Effect of hydrogen peroxide treatment on the concentration of volatile compound in coriander seeds oil

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ABSTRACT
Coriander seeds essential oil have shown some remarkable biological properties and health benefits. The coriander seeds used in Indonesia are imported and also treated with hydrogen peroxide before reaching consumers. Hydrogen peroxide is known to be a strong oxidizer, but so far, there has been no information that explains its effects on the essential oil composition and concentration in coriander seed. This study aims to determine the effect of using hydrogen peroxide and the drying method on the composition of essential oils in coriander seeds. Yield and volatile oil compounds were measured, and the results were compared between the hydrogen peroxide concentration (0.35 and 50%) and drying method (mechanical drying (50 °C) and room temperature drying). GC-MS analysis detected linalool as the most common volatile constituent in all treatments. The highest concentration of linalool compounds (70.16%) was found in seeds without hydrogen peroxide (0%) treatment combined with mechanical drying, followed by without hydrogen peroxide (0%H2O2) combined with room drying (23.74%), then by 35% hydrogen peroxide combined with room drying (18.71%), 35% hydrogen peroxide combined with mechanical drying (18.84%), 50% hydrogen peroxide combined with room drying (22.18%) and by 50% hydrogen peroxide combined with mechanical drying (15.45%). Therefore, the yield was clearly affected only by hydrogen peroxide treatment where no hydrogen peroxide treatment gave the highest yield. The drying method did not have any significant effect on yield.

KEYWORDS
Bleaching
Coriander Seeds
Essential Oil
GC-MS
Hydrogen Peroxide

Introduction
Coriander (Coriandrum sativum L.) belongs to the family Umbelliferae (Apiaceae) native to the Mediterranean region. In Indonesia, coriander is not only used as a spice for cooking but also as herbal medicine for treating various diseases. This pattern of coriander usage can be attributed to an increase in health awareness among consumers coupled with changing trends in public consumption. Moreover, coriander seeds contain up to 1% essential oil. The International Organization of Standards (ISO) standard for coriander essential oil is α-pinene (3.0%-7.0%), myrcene (0.5%-1.5%), limonene (2.0%-5.0%), γ-terpinene (2.0%-7.0%), linalool (65.0%-78.0%), camphor (4.0%-6.0%), α-terpineol (0.5%-1.5%), geraniol (0.5%-3.0%), and geranyl acetate (1.0%-3.5%) (ISO, 1997). Those compounds are known to have antioxidant, antibacterial, anticancer, neuroprotective, anxiolytic, hypnotic, anticonvulsant, analgesic, anti-inflammatory, antidiabetic, activities, digestive, respiratory, and urinary systems (Beyzi et al., 2017; Prachayasittikul et al., 2018; Micić et al., 2019; Weisany et al., 2019; Nguyen et al., 2020; Weisany et al., 2021).

Despite the high demands of coriander seed in Indonesia, its cultivations are limited to small-scale production. This situation has made Indonesia as an importer of coriander seeds from India, Bulgaria, and China (Wei et al., 2019; Fukushima et al., 2020). The imported coriander seeds are, however, are dull brown and dirty in appearance. This condition has become a weakness that makes consumers have less interest. Therefore, the situation has compelled coriander importers to improve the physical quality, especially in color, by treating it with hydrogen peroxide as a bleaching agent.
Hydrogen peroxide is widely used in many households in low concentrations (3-9%) for medicinal applications and bleach for clothes and hair bleach. The choice of hydrogen peroxide as a cleaning agent is based on the nature of hydrogen peroxide as an environmentally friendly oxidizer that can break down into water and oxygen (Singh and Kumar 2018; Al-Saikhan and Shalaby 2019). In general, previous studies only looked at the effect of hydrogen peroxide on physical quality regarding its use as a bleaching agent, oxidizer, and antimicrobial agent. However, no studies have examined the impact of using hydrogen peroxide on commodities that produce essential oils.

