Pyrethroid analysis in fresh tea leaves: preliminary study in building the low volume extraction

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Abstract. Low volume extraction using rotary agitator was used in order to develop method analysis for simultaneous determination of five pyrethroids (lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate, and deltamethrin) in fresh tea leaves. The application of n-hexane, n-hexane with some modifications, acetonitrile, and ethyl acetate were tested to extract the pyrethroids before being injected to gas chromatography coupled with electron capture detector. The experiment was conducted in spike-experiment where known amount of pyrethroids were fortified into the fresh tea leaves and the results were compared to the no-spike-experiment where no pyrethroids were added. Result showed that n-hexane was able to extract all target pyrethroids from fresh tea leaves but the recovery values were low. Meanwhile, acetonitrile and ethyl acetate were able to extract pyrethroids with higher recovery values compare to n-hexane but only acetonitrile gave recovery values that were in the range suggested by European Union. Therefore acetonitrile is suggested as a solvent for low volume extraction of pyrethroids from fresh tea leaves.

Keywords: pyrethroids, tea infusion, low volume liquid-liquid extraction, green analytical chemistry

1. Introduction
As the most valued item in tea plantation, tea leaves are vulnerable to insects such as mites, beetles, and caterpillars [1]. Meanwhile, pyrethroids are capable to wide-spectrum of insects and are known to be very effective for sucking pests in tea, mostly by attacking the nerves of the pests [1-4]. These have led to the application of pyrethroids in tea plantation.

Pyrethroids were favourable compare to the more toxic organochlorin [3] thus replaced the application of organochlorin that was banned due to its toxicity. However, they are still nerves poisons [3] and some recent studies show their neurotoxicity in mammals [5,6] in addition to other adverse effect on human [7].

Based on their structures, pyrethroids are classified as esters and divided in two types, depend on whether they have cyano groups or not [7,8]. Permethrin, which does not contain a cyano group, is an example of type I, while cypermethrin, deltamethrin, and fenvalerate are type II with cyano groups [8]. This cyano groups are believed to increase the capability of pyrethroids in inhibiting the pests [3,7]. Structure of some pyrethroids with the highlighted cyano groups are given in Figure 1.
Figure 1. Structures of some pyrethroids.

Since pyrethroids are usually applied in tea plantation, there is a possibility of pyrethroids residue in tea leaves. Studies have linked the application of pyrethroid in agriculture and their residues [7]. Moreover, as previously mentioned, pyrethroids may cause negative effects on human health thus their occurrence in tea leaves are of concerns. In addition, pyrethroid detections in tea samples especially dried tea have been reported considerably [9-13].

In our previous report [13], n-hexane was able to extract pyrethroids from commercial tea samples and primary secondary amine (PSA) was used to remove interferences. This paper report the preliminary study in building the method for analysis of pyrethroids in fresh tea leaves. Several procedures including the application of n-hexane, n-hexane with some modifications, acetonitrile, and ethyl acetate were tested to extract the pyrethroids before being injected to gas chromatography coupled with electron capture detector.

2. Experimental

2.1. Chemicals and reagents

Five pyrethroids, lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate, and deltamethrin, were used in this study. These pyrethroids were purchased as separated standards from Chem Service, USA, with purity >99.2 %. Stock solutions were prepared separately for each pyrethroid at around 100 mg/L in n-hexane. Mix stock solution was prepared from the stock solutions so the concentration of each pyrethroid was around 5 mg/L. The mix stock solution was then diluted in n-hexane to obtain standard solutions at desired concentrations. The mix stock solution was also used for the spike-experiment when pyrethroid fortification was needed. Primary secondary amine (PSA) and graphitized carbon black were sourced from Agilent, USA. Solvents and other chemicals were sourced from Merck, German, unless otherwise stated. The fresh tea leaves used in this study were collected from a tea plantation in West Java area with no history of pyrethroids application. The fresh tea leaves were kept at -4°C and were processed using food blender (Panasonic) just before the experiment.
2.2. Apparatus
The pyrethroid quantification was performed on an Agilent 7890B gas chromatography (GC) equipped with a micro electron capture detector (ECD) from Agilent at chromatographic conditions as explained in Pitoi et al. [13,14], except for the detector temperature that was set at 300 °C. A rotary agitator was used to perform the low-volume extraction. A rotary agitator was used to perform the low-volume extraction while EBA-12 and Vortex Genius-3 were used for centrifugation and vortex respectively during the extraction.

