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Identification of pesticide residues in harvested edamame soybeans and application of the water ozonation technique

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Abstract The results showed that four types of active compounds in the pesticide including Captan, Methyl thiophanate, Imidacloprid, and Cypermethrin which were used as standards for detecting residues in Edamame, the Imidacloprid, and Cypermethrin types were not found in either the soybeans harvested from the farmer or the company fields. On the other hand, Methyl thiophanate residue was found in the fresh shells of soybeans harvested from the company, while Captan residue was found in soybean intact pods harvested from the farmer's field. Captan and Methyl thiophanate have different properties when applied in the field. Captan is a type of pesticide compound that dissolves easily during the washing process, but using it for a long period causes accumulation on tissues and it is difficult to remove. Meanwhile, Methyl thiophanate residue is included in the insoluble category or does not easily decompose when washed, and it can permeate into all parts of Edamame. The ozonated-water technique with a dose of 0.12 ppm for 1 h was able to reduce the residue of Captan pesticide by 30.13% on the shells and intact pods of Edamame, while Methyl thiophanate residue can be reduced up to 80.97% on the shells of Edamame seeds.

Keywords: Decleaning Residue, Edamame soybean, Pesticides residue, Water Ozonating treatment

Introduction

Pesticide residues found in Edamame are caused by the use of drugs such as insecticides and fungicides during plant maintenance (on farm). Plants need to be protected from damage caused by pests and diseases can reduce productivity, and crop failure if there are pods that are deformed by pests, the product becomes cheap or cannot be sold. The use of pesticides for pest and disease control must be carried out with care, ensuring that there are no pesticide residues when the pods are harvested, considering that the residue threshold requirements set for exported Edamame are very strict.

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Pest and disease control starts from seed planting and sometimes even until the plants are ready to be harvested. The selection of pesticides is an activity that is routinely carried out by farmers before applying them to plants because it affects the efficiency and effectiveness of their use. Every pest shows a typical symptom, or specific attack. Farmers must be able to recognize symptoms that arise and distinguish the types of pest attacks on plants, among others due to pests, diseases, or other organisms. Determine the specific cause of the problem that occurs in the plant, select and adjust the type of pesticide as needed.

Edamame soybean is a plant that in its cultivation process requires more attention, starting from fertilizing, irrigation and controlling pests and diseases to get high production. Excessive use of pesticides is often found in controlling pests and diseases (Chiabrando *et al*., 2017). However, the development of the international market demands the fulfillment of quality standards, especially the use of chemical pesticides and their residue levels in export products. One of the new developments in food preservation technology is the use of Ozone, which is a reactive form of oxygen (O_2) and is capable of destroying a large number of toxic particles through the oxidation process. Several studies have developed ozonated water treatment as a substitute for chemicals to suppress microorganism decontamination as well as suppress pesticide residues on several types of fruits and vegetables (Brandao *et al*., 2016; Papachristodoulou *et al*., 2017; de Souza Ferreira *et al*., 2017).

Trombete *et al*. (2016) reported that the rate of degradation of Methyl parathion increased in line with the increasing concentration of Ozone. Ozone treatment was effective in reducing residual concentrations to 72% Captan, 75% Methyl azinphos, and 46% in post-harvest apples. Research by Lozowicka and Jankowska (2016) shows that the Ozonated water used for washing vegetables depends on the level of Ozone concentration and temperature. The results showed that at a temperature of 14^{o}C, within 30 min, 36.2% of Diazinon residue could be removed; 24.8% Methyl parathion; 19.7% Paration, and 44.3% Cypermethrin in vegetables using Ozonated water in 2 mg L^{-1} Ozone solution. Efficiency increased at 24^{o}C at the same Ozone concentration and decreased to 53.4% diazinon residue; 47.9% Methyl parathion; 55.3% Paration and 61.1% Cypermethrin in vegetables. The four organophosphate pesticides, namely Methyl parathion, Paration, Diazinon, and Cypermethrin, are used in a broad spectrum to control pests and cause high residue concentrations in vegetables (Łozowicka and Jankowska, 2016).

Jember is central for Edamame soybeans in East Java Province. Most of the soybeans produced are to meet export markets, especially Japan. Preliminary studies that have been conducted obtained data that most Edamame soybean farmers use pesticides with the active compounds of Captan, Methyl thiophanate, Cypermethrin, and Imidacloprid to control pests and diseases. Based on the preliminary study, research is needed regarding the use of pesticides and residues left behind in soybean harvests which are closely related to consumer health. The objectives were to identify pesticide residues on fresh Edamame harvested by the company and farmer, to know the types of exposure to pesticide residues and determine the proper method of declining or removing residues on the pods and seeds of Edamame soybean, and to determine the effectiveness of the Ozonated water technique in reducing pesticide residues on the shell, seeds, and whole Edamame soybean pods.