Problems that arise in the process of cleaning cilantro using hydrogen peroxide bleaching agents, among others, cause the essential oil content to be oxidized because hydrogen peroxide is very reactive. Moreover, after going through the bleaching process, coriander seeds will also become wet and end up with higher moisture content. This condition causes coriander seeds to become unattractive when they are directly packaged and distributed to the market. Therefore, the market needs to dry it again to reach a moisture content of 9% as required by the market (Carrubba and Lombardo 2020).

The drying method used in this study was drying at room temperature. Drying seeds at room temperature is feared that the moisture content of the seeds will not be evenly distributed so that in this study, it is combined with mechanical drying. On the other hand, the temperature often used in mechanical drying is 50 °C (Wang et al., 2021). To determine the effect of hydrogen peroxide as a bleaching agent and a method for drying coriander seeds, an internal quality analysis is needed that evaluates the composition and concentration of compounds in the essential oil of coriander seeds. This study aims to determine the effect of using two different concentrations of hydrogen peroxide as a bleaching agent and drying methods on the composition and concentration of chemical compounds in the essential oil of coriander seeds according to the standards issued by ISO, especially linalool as the main compound.

Research Methods

Tools and Materials

The materials used in this study were 54 kg of imported coriander seeds from Bulgaria, obtained from an importer in Tangerang, West Java (Figure 1), hydrogen peroxide with 35% and 50% concentrations as a bleaching agent, sodium bicarbonate as catalyst for bleaching reaction and natrium sulphate for coriander seeds oil extraction (PT. Evonik Degussa Peroxide, Indonesia).

Tools used were a tailor-made mechanical drying with temperature control (Figure 2), electric fan, measuring cup, glass funnel, basin, glove, sealer, analytical balance (Latina DST 3000, China), a set of distillation equipment, and GC-MS analyzer (Agilent 7890B, Avondale, PA, USA).

Research Procedures

The experiment was structured using a two-factor, completely randomized design. The first treatment factor is hydrogen peroxide concentration; H0 (0%), H1 (35%), and H2 (50%). The second factor is the drying method; mechanical drying 50 °C (P1) and room temperature drying 27 ± 2 °C (P2). Each treatment was prepared in triplicate.

Hydrogen Peroxide Addition Treatment and Drying Method

For one sample, a total of 3 kg coriander seed was put into a basin as a container for the bleaching process. Hydrogen peroxide (in three concentration levels as mentioned above) and 9 mg (3 mg/kg seeds) sodium bicarbonate (NaHCO3) poured into the basin containing the seeds and stirred for ~5 minutes, then dried with a mechanical drying at 50 °C or room temperature 27 ± 2 °C. An electric fan was used in the room temperature drying process for better air circulation. Drying was carried out to reduce the

Figure 1. Coriander seeds imported from Bulgaria

Figure 2. Setup of mechanical drying
moisture content of the seeds until it reaches a 7% wet base, which was 30 minutes for mechanical drying and 8 hours for ambient temperature air drying. The cleaned and dried coriander seeds, 250 g for each combination treatment, were stored in polypropylene plastic bags for further analysis (Figure 3).

![Figure 3. Coriander seeds before and after bleaching with H2O2](image_url)

![Figure 4. The appearance of essential oil extracted from coriander seeds before and after bleaching with H2O2](image_url)

**Coriander Essential Oil Extraction**

Distillation of essential oil was carried out at the Agricultural Research and Development Agency in Cimanggu. The essential oil was extracted using the steam distillation method. First, the sample was powdered to 4 mm particle size. Then, put into a distillation kettle filled with 3 liters of water. This was later heated to a temperature of 100 °C for 6 hours. The distilled essential oil was separated from the remaining water using 2% Na2SO4 (Figure 4). The extracted essential oil was stored in glass bottles covered with aluminum foil and stored at -4 °C (Weisany et al., 2021).