2.3. Low volume extraction
For the low volume extraction, fresh tea leaves and solvent were added into a capped centrifuge tube and extracted in a rotary agitator. For the spike experiment, certain amount of mix stock solution was added into the fresh tea leaves so the final concentration of each pyrethroid in the fresh tea leaves was 0.5 ppm, except for the experiment where different concentrations of pyrethroids were applied. The no-spike experiment was also conducted in parallel to the spike-experiment in which no pyrethroid was added into the fresh tea leaves. All experiment was conducted in 3 replicates. For the extraction using n-hexane, the procedure is as explained in the previous paragraph. The sample was rest for an hour after the addition of pyrethroids and then n-hexane was used as a solvent during the low volume extraction.

For the modification of n-hexane extraction, there were four procedures tested: the sample was directly extracted after the addition of pyrethroids (no rest hour); the extraction was done twice (double) in which fresh n-hexane was used after the first extraction and n-hexane phase from both extractions was combined; acetonitrile was mixed with n-hexane and used as the solvent; and Milli-Q water was added to the fresh tea leave after the fortification and the sample was left for 30 minutes before the extraction. For the extraction using acetonitrile and ethyl acetate as solvents, the extraction was similar to the n-hexane except for the type of solvent that was used.

3. Result and discussion

3.1. Extraction using n-hexane
In our previous study, n-hexane was able to extract pyrethroids from dried tea [13] and infusion tea [14]. Therefore in this study, n-hexane was initially chosen to extract pyrethroids. Preliminary qualitative experiment showed that n-hexane, without any clean up, was able to extract the pyrethroids (Figure 1) and its chromatogram showed that all pyrethroids and their isomers are well separated. Moreover, as shown in Figure 2, other compounds did not interfere with the target analytes which benefited the extraction. Therefore, further quantitative experiment was then performed.

Three spike-concentrations, 0.05, 0.1, and 0.5 mg/kg, were used for the experiment in triplicates. This experiment was compared to the control where no pyrethroid was added into the fresh tea samples. The recovery values were then calculated and the result is given in Figure 3.

![Figure 2. Chromatogram of pyrethroids extracted by n-hexane](image-url)
Figure 3 shows that, although n-hexane is able to extract all pyrethroids, the recovery values are poor for all tested concentration and for all pyrethroids except for permethrin. Moreover, the concentrations of pyrethroids do not comparably affect the recovery values in the range of 0.05 to 0.5 mg/kg since the values for each pyrethroid for the different concentrations are quite similar. The suggested recovery values for residue analysis is 70 to 120% [15] as suggested by European Union (EU). Thus based on recovery data, the utilization of n-hexane alone during extraction of pyrethroids can only be used to extract permethrin (at 0.05 and 0.1 mg/kg) from fresh tea leaves.

This result is quite surprising. Theoretically, non-polar compounds with high log K_{ow} values such as pyrethroids [16-20] will be soluble in organic solvent like n-hexane thus it was predicted that the recovery values will be high as in dried tea [13]. However, this study suggests that n-hexane is not suitable to extract pyrethroids from fresh tea leaves.

![Figure 3. Recovery of pyrethroids extracted by n-hexane at 0.05, 0.1, and 0.5 mg/kg.](image)

### 3.2. Modification of extraction procedures for n-hexane
Modification of the extraction procedures was applied to increase the recovery of the extraction. No-rest hour was conducted to eliminate the matrix-pyrethroids bond during rest hour (procedure 2). Double extraction (procedure 3) was conducted to see the effect of number of extraction, procedure 4 was conducted to see the effect of more polar extraction solvent, acetonitrile was added to n-hexane, and procedure 5 was conducted to see the effect of hydration. The recovery values for procedures 2-4 was then compared to when n-hexane was used as sole solvent to extract (procedure 1) and the comparison is given in Figure 4.

Similar to the extraction with n-hexane alone, the four tested procedures failed to extract pyrethroids with recovery of 70 to 120% except for permethrin (procedure 2 and 3 only). This implies that rest hour, number of extraction, more polar solvent, and hydration, do not increase the recovery if n-hexane is used as the solvent in fresh tea leaves extraction as suggested in previously.

### 3.3. Extraction using acetonitrile and ethyl-acetate
Further study was then conducted to extract pyrethroids from fresh tea leaves using acetonitrile and ethyl acetate and the recovery result is given in Figure 5. Figure 5 shows that the recovery of pyrethroids extracted by acetonitrile and ethyl acetate are very much better than extracted by n-hexane.

Recovery values of acetonitrile for all pyrethroids fall in the range of 70 to 120%. This suggested that acetonitrile can be used to extract all pyrethroids from the fresh tea leaves. However, although ethyl acetate gives recovery higher than n-hexane, its values for permethrin, cypermethrin, and fenvalerate exceed the recovery guideline.
Figure 4. Recovery of pyrethroids extracted at different procedures.