Materials and methods

The research was carried out from March 2020 to March 2021 at the Laboratory of Bioscience; Laboratory of Food Analysis, and Laboratory of Food Processing; Polytechnic of Jember, Jember, East Java Province, Indonesia. The Edamame soybean used were those that had just been harvested, and sourced from two locations; fields owned by individual farmers and farmers' fields in partnership with the company (PT Gading Mas Jember). Captan and Methyl thiophanate pesticides were prepared with a concentration of 2.5 ppm each and used for spraying on de-cleaning effectiveness analysis. The CRM pesticides standard (Supelco, Sigma Aldrich) was used for quantitative analysis.

Methods of research

The research was conducted using a mathematical model design (Steel and Torrie, 1991). The research was conducted in 2 stages. The first stage is the identification of the pesticide residue content of Edamame soybeans comes from the farmer and the company, including the shells, seeds and pods of Edamame. Identification of pesticide residues which includes Captan, Methyl thiophanate, Cypermethrin, and Imidacloprid was carried out on several parts of soybeans.

The second stage is to analyze the effect of the Ozonated water technique in reducing pesticide residues on Edamame soybeans, by comparing the identified pesticide residues in samples of the shells, seeds, and pods of Edamame soybeans that have been treated with the Ozonated water technique and without ozone. Ozonated water washing technique was treated using an Ozone Generator machine (Elitech, Ozone Technology Co. Ltd) with a concentration of 0.12 ppm for 1 h. To determine the characteristics of pesticide properties and ozonation effects, soybean samples were compared between those obtained directly and those sprayed with the type of pesticide identified. All experiments were repeated three times.

Analysis of pesticide residue

The sample for each treatment was prepared from 1 kg of fresh Edamame soybeans. Analysis of pesticide residues was observed on direct samples which come from the fields and samples that were treated by sprayed for each residue of Captan, Methyl thiophanate, Cypermethrin, and Imidacloprid pesticides. The samples were then washed and air-dried for analysis of pesticide residues

Isolation of pesticide residues in Edamame samples was carried out following the QuEChERS method (Anastassiades *et al*., 2003). After the sample was prepared according to the treatment, then the extraction process was carried out by homogenizing 15 g of the sample with a blender until smooth, then transferring it to a 50 ml centrifuge tube and adding 15 ml of Acetonitrile. Then the sample was sonicated for 2 min, vortexed for 5 min, added 6 g of anhydrous $MgSO_4$ and 2.5 g of Sodium acetate trihydrate. The sample was vortexed/shaken vigorously for 5 min, then centrifuged at 4000 rpm for 5 min. The supernatant obtained was transferred to a new centrifuge tube with the addition of 1.8 g anhydrous $MgSO_4$ and 0.3 g PSA. The extract was shaken using a vortex mixer for 20 sec and centrifuged at 4000 rpm for 5 min. Approximately 3 ml of the supernatant was filtered through a 0.45 mm d 13 mm PTFE filter (Sartorius, France). A total of 800 µl of the filtrate was transferred to an Autosampler bottle.

The parent standard solution of pesticides Captan, Methyl thiophanate, Cypermethrin, and Imidacloprid (Supelco, Sigma Aldrich) was dissolved in double distillate water: acetonitrile in a ratio of 25/75 (v/v) with each concentration reaching 100 mg L^{-1}. From this master standard, a series of standards is then made for sample analysis.

Samples were analyzed using LC-MS 2020 (Shimadzu, Japan), with a mass spectrometer detector QTRAP 3200, column C18 5 m (150mm x 4.6mm), at a temperature of 25 ^{o}C. The mobile phase used was eluent A: distilled water and 0.1% formic acid, and eluent B: acetonitrile. The flow used gradient elution starting from 20% - 100% eluent B. The sample injection volume was 10 µl with a flow rate of 0.6 ml/min and an analysis time of 12 min.

Statistical analysis

Data were analyzed statistically using Two-Way Traffic Analysis of Variance (ANOVA), and further statistical tests to assess differences between interrelated treatment pairs were analyzed using the Paired sample T-test, to compare the reduction in pesticide residues in each part of Edamame whether with ozone treatment or not. The level of significance was determined at $p<0,05$.

Results

Identification of four types of pesticide residues in Edamame soybeans

Based on the preliminary study, there are four types of pesticide-active compounds that are commonly used by farmers in controlling pests and diseases in soybean plants, including Captan, Methyl thiophanate, Imidacloprid and Cypermethrin. Based on this information, samples of soybeans from the field were prepared from two sources: soybeans cultivated by the farmer and soybeans owned by farmer who partnered with the company, ready to be identified.

