Effect of chopping on yield, physico-chemical properties, and chemical composition of Clove (Syzygium aromaticum L.) leaf essential from three varieties

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Abstract. Clove oil is one of the highest potential essential oil to be developed and traded in Indonesia. Several factors that can affect the yield and quality of the clove oils such as material pretreatment and plant variety. The purpose of this study was to assess the yield and quality of clove leaf oil between three plant varieties (var. A, var. B and var. C) without leaf chopping and with leaf chopping (chopped with a size of diameter ± 0.5-1cm). Chemical compositions of the oil were analyzed using GC-MS and the physico-chemical properties and measured based on SNI 06-2387-2006. The results showed var. C with chopping treatment produced the highest yield (2.67%). GC-MS analysis showed that the main compound of clove oil was eugenol (66.10 – 72.29%), which the highest content obtained at whole leaves var. A. Varieties of clove plants and leaves chopping have effects on yield, amount and percent of chemical compounds, but no significant effect on physicochemical properties. Var. A has best clove leaf oil quality, whereas var. C with chopped treatment produced the highest yield.

Keywords: plant variety, leaves chopping, clove leaf oil, physico-chemical properties, chemical compound.

1. Introduction

Indonesia is currently one of the biggest suppliers of essential oil in the world [1]. One of the essential oils with high potential to be developed and traded is clove oil [2]. According to [3], there are four varieties of clove i.e. Siputih, Zanzibar, Ambon, and Zambon (composite clove). The difference between these clove variety might affect the yield and quality of the essential oil produced. Previous research conducted by [4] showed that variety difference in clove leaf was affecting its chemical composition. The highest eugenol yield was produced by Siputih variety (80,15%), while Sikotok variety produced the lowest eugenol yield (48,33%).

In addition to variety, pre-treatment was also known to affect the yield of clove essential oil. Chopping the material into small pieces was done to accelerate the evaporation of essential oil that stored in oil pockets between cell cavity in the tissue [5]. Previous research by [6] showed that the essential oil yield was increased between 0.19% to 0.6% by chopping the clove into small pieces for steam-hydro distillation using the microwave.

Studies about the effect of clove variety and pre-treatment to yield and quality of clove essential oil and its interaction is still limited. The objective of this study was to assess the yield and quality of clove leaf oil between three plant varieties (var. A, var. B and var. C) with whole and chopped clove leaf.
2. Materials and Methods

2.1. Leaves collection and distillation
Dried clove leaves were obtained from community plantation in Craken Village, Munjungan, Trenggalek, East Java. Fallen leaves were collected from 3 clove trees of each clove variety (Variety A, B, C). Every tree was marked with labels and fallen leaves were collected after 3 months. Only the top layer of leaves was collected. The bottom layer of leaves that directly touched the ground were not collected. As much as 1.25 kg of leaves from each variety were distilled with water-steam distillation method for 8 hours. Before distillation, leaves were divided by two factors, namely pre-treatment (whole leaves and chopped with a size of leaf width± 0.5-1cm) and plant varieties (varieties A, B, and C) with three replicates per treatment. The yield of essential oil clove was calculated with formula:

\[ \text{Oil yield (\%) = } \frac{\text{specific gravity of oil} \times \text{volume of oil}}{\text{dry air weight of bud}} \times 100 \]

2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
Chemical composition of clove leaf oil from each treatment was analyzed with Gas Chromatography-Mass Spectrometry (GC-MS) QP2010S SHIMADZU with the column specification of Rtx 5MS (30 m x 0.25 mm, film thickness 0.25 µm), using helium as the mobile phase with flow speed of 3 ml/minute, column temperature of 70°C, injection temperature of 290°C, split injection method and flow control mode of 108 kPa pressure. Initial temperature was 70°C for 5 minutes and the final temperature was 285°C for 20 minute, temperature increment speed of 6°C/minute. NIST database library was used to determine the chemical compound. The result was then compared with other literatures [7].

2.3. Physico-Chemical Properties Analysis
The physico-chemical properties were analyzed based on SNI 06-2387-2006 which have several parameters such as (color and aroma), specific gravity, refractive index, and miscibility in 70% ethanol (v/v).

Color observation was done by taking 10 ml of oil sample into reaction tube. The reaction tube was then leaned on a white sheet of paper for comparison. The color was visually observed in 30 cm of observation range. Meanwhile, the aroma observation was based on organoleptic test with nose.