Analysis of volatile compounds was carried out using GC-MS at the National Police-Criminal Investigation Agency, Central Forensic Laboratory, Sentul, West Java. Essential oil yield (%) was measured using the following formula (1).

\[
\frac{V}{W} = \frac{W_t}{W_k} \times 100 \quad (1)
\]

Where:
- \( V \) = volume
- \( W \) = weight
- \( W_t \) = the mass of essential oil obtained
- \( W_k \) = the mass of dry matter

**Identification of Compounds with Gas Chromatography-Mass Spectrometer**

The chemical composition of coriander essential oil was determined using a GC-MS analyzer following the procedure used by (Beyzi et al., 2017) with slight modifications on held time. GC-MS analysis was carried out using the Agilent 7890B gas chromatography system (Agilent Technologies, Avondale, PA, USA) equipped with a mass spectrometer detector and an HP-5 MS column (30 m x 0.25 mm, 0.25 μm film thickness) with a pressure of 90 kPa, split 1:25 and injection volume is 0.6 μL. The oven temperature was set at 50 °C for 5 minutes, then
increased from 50 to 150 °C at a rate of 3°C/minute, then increased again to 230 °C / minute for 5 minutes. The flow rate of helium as carrier gas was set at 1.6 mL/minute. The qualitative analysis was based on comparing the retention time with the mass spectrum library using Wiley10n1.

Table 1. Essential oil yield

| [H₂O₂] (%) | The yield (%) |
|------------|--------------|
| 0          | 0.97a        |
| 35         | 0.88ab       |
| 50         | 0.78b        |

*Note: Value in the same column with different letters (a-b) are significantly different at p < 0.05.

**Statistical Analysis**
The data were analyzed using SAS 9.4 software. Analysis of variance was carried out and means separated at 5% using Tukey's Honest Significant Difference test. Samples were grouped based on the concentration of hydrogen peroxide and the drying method. Principal Component Analyses (PCA) were done using Orange 2020 software version 3.28 (Demsar et al., 2013).

**Results and Discussion**

**The Yield of Essential Oil**
The yield of coriander seed essential oil is shown in Table 1. H₂O₂ treatment affected the yield of essential oil (p> 0.05). The yield of essential oil without H₂O₂ treatment was 0.97%. This result was significantly higher than those treated with H₂O₂ of concentrations 35 and 50%, namely 0.88 and 0.78%, respectively. The difference test showed that H₂O₂ 35% concentration did not show any significant difference in yield results from without H₂O₂ treatment. Based on the results obtained, a higher H₂O₂ concentration causes the resulting yield to decrease. Hydrogen peroxide is a reactive oxygen species (ROS) that can cause damage to coriander cell walls. The oxidation process starts with the oxygen atom pulling electrons in the coriander cell (Lin et al., 2020). With fewer electrons, the cell wall becomes damaged and even destroyed. Thus, the volatile compounds will come out and evaporate. Mani-López et al. (2016), explained that hydrogen peroxide affects physical properties such as texture because of its ability to change the structure of lipids and proteins, the primary constituents of cell membranes, increasing the quality degradation associated with plant tissue firmness. The yields obtained from all three concentration rates of H₂O₂ did fall within the range recorded by previous research (0.3- 1.1%) (Beyzi et al., 2017).

**Essential Oil Composition of Coriander Seed Oil**
The composition of the volatile compounds of coriander seeds is shown in Table 2. 36 volatile compounds were identified from the H0P1 treated samples significantly different from the H0P2, H1P1, H1P2, H2P1, and H2P2 treatments. The combination of treatment without the addition of H₂O₂ with the mechanical drying resulted in a higher concentration of volatile compounds than others. In comparison, the combination of 50% H₂O₂ treatment with mechanical drying resulted in a lower concentration of volatile compounds than other treatments. The major volatile compound identified in all treatments was linalool. This result is in agreement with the results of previous studies by Beyzi et al. (2017) and Ghazanfari et al. (2020), there was a difference in the percentage of linalool peak area in each treatment, with the highest percentage (70.16%) recorded in H0P1. For other treatments, the percentage of the peak areas obtained was 23.74% for H0P2, 18.71% for H1P2, 18.84% for H1P1, 22.18% for H2P2, and 15.45% H2P1.