Identification of the presence of pesticide residues in soybeans was carried out using analytical chromatography, in which intact soybean pod samples were separated into shells, seeds, and pods. This sample separation aims to determine the type of pesticide used and its distribution on the object, in this case the soybean pods.

Table 1. Identification result of four types of pesticide residues on seeds, shells, and pods of Edamame harvested from the company and farmer fields

Type of sample	**Methyl thiophanate (ppm)**	**Imidacloprid (ppm)**	**Captan (ppm)**	**Cypermethrin (ppm)**
Company (Seeds)	*nd*	*Nd*	*nd*	*nd*
Company (Shells)	5.37 ±0.01	*Nd*	*nd*	*nd*
Company (Pods)	*nd*	*Nd*	*nd*	*nd*
Farmer (Seeds)	*nd*	*Nd*	*nd*	*nd*
Farmer (Shells)	*nd*	*Nd*	3.39 ±0.07	*nd*
Farmer (Pods)	*nd*	*Nd*	3.11 ±0.07	*nd*

nd = not detection

The results of the analysis showed that the Edamame soybean cultivated by the company contained Methyl thiophanate residue, while the other 3 types of pesticide residues (Imidacloprid, Captan and Cypermethrin) were not found. On the other hand, Captan residue was found in Edamame soybeans cultivated by the farmer (Table 1 and Figure 1). The Methyl thiophanate residue present in soybean shells from the company is a higher concentration than the Captan residue found in soybeans from the farmer in the same tissue, with the Methyl thiophanate and Captan concentrations of 5.37 ppm and 3.39 ppm, respectively (Table 1). The concentration of Captan contained in the pods which the sample came from a mixture of seeds and shells, showed lower concentrations compared to those found in the shells.

Based on the results obtained, the further analysis is focused on residues of Methyl thiophanate and Captan found in soybeans from company and farmer, especially to determine the effectiveness of the method used to reduce the residues, in this case, the water ozonation technique, and to determine the character of pesticide residues.

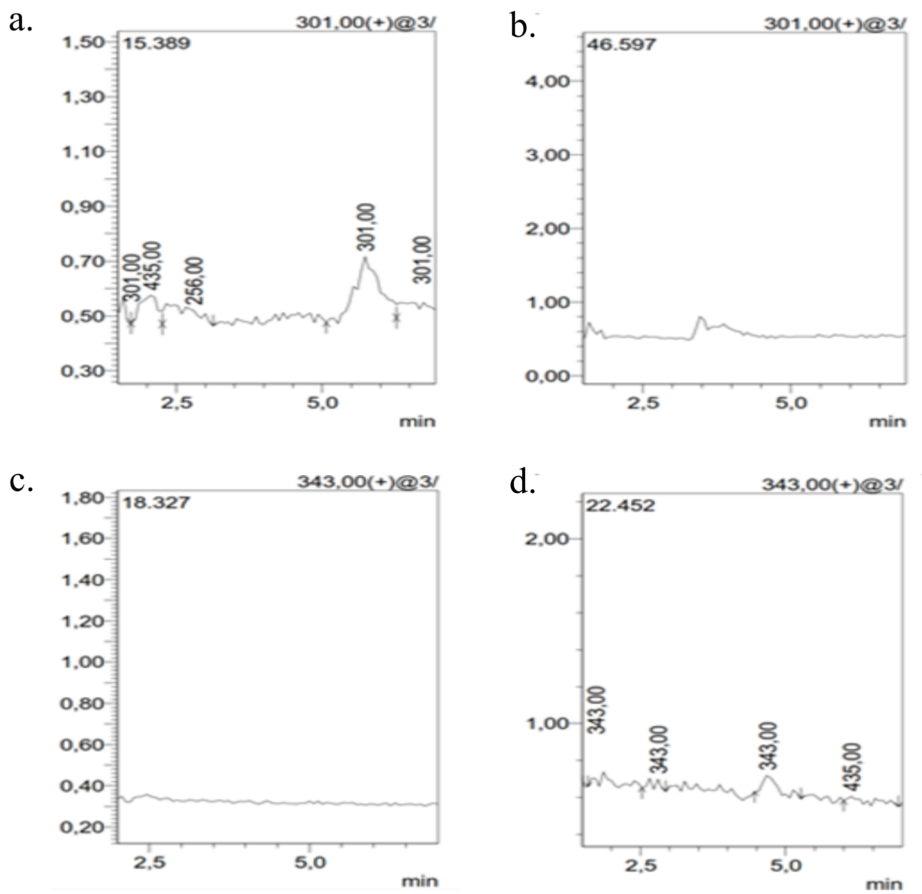

Figure 1. Chromatography analysis of Captan and Methyl thiophanate residues in Edamame shells. Chromatogram analysis of Captan (a) and Methyl thiophanate (c) is prepared from farmer's soybeans samples, while the chromatogram analysis of Captan (b) and Methyl thiophanate (d) is the result of soybean samples from the company

