14.476                     | 72.381 |        |        |          |
|                             | 17 718.8   | 16.857                     | 84.283 |        |        |          |
|                             | 16 966.3   | 16.148                     | 80.740 |        |        |          |
| 30                          | 23 586.5   | 22.383                     | 74.611 | 78.605 | 1.929  | 2.454    |
|                             | 23 213.1   | 22.032                     | 73.439 |        |        |          |
|                             | 24 066     | 22.835                     | 76.116 |        |        |          |
|                             | 28 829.8   | 27.322                     | 91.073 |        |        |          |
|                             | 24 407     | 23.157                     | 77.187 |        |        |          |
|                             | 25 050.3   | 23.762                     | 79.206 |        |        |          |

Table 8: Analyses of fenpropathrin, lambda-cyhalothrin, and chlorothalonil contents in tomatoes and potatoes

| Commodity | Notes | Fenpropathrin | Lambda-Cyhalothrin | Chlorothalonil |
|-----------|-------|---------------|--------------------|----------------|
| Potatoes  |       |               |                    |                |
|           | Depok | Undetected    | Undetected         | Undetected     |
|           | Jakarta | Undetected | Undetected         | Undetected     |
|           | Tangerang | Undetected | Undetected         | Undetected     |
|           | Organic B | Undetected | Undetected         | Undetected     |
|           | Organic H | Undetected | Undetected         | Undetected     |
|           | Organic S | Undetected | Undetected         | Undetected     |
| Tomatoes  |       |               |                    |                |
|           | Depok | Undetected    | Undetected         | Undetected     |
|           | Jakarta | Undetected | Undetected         | Undetected     |
|           | Tangerang | Undetected | Undetected         | Undetected     |
|           | Organic O | Undetected | Undetected         | Undetected     |
|           | Organic P | Undetected | Undetected         | Undetected     |
|           | Organic S | Undetected | Undetected         | Undetected     |

observed from several of the tomato and potato samples. This retention time range was similar to that of chlorothalonil (10.82–10.84) but was sufficiently different to conclude that the undefined peaks were not chromatogram peaks of chlorothalonil and were likely due to matrix components.

CONCLUSION

Our linearity tests showed that all standards met the requirement of r≥0.9990, and these were applicable to fenpropathrin, lambda-cyhalothrin, and chlorothalonil concentration ranges of 20–45 ppb,
10–40 ppb, and 5–30 ppb, respectively. LOD and LOQ values for fenpropathrin, lambda-cyhalothrin, and chlorothalonil were similar to those reported by Lozano et al. and indicated high sensitivity of analyses [12,13]. Accuracy and precision values also fulfilled the requirements, further validating of the methods used in this study. Finally, all samples fulfilled the requirements of SNI 7313: 2008 “Maximum Limit of Pesticides Residual Products in Harvest Commodities” and the other terms and conditions of Japanese standards.

CONFLICTS OF INTEREST
All authors declare that they have no conflicts of interest.

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