Lane and Burgess (2001) have reported that using a bicarbonate activator in hydrogen peroxide solution reacts to several alkenes, including citronellal, α-pinene, and linalool. In this study, the catalyst used was NaHCO₃ (sodium bicarbonate), successfully oxidized monoterpen compounds including linalool, limonene, geranyl acetate, and γ-terpinene. The application of the NaHCO₃ catalyst aims to make the pH of the acidic hydrogen peroxide solution change to alkaline so that more reactive oxidants are formed. This is consistent with the statement of Pan et al. (2021) and Trautmann et al. (2021) that the use of NaHCO₃ in addition to adjusting the pH into the alkaline area is also to form peroxymonocarbonate ions.
Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide. It has been reported by Singh and Kumar (2018) that the optimal activity of the enzyme is at 48 °C and stops at 55 °C. In this study, 50% H$_2$O$_2$ concentration treatment had the least amount of yield and volatile compounds. This is thought to have a strong relationship with the denaturation of the enzyme that should catalyze H$_2$O$_2$. When the coriander seeds were stirred, the reaction temperature reaches 64 °C. Thus the enzyme which previously had a role in catalyzing H$_2$O$_2$ has been denatured.

Table 2. The essential oil composition of coriander

| No | Compounds             | Treatments |  |  |  |  |  |  |
|----|-----------------------|------------|---|---|---|---|---|---|
|    |                       | H0P1 (%)  | H0P2 | H1P1 (%) | H1P2 | H2P1 (%) | H2P2 (%) | H2P3 (%) |
| 1  | α-Pinene*             | 5.13a      | 2.08b | 1.67b     | 1.55bc | 1.43bc    | 1.31c     |
| 2  | Camphene*             | 1.22a      | 0.37b | 0.30b     | 0.30bc | 0.26bc    | 0.22c     |
| 3  | β-Pinene*             | 2.16a      | 0.62b | 0.53b     | 0.51bc | 0.46bc    | 0.31c     |
| 4  | β-Myrccene*           | 2.15a      | 0.55b | 0.49b     | 0.46bc | 0.42bc    | 0.30c     |
| 5  | Benzene               | 0.57       | 0.95  | 0.70      | 0.76   |           | 0.56      |
| 6  | Limonene*             | 7.10a      | 1.19b | 1.12b     | 0.97bc | 0.93c     | 0.66d     |
| 7  | γ-Terpinene*          | 7.86a      | 2.53b | 2.68b     | 2.26bc | 2.40bc    | 1.69c     |
| 8  | α-terpinolene*        | 1.95a      | 0.39b | 0.28b     | 0.34b  | 0.36b     | 0.23b     |
| 9  | Linalool*             | 70.16a     | 23.74b| 18.71c    | 18.84bc| 22.18bc   | 15.45c    |
| 10 | Camphor               | 5.66       | 2.41  | 1.76      | 1.98   | 2.31      | 1.50      |
| 11 | Terpene-4-ol*         | 1.15a      | 0.19c | 0.28b     | 0.21c  | 0.31b     | 0.20c     |
| 12 | Borneol*              | 0.57a      | 0.12b | 0.13b     | 0.08b  | 0.12b     | 0.11b     |
| 13 | α-Terpineol*          | 1.82a      | 0.37b | 0.29c     | 0.34b  | 0.45b     | 0.32c     |
| 14 | Geraniol*             | 8.08a      | 1.96b | 1.40c     | 1.68b  | 2.26b     | 1.44c     |
| 15 | Geranyl acetate*      | 12.19a     | 3.30b | 2.73b     | 2.25b  | 0.07d     | 1.82c     |
| 16 | Caryophyllene*        | 1.06a      | 0.16b | 0.27b     | 0.13b  | 0.18b     | 0.09c     |
| 17 | β-Citronellol*        | 0.90a      | 0.18b | 0.21b     | 0.10b  | 0.19b     | 0.05c     |
| 18 | Decanal*              | 0.25a      |       |           | -      |           | -         |
| 19 | Z-Citral*             | 0.14a      |       |           | -      |           | -         |
| 20 | Cyclooctane*          | 0.15a      |       |           | -      |           | -         |
| 21 | 3-dodecen-1-ol*       | 0.15a      |       |           | -      |           | -         |
| 22 | Tetradecanoic acid*   | 0.20a      |       |           | -      |           | -         |
| 23 | 2-7 Pentadecanol*     | 0.16a      |       |           | -      |           | -         |
| 24 | 2-Pentadecanone*      | 0.17a      |       |           | -      |           | -         |
| 25 | β-Citronellol*        | 0.34a      |       |           | -      |           | -         |
| 26 | Myrtenyl acetate*     | 0.25a      |       |           | -      |           | -         |
| 27 | Neryl acetate*        | 0.22a      |       |           | -      |           | -         |
| 28 | n-Hexadecanoic acid*  | 0.20a      |       |           | -      |           | -         |
| 29 | Citronellol*          | 0.16a      |       |           | -      |           | -         |
| 30 | 4-vinylguaiacol*      | 0.15a      |       |           | -      |           | -         |
| 31 | Nerolidol*            | 0.11a      |       |           | -      |           | -         |
| 32 | Tetradecanal*         | 0.10a      |       |           | -      |           | -         |
| 33 | Caryophyllene oxide*  | 0.12a      |       |           | -      |           | -         |
| 34 | Dodecanoic acid*      | 0.11a      |       |           | -      |           | -         |
| 35 | Cyclooctadecane*      | 0.11a      |       |           | -      |           | -         |
| 36 | Phenol*               | 0.24a      |       |           | -      |           | -         |