Reduction of pesticide residue after ozonated water-treated

Residue of Captan

To determine the effectiveness of the de-cleaning method with ozone treatment and the process of penetration of the Captan pesticide after it was applied to soybeans, the soybean samples from two different sources were divided into the seeds, shells and intact pods which were then sprayed with Captan pesticide. Spraying with Captan is intended to represent samples of newly harvested soybeans that were sprayed with pesticides excessively, especially after approaching harvest age. It is expected that by comparing Captan residue in Edamame pods between field applications and spraying treatments, the penetration and decomposition characteristics of Captan residue can be identified.

Captan spraying was carried out on fresh soybeans followed by wind-drying and washing with water before the residue content was analyzed. The stages were the same for the control, but Captan was not sprayed. As shown in Table (2), the decrease in the concentration of Captan residue before treatment (control) and after the ozone treatment was used to calculate the percentage reduction in residue after the ozone treatment.

Table 2. Analysis residue of Captan pesticides on several parts of Edamame soybeans after ozone treatment

Type of sample	Control (ppm)	Ozone-treated (ppm)	Reduction of residue (%)
A. Company's harvest			
Seeds	*Nd*	*nd*	-
Shells	*Nd*	*nd*	-
Pods	*Nd*	*nd*	-
Spraying Captan : Seeds	*Nd*	*nd*	-
Spraying Captan : Shells	*Nd*	*nd*	-
Spraying Captan : Pods	*Nd*	*nd*	-
B. Farmer's harvest			
Seeds	0	0	-
Shells	3.39 ±0.01	2.67 ±0.03*	21.39 ±0.80
Pods	3.11 ±0.07	2.34 ±0.03*	24.65 ±1.00
Spraying Captan : Seeds	0	0	-
Spraying Captan : Shells	3.87 ±0.04	2.71 ±0.05*	30.13 ±1.14
Spraying Captan : Pods	2.80 ±0.01	2.49 ±0.03*	10.97 ±0.77

nd = not detection
* = Significant by $t_{\alpha=0.05; 2} = 4.303$

It showed that Edamame soybeans from the company did not detect Captan residue on the seeds, shells and intact pods, both samples taken directly from the field and samples treated with Captan pesticide spraying (Table 2A). In contrast to Edamame soybeans originating from farmer, Captan residue was detected in Edamame shells and pods samples, but not in seeds (Figure 2). Captan residue found on the shells was higher than in the intact pods, both in soybean samples from the field and the spraying treatment of 3.39 ppm and 3.87 ppm, respectively.