Figure 5. Recovery of pyrethroids extracted by different solvents.
The low recovery values of low volume extraction using n-hexane as sole solvent or n-hexane with some modifications is probably due to the complicated tea matrix. Tea, including tea leaves, consists of so many components that may co-extracted by n-hexane and thus decreases the recovery values [21, 22]. Similarly, the high recovery values observed for ethyl acetate is probably contributed to signal enhancement that may be caused by interferences of the complicated tea matrix. These are needed to be further investigated.

4. Conclusion
Although n-hexane was able to extract all target pyrethroids from fresh tea leaves, the recovery values were below the minimum value suggested by EU. Meanwhile, acetonitrile and ethyl acetate were able to extract pyrethroids with higher recovery values compare to n-hexane but some recovery values for ethyl acetate were above the suggested recovery of EU. Therefore, only acetonitrile is suggested as a solvent for low volume extraction of pyrethroids from fresh tea leaves.

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References
[1] Chen G, Cao P and Liu R 2011 A multi-residue method for fast determination of pesticides in tea by ultra performance liquid chromatography–electrospray tandem mass spectrometry combined with modified QuEChERS sample preparation procedure Food Chem 125 1406-1411
[2] Mochizuki M 2003 Effectiveness and pesticide susceptibility of the pyrethroid-resistant predatory mite Amblyseius womersleyi in the integrated pest management of tea pests BioControl 48 207-221
[3] Casida J E 1980 Pyrethrum flowers and pyrethroid insecticides Environ. Health Perspect. 34 189-202
[4] Esteve-Turrillas F A, Pastor A, and Guardia M 2005 Determination of pyrethroid insecticide residues in vegetable oils by using combined solid-phases extraction and tandem mass spectrometry detection Anal. Chim. Acta 553 50-57
[5] Boonchianma S, Ngeontae W, and Srijaranai S 2012 Determination of six pyrethroid insecticides in fruit juice samples using dispersive liquid–liquid microextraction combined with high performance liquid chromatography Talanta 88 209-215
[6] Yu X, Ang H C, Yang H, Zheng C, and Zhang Y 2017 Low temperature cleanup combined with magnetic nanoparticle extraction to determine pyrethroids residue in vegetables oils Food Control 74 112-120
[7] Ogaly H A, Khalaf A, Ibrahim M A, Galal M K, and Abd-Elsalam R M 2015 Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain Neurotoxicol. Teratol. 50 23-31
[8] Kim K B, Anand S S, Kim H J, White C A, Fisher J W, Tornero-Velez R, and Bruckner J V 2010 Age, dose, and time-dependency of plasma and tissue distribution of deltamethrin in immature rats Toxicol. Sci. 115 354-368
[9] Cho S K, Abd El-Aty A M, Rahman M M, Choi J H, and Shim J H 2014 Simultaneous multi-determination and transfer of eight pesticide residues from green tea leaves to infusion using gas chromatography Food Chem. 165 532-539
[10] Paramasivam M and S Chandrasekaran 2014 Persistence behaviour of deltamethrin on tea and its transfer from processed tea to infusion Chemosphere 111 291-295.
[11] Li B, Zeng F, Dong Q, Cao Y, Fan H and Deng C 2012 Rapid Determination Method for 12 Pyrethroid Pesticide Residues in Tea by Stir Bar Sorpitive Extraction-Thermal Desorption-Gas Chromatography Phys. Procedia 25 1776-1780
[12] Wu C C 2017 Multiresidue method for the determination of pesticides in Oolong tea using QuEChERS by gas chromatography-triple quadrupole tandem mass spectrometry Food Chem. 229 580-587
[13] Pitoi M M, Ariyani M, Koemawati T A, and Yusiasih R 2019 Pyrethroids residues analysis in Indonesian commercial tea by GC-ECD AIMS Agric. Food 4 447-457
[14] Pitoi M M, Ariyani M, Rosmalina R T, and Koesmawati T A 2019 Simultaneous determination of deltamethrin and 4 other pyrethroids residues in infusion tea: Preliminary study *IOP Conference Series: Earth and Environmental Science* **277** 012021

[15] European Commision 2010 Guidance document on pesticide residue analytical methods

[16] HSDB 2012 Hazardous Substance Data Bank: Cyhalothrin U.S. National Library of Medicine

[17] HSDB 2012 Hazardous Substances Data Bank: Cypermethrin U.S. National Library of Medicine

[18] HSDB 2018 Hazardous Substances Data Bank: Fenvalerate U.S. National Library of Medicine

[19] HSDB 2012 Hazardous Substances Data Bank: Deltamethrin U.S. National Library of Medicine

[20] HSDB 2014 Hazardous Substances Data Bank: Permethrin U.S. National Library of Medicine

[21] Kanrar B, Mandal B, and Bhattacharyya A 2010 Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography–tandem mass spectrometry *J Chromatogr A* **1217** 1926-1933

[22] Maštovská K and Lehotay S J 2004 Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues *J Chromatogr A* **1040** 259-272