Note: Value in the same column with different letters (a-d) are significantly different at p < 0.05. (-): not identified. (*) is statistically significant at p <0.05.
On the other hand, 35% $\text{H}_2\text{O}_2$ treatment had a yield that was not significantly different from 0% $\text{H}_2\text{O}_2$ treatment. This was because the reaction temperature whiles stirring coriander seeds only reached 45 °C, so that the enzyme activity was still in good condition. In addition to temperature, enzyme activity is also influenced by pH. It has been reported by Singh and Kumar (2018) that the enzyme will work optimally at pH 7. In this study, the pH of the hydrogen peroxide solution catalyzed by NaHCO₃ becomes alkaline so that the pH of the catalase enzyme in coriander seeds is beyond its optimum pH.

The concentration of volatile compounds identified in the H2P2 treatment was higher than that of H2P1. Coriander seeds are dried at room temperature (~27 °C), with the help of a fan. This causes the bleaching activity to last long, and the reaction temperature gradually drops. Therefore, the oxidation process also does not last long because it is outside the optimum temperature (Lin et al., 2020). The same thing happened in the H1P2 treatment: a combination of 35% $\text{H}_2\text{O}_2$ and drying at room temperature with the reaction temperature at 45 °C of stirring the seeds. According to Kan et al. (2020) and Khodaei et al. (2020), bleaching activity using hydrogen peroxide as an oxidant at temperatures below 40 °C is unstable even with the addition of a catalyst.

H0P1 treatment resulted in the peak area of major volatile compounds such as linalool (70.16%), limonene (7.10%), geranyl acetate (12.19%), $\alpha$-pinene (5.13%), $\gamma$-terpinene (7.86%), geraniol (8.08%), camphor (5.66%), and $\alpha$-terpinolene (1.95%). In addition to this, a yield of 0.97% was obtained from 3 kg of dried coriander seeds. These results are not much different from the research of Beyzi et al. (2017), who used similar distillation methods (steam distillation), to derive linalool (89.46%), limonene (0.27%), geranyl acetate (1.88%), $\alpha$-pinene (0.47%), $\gamma$-terpinene (1.99%), geraniol (1.84 %), camphor (2.79%), $\alpha$-terpinolene (0.28%), and yield of 0.30% from 100 g fresh coriander seeds.