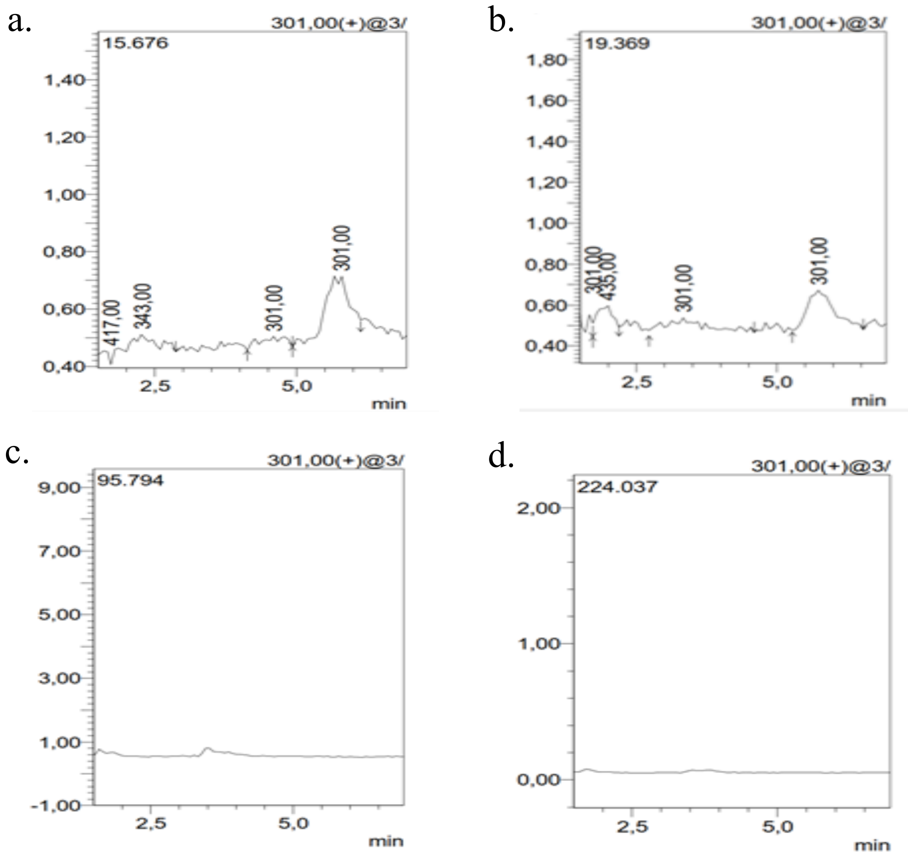

Figure 2. Analysis of Captan pesticide residue on Edamame shells obtained from the Farmer (a) and Company (c). Edamame shells sample was sprayed with Captan pesticide and compared the results between the treatment without Ozone (a and c), and with Ozone (b and d). Chromatogram obtained using Chromatography Mass Spectrometry analysis

The ozone treatment was able to significantly reduce the Captan residue contained in the shells and intact pods, both in soybean samples from direct fields or soybeans by spraying treatment. The content of Captan residue was found to decrease to 21.39% in soybean shells taken directly from the field, after being treated with ozone, while intact pod sample taken from the same source decreased by 24.65%. In Edamame soybean samples from farmer which additional treatment with Captan pesticide spraying, ozone treatment was able to reduce the residues on shells and intact pods by 30.13% and 10.97%, respectively (Tabel 2B). Additional Captan pesticide that is applied by spraying does not affect the accumulation of Captan residue in the shells and intact pods as well.

Residue of Methyl thiophanate

In the same case as the Captan pesticide, the residual content of Methyl thiophanate was measured to determine the character and effectiveness of the de-cleaning method with ozone treatment of the pesticide after application, especially for crops that also have the potential to be used as feed. The soybean samples from two different sources were divided into seeds, shells and intact pods which were then sprayed with Methyl thiophanate pesticide. The percentage reduction of Methyl thiophanate residue is the percentage difference between the concentrations of residue in Edamame without ozone treatment and that of residue treated with ozone.

Table 3. Analysis residue of Methyl thiophanate pesticides on several parts of Edamame soybeans after Ozone treatment

Type of sample	Control (ppm)	Ozone-treated (ppm)	Reduction of residue (%)
A. Company's harvest			
Seeds	0	0	-
Shells	5.37 ±0.10	0	100
Pods	0	0	-
Spraying Methyl thiophanate : Seeds	4.14 ±0.12	1.73 ±0.03*	58.28 ±0,85
Spraying Methyl thiophanate : Shells	21.23 ±0.31	4.04 ±0.14*	80.97 ±0.88
Spraying Methyl thiophanate : Pods	8.81 ±0.05	2.98 ±0.03*	66.19 ±0.30
B. Farmer's harvest			
Seeds	0	0	-
Shells	0	0	-
Pods	0	0	-
Spraying Methyl thiophanate : Seeds	4.23 ±0.16	2.18 ±0.01*	48.52 ±1.78
Spraying Methyl thiophanate : Shells	25.79 ±0.11	20.50 ±0.31*	20.51 ±0.84
Spraying Methyl thiophanate : Pods	12.46 ±0.21	4.69 ±0.12*	62.37 ±1.56

* Significant by t $\alpha_{0.05;2}$ = 4.303

Methyl thiophanate residue was detected in all Edamame tissues to which Methyl thiophanate pesticide sprays were added, both from company and farmer as shown in Table 3. The residue was also detected on soybean shells taken directly from the field without being treated with pesticide spraying. Methyl thiophanate residue showed high accumulation in shells and intact pods (Figure 3), and the concentrations in shells were higher than in intact pods. Methylthiophanate residue was also detected in Edamame seeds, from both sample sources.

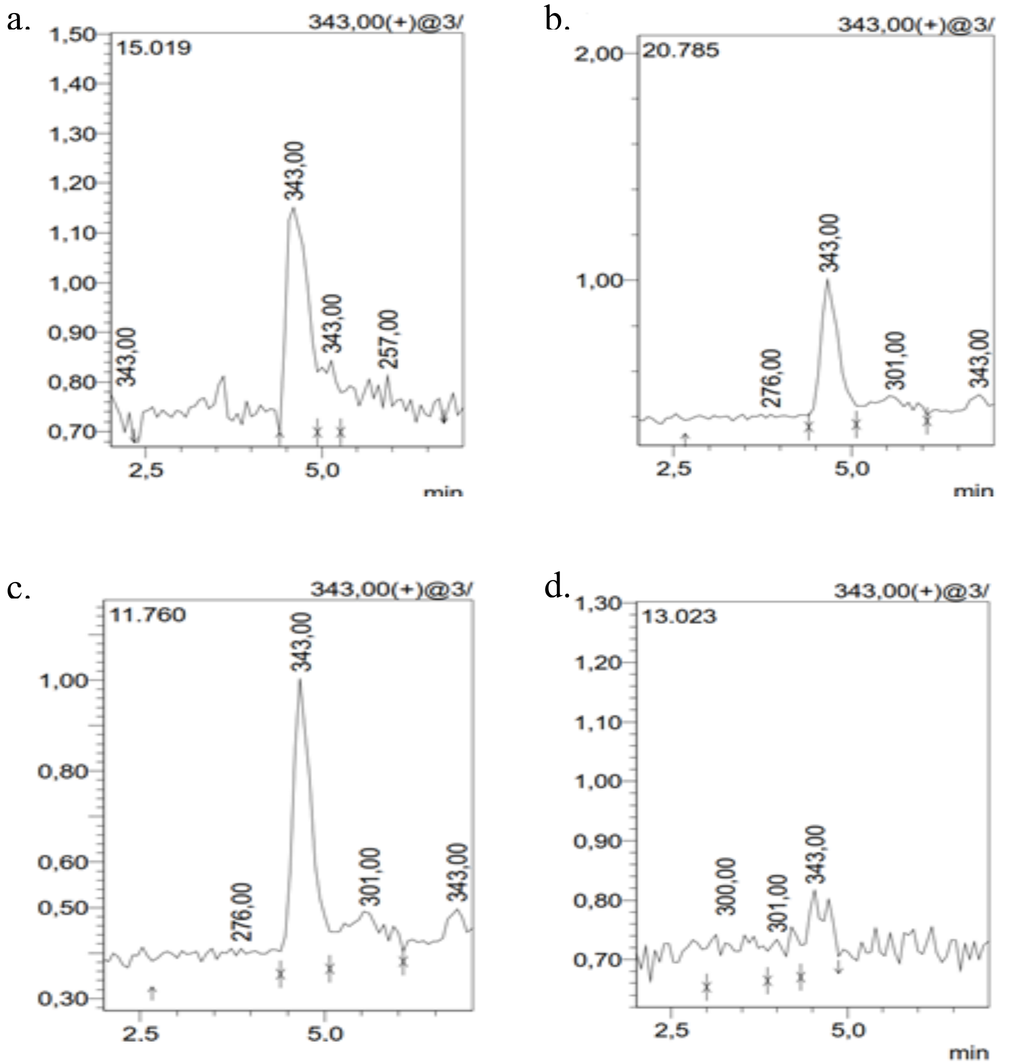

Figure 3. Analysis of Methyl thiophanate pesticide residue on Edamame shells obtained from the Farmer (a) and Company (c). Edamame shells sample was spraying with Methyl thiophanate pesticide and compared the results between the treatment without Ozone (a and c), and with Ozone (b and d). Chromatogram obtained using Chromatography Mass Spectrometry analysis

Result showed that the ozone treatment applied to the soybeans from the company showed a significant reduction in the levels of residual Methyl thiophanate in all tissues compared to the control. The residual content of Methyl thiophanate on soybean shells from a company that was not treated with pesticide spraying decreased by 100% after ozone treatment, from a concentration of 5.37 ppm to 0 ppm. Meanwhile, Edamame soybeans from the company that was treated by additional pesticide spraying and subjected to ozone treatment showed a significant decrease in residual levels of Methyl thiophanate compared to controls or without ozone treatment, in all tissues analyzed. The residual content of Methyl thiophanate found in Edamame soybeans after being treated by additional pesticide spraying on seeds, shells and intact pods were 4.14 ppm, 21.23 ppm and 8.81 ppm, respectively. After ozonated-water treatment, residues present in all tissues decreased significantly from intact seeds, shells and pods by 1.73 ppm, 4.04 ppm and 2.98 ppm, respectively. These results showed that the ozonated water treatment was able to reduce the levels of Methyl thiophanate pesticide residues in all parts of the Edamame soybean tissue from the seeds, shells and intact pods about 58.28%, 80.97%, and 66.19%, respectively (Table 3A).