**Sample Grouping**

GC-MS data were subjected to multivariate analysis so that they could be visualized. The sample grouping was based on the volatile compounds of coriander seeds treated with $\text{H}_2\text{O}_2$ and different drying methods, using PCA with major peak area as the variables. The PCA results were displayed in the form of a score plot, as shown in Figure 5. Before being grouped with PCA, data pretreatment was first carried out on the variables used. Appropriate data pretreatment is an essential step in data analysis using chemometrics such as PCA to obtain clean data. The pretreatment of data used in this study were centering (mean) and scaling (standard deviation). The plot of scores from the PCA analysis aims to determine whether there were changes in the composition and concentration of volatile metabolites due to the treatments applied, namely the addition of $\text{H}_2\text{O}_2$ and different drying methods. The total diversity of data from the two PCs is 81% in the score plot, meaning that 81% of the data diversity can be explained by the variable peak area of volatile compounds. According to Varmuza et al. (2002), if the total diversity of PC1 and PC2 is greater than 70%, then the score plot shows good visualization.
The composition and concentration of coriander seeds volatile compounds with H0P1 treatment (Figure 5) showed that samples had identical metabolite profiles. The group of volatile compounds of coriander seeds in treatments H0P1, H0P2, H1P1, H1P2, H2P1, and H2P2 became more distinguished by increasing the distance between the groups. The properties of H2O2 as an oxidizing agent and the drying method at different temperatures cause significant changes in the composition and concentration of the identified volatile compounds.

The PCA biplot is another form of PCA analysis that combines score plots and loading plots. This is done to identify the similarity in characteristics between treatments. Figure 6 shows that the H2P2 and H1P1 treatments were close to each other, it shows that the concentrations of volatile compounds identified in the two treatments have a high similarity compared to other treatments. On the other hand, H0P1 forms a group that is quite far from H2P1 and H2P2, this means that the volatile compounds identified in H0P1 were much different from the H2P1 and H2P2 treatments. The loading plot depicts the measurement variables, namely the peak area of α-pinene, camphene, β-pinene, β-myrcene, benzene, geranyl acetate, γ-terpinene, α-terpinolene, linalool, camphor, terpene-4-ol, α-terpineol, and limonene. The PCA biplot showed that the variables that affected the grouping of H0P1 samples were the peak area of α-pinene, camphene, β-pinene, β-myrcene, benzene, geranyl acetate, γ-terpinene, α-terpinolene, linalool, camphor, terpene-4-ol, α-terpineol, while the grouping of H0P2 samples was influenced by the peak area of the limonene compound. The influencing variable can be seen from the angle formed on the biplot against sample grouping.

Several pretreatment PCA analyses have been reported to identify better variables indicated by the point between the components being closer (van den Berg et al., 2006). The pretreatment data used in this study were centering (mean) and scaling (standard deviation), which succeeded in making the points between the same treatments close together. The pretreatment of each research data will be different based on the characteristics of the data generated.

The closeness between variables in the score biplot (Figure 6) can provide information regarding the correlation between variables to treatment. The narrower the angle formed by the volatile compound line to the treatment, the better the response has shown and vice versa. The correlation between variables in the PCA biplot aims to find out how a variable affects or is influenced by other variables. Two variables with a high and positive correlation value will be described as two vector lines in the same direction.
and forming a narrow-angle. If the angle formed is close to 90°, then the correlation between the two variables is low. Meanwhile, if the angle is obtuse and opposite, the correlation is negative or uncorrelated. The volatile compounds of coriander seeds are generally close to one another. However, camphene and β-pinene form a narrower angle, the same thing is found in the variables α-terpinolene and terpene-4-ol, geranyl acetate and α-pinene, β-myrcene and β-pinene, etc.

Conclusion
In this study, hydrogen peroxide treatment coupled with drying method was found to significantly affect percentage yield and composition of coriander seed essential oil. Linalool, as a major essential oil component, was found to be the most abundant compound without the hydrogen peroxide and mechanical drying treatment (H0P1). On the other hand, the percentage yield was only affected by the hydrogen peroxide treatment. The sample without hydrogen peroxide treatment recorded the highest yield. GC-MS analysis of the coriander seed essential oil also identified 36 chemical compounds.

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