Edamame soybeans from the farmers treated by additional pesticide spraying of Methyl thiophanate showed the residue content of pesticide following results in seeds, shells and intact pod, were 4.23 ppm, 25.79 ppm, and 12.46 ppm, respectively (Table 3B), while after ozonated water treatment the residue content of Methyl thiophanate reduces by 2.18 ppm, 20.50 ppm, and 4.69 ppm, in seeds, shells and intact pods, respectively. This result showed that ozonated water treatment on soybeans from farmer that were treated by pesticide spraying effects a significant reduction in pesticide residues on seeds and intact pods about 48.52% and 62.37%, respectively. However, the decrease in residual content in soybean shells from farmer was much smaller compared to soybean shells from company after ozone treatment. The reduction of Methyl thiophanate residue on soybean shells from farmer and company after ozone treatment was 20.10% and 80.97%, respectively.

Discussion

The accuracy of pest and disease control in cultivation systems will have an impact on production and crop quality. Accuracy in controlling pests and diseases includes the use of pesticide types, dosages and timeliness of application. Farmers or soybean-producing companies in the Jember area use different types of pesticides, depending on the needs and intensity of pest and disease attacks that threaten Edamame soybean production. The company emphasizes the use of Methyl thiophanate, while farmers use Captan. The results obtained also contribute to companies engaged in the food and feed sector, especially those that use soybean raw materials and soybean waste to carry out the process of de-cleaning pesticide residues before further processing for food or feed ingredients. The shells and intact pods of fresh soybeans from farmers detected Captan, while in soybean shells from companies that detected methyl thiophanate, it showed an indication that in order to maintain the health of livestock, farmers should not use Edamame shells directly for use as animal feed but must be processed first to ensure that it is free from pesticide residues. The content of Captan residue on the shells and intact pods of soybean from the farmer's fields and soybeans that were treated by additional spraying of pesticide was not significantly different. This indicates that Captan is a type of pesticide that is often used by farmers and easily decomposes or dissolves during plant maintenance or perhaps by washing after harvest. It is also possible that farmer used Captan continuously for a long period resulting in the accumulation of pesticide residue on the shells or intact pods of Edamame soybean.

Captan is a contact fungicide with a broad spectrum, the active ingredient contact activity has many targets. Generally considered to be a low-risk fungicide group without any resistance. Contact pesticides are pesticides that can kill or interfere with the proliferation of pests or insects, working when in direct contact with the body of the targeted pest. This type of pesticide is not effective for pests that move frequently, but if there are still contact pesticide residues on the plant, the plant pest organisms can still be killed (Hudayya and Jayanti, 2013).

The content of Methyl thiophanate residue in edamame obtained from the farmer fields compared to that added with Methyl thiophanate spray showed a significant difference. This result indicates the character of methyl thiophanate, which is a group of pesticides that are not easily soluble or decompose by washing. Methyl thiophanate is a systemic fungicide that is applied to a broad-spectrum antifungal, recommended against rotting soybean charcoal (*Macrophomina phaseolina*) at longer intervals than that used for contact fungicides (Laksmi *et al*., 2000). Systemic pesticides are toxic pesticides that enter the plant tissue system and are translocated to all parts of the plant, or hit the target and provide a toxic effect (Hudayya and Jayanti, 2013). In addition, the use of Methyl thiophanate with low operating costs because it can save on energy use and labor costs (Laksmi *et al*., 2000).

The results of the analysis showed that all parts of Edamame including seeds, shells and intact pods from company and farmer with the addition of spraying treatment detected Methyl thiophanate residue. This can be a serious concern because products that can be consumed by humans or livestock are not completely free of pesticide residues, and it is possible that they have the potential to endanger health due to the excessive use of pesticides.

The United States Environmental Protection Agency (US EPA) issued a Re-registration Eligibility Decision (RED) regarding the use of Methyl thiophanate and the primary metabolite Carbendazim concerning the results. In rabbits, Methyl thiophanate showed an increase in the supernumerary ribs and a decrease in fetal weight. Methyl thiophanate has also indicated an increased incidence of liver tumors in mice. In addition, Methyl thiophanate was found to exhibit low acute oral, dermal, and inhalation toxicity. Methyl thiophanate is classified by the US EPA as possibly carcinogenic to humans based on increased doses causing liver tumors in males and females (The Scientific Committee on Plants, 2001).

De-cleaning with the ozonated water technique at a concentration of 0.12 ppm for 60 minutes has proven effective in reducing pesticide residues on Edamame soybeans, especially Captan residues up to 39.13% and Methyl thiophanate residues up to 80.97%. Previous studies reported that the application of 0.4 ppm ozonated water for 30 minutes on Kumquat fruit can reduce 10 types of pesticides residues including Imidacloprid (Wu *et al.,* 2019). Furthermore, the application of ozonated water on Apples with a concentration of 2 ppm for 30 minutes can reduce Captan pesticide by 81% (Sadlo *et al*., 2017). This is consistent with the results of the study conducted by Wang *et al*. (2021), in which 2.0 ppm of ozonated water for 15 min was used to degrade the residual malathion and carbosulfan by 53% and 33%, respectively.

The application of ozonated water technology to soybean Edamame is proven to be effective in reducing pesticide residues. Several factors may affect the effectiveness of the ozonated water technique to reduce pesticide residues including media temperature, media pH, the concentration of ozone solution, type of pesticide and the characteristics of the materials handled which greatly affect the process of ozone oxidation mechanisms to against the active components of pesticides (Lozowicka *et al.*, 2016; Wang and Wu, 2018). Industrial Washing Application such as Electrolyzed Water and Ozone Technology Industrial Washing Technology such as Electrolyzed Water and Ozone Applications is an effective method of removing pesticide residues depending on several factors, including nature of fruits and vegetables, physicochemical properties of pesticides, solubility of pesticides in water, contact time with washing solutions and types of washing methods (Acoglu and Omeroglu, 2021) is an effective method of removing pesticide residues depending on several factors, including nature of fruits and vegetables, physicochemical properties of pesticides, solubility of pesticides in water, contact time with washing solutions and types of washing methods (Acoglu and Omeroglu, 2021).

Pesticide degradation in water can occur through hydrolysis, photolysis, and oxidation-reduction. When Ozone reacts with organic pesticides, there is disruption of unsaturated aliphatic compounds, such as alkenes and alkynes, in the molecular structure of pesticides by splitting the carbon chain and opening the benzene ring as well as oxidation of discolor vinyl, nitro, methoxy, amino, and other functional groups. Oxidative cleavage radically changes the molecular structure of organic pesticides and causes them to lose their potency. The small molecule components are further produced by the reaction between ozone and the unsaturated carbon chains in pesticide molecules, such as acids, alcohols, amines, carbonyls, carboxylates, and their oxides, especially those that are water-soluble. Thus, these compounds can be washed further by being bound to water, contributing to the degradation and removal of pesticide residues (Wang and Wu, 2018). Ozone oxidation mechanisms of organic targets include direct oxidation by oxygen atoms and indirect oxygen controlled by hydroxyl radicals, which are triggered by the decomposition of ozone molecules themselves. The speed of the direct oxidation reaction is lower than that of the indirect oxidation. Ozone (O_3) is a strong oxidant having an oxidative potential of 2.07 Volts and if ozone reacts with dissolved organic components it can generate radical species such as hydroxyl radicals which have a higher oxidative potential (Megahed *et al.*, 2010). The oxidative strength of ozone is responsible for degrading molecules such as pesticides. However, this ability can affect other nutraceutical and bioactive components (Lv *et al.*, 2019), such as antioxidant compounds in Blueberry (Piechowiak *et al.*, 2019), ascorbic acid and polyphenols in Grapes (Swami *et al.*, 2021). There is a need to define the optimum duration and concentration of the ozonation treatment so that a good balance can be achieved between the removal of pesticides and the nutraceutical quality of soybeans. Further studies are needed on degradation products formed by ozonation to ensure the quality of Edamame soybeans.

In conclusion four types of active compounds in the pesticide including Captan, Methyl thiophanate, Imidacloprid, and Cypermethrin which were used as standards for detecting residues in Edamame, the Imidacloprid, and Cypermethrin types were not found in either the soybeans harvested from the farmer or the company fields. On the other hand, Methyl thiophanate residue was found in the fresh shells of soybeans harvested from the company, while Captan residue was found in soybean shells and intact pods harvested from the farmer's field. In Addition, in order to maintain the health of livestock, farmers should not directly use edamame shells as animal feed but must be processed first to ensure that they are free from pesticide residues. Captan is a type of pesticide compound that dissolves easily during the washing process, but using it for a long time causes accumulation on tissues and it is difficult to remove. Meanwhile, Methyl thiophanate residue is included in the insoluble category or does not easily decompose when washed, and it can permeate into all parts of Edamame. This can be a serious concern because products that can be consumed by humans or livestock are not completely free of pesticide residues, and they have the potential to endanger health due to the excessive use of pesticides. The ozonated-water technique with a dose of 0.12 ppm for 1 h was able to reduce the residue of Captan pesticide by 30.13% on the shells and intact pods of Edamame, while Methyl thiophanate residue can be reduced up to 80.97% on the shells of Edamame seeds.

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