Specific gravity was measured with pycnometer based on the ratio between oil weight in a certain volume with aquadest weight in the same volume and temperature. Clove oil was cooled down to 20°C by placing the bottle between ice. Empty flask was weighed (Mb) with analytical scale. An amount aquadest were put inside and weighed (M1). A total of 5 ml of clove essential oil with an amount of 5 ml was put into an empty flask (Ma) and flask with oil was weighed (M2). The specific gravity at room temperature was calculated using the following formula:

\[ \text{Specific gravity} = \frac{(M2-Ma)}{(M1-Mb)} \]

Refraction index was measured with a refractometer (2WAj series, ABBE brand). The oil was flowed to refractometer in same temperature with the measurement temperature. Refractometer scale reading was done by observing the clear border between light and dark spots. Refraction index was then calculated with the following formula:

\[ \text{Refraction Index (IB)} = \text{IBo} + 0,0004 (t_1 - t) \]
Miscibility in 70% ethanol was measured based on the volume ratio of oil in it. As much as 1 ml of clove oil was continuously added with 1 ml of ethanol 70% and then shaken. After the oil has been dissolved, the addition of ethanol then stopped, and the amount of added ethanol was noted. Miscibility in 70% ethanol value was calculated using the following formula:

$$\text{Miscibility in 70\% ethanol} = \frac{\text{1 ml oil}}{\text{ml 70\% ethanol}}$$

2.4. **Statistics Analysis**

The collected data were analyzed using ANOVA and descriptive analysis. Factors with significant differences were further tested with Honestly Significant Difference (Tukey HSD) with a 5% test level using SPSS software.

3. **Results and Discussions**

3.1. **Oil yield**

Oil yields of clove leaf with chopping treatment factor in variety A, B, and C are shown in Figure 1. There was an interaction between the two factors and significant effect was found to the oil yield of clove oil ($p=0.002$). The chopped leaves of variety C showed the highest yield value (2.67%). Chopped variety A leaves were also produced yield above 2%. Whole leave of variety C leaves showed the lowest yield value (1.4%).

![Figure 1](image)

**Figure 1.** Yield of clove oil with interaction of variety (Var. A; Var B; and Var. C) and treatment (P1=whole leaves; P2=chopped leaves).

The increase of yield value in chopped variety C leave indicating that the chopping treatment on clove material increase the amount of oil yielded by breaking the oil cells. [8] reported that pre-distillation chopping on the material, made the evaporation of the oil easier. The easiness of oil evaporation from those material was resulted from the broken oil cells. Chopping treatment before the distillation process also increase the surface area width so the oil can get out from the cell easier [8].

[5] also reported that rough chopped leaves produced higher yield (0.752%) compared to whole leaves (0.165%). This increase in yield indicating that using smaller material for distillation process can result in higher yield of essential oil. Smaller material size might be affected by hydrodiffusion process.
on clove leaves during the distillation process. According to [9], hydrodiffusion is a process of essential oil that pushed out of the plant material with water that plays a role in heat transmission into the material. This process runs very slowly in whole leaves material [5].

3.2. Chemical Composition

GC-MS results showed that there were 4 – 6 chemical compounds that identified in chopped leaves from clove oil distillation in all varieties. In whole leaf, 4 – 8 compounds were detected. Oil from both whole and chopped leaves had 2 main chemical compound that is eugenol and caryophyllene with percentage of 66.10% - 72.29% and 22.46% - 28.56% respectively (Table 1).

| No. | Retention time (minute) | Name of compound | Chemical formula | Percentage (%) | V1P 1 | V2P 1 | V3P 1 | V1P 2 | V2P 2 | V3P2 |
|-----|-------------------------|-------------------|------------------|----------------|-------|-------|-------|-------|-------|-------|
| 1.  | 15.923                  | α-Cubebene        | C15H24           | 0.77           |      | -     | -     |      | -     | -     |
| 2.  | 16.239                  | Eugenol           | C10H16O2         | 72.29          | 66.4  | 66.10 | 71.71 | 68.97 | 69.15 |       |
| 3.  | 16.592                  | α-Copaene         | C15H24           | 0.87           | 1.14  | -     | 1.19  | 0.30  | -     | -     |
| 4.  | 17.652                  | Caryophyllene     | C15H24           | 22.46          | 27.5  | 28.56 | 22.60 | 25.76 | 26.34 |       |
| 5.  | 18.424                  | Humulene          | C15H24           | 2.47           | 3.23  | 3.70  | 2.68  | 3.14  | 3.54  |       |
| 6.  | 19.933                  | δ-Cadinene        | C15H24           | 0.47           |      | -     | 0.45  | -     | -     | -     |
| 7.  | 21.275                  | Caryophyllene oxide | C15H24O         | 0.67           | 1.59  | 1.65  | 1.37  | 1.83  | 0.96  |       |

Total 100 100 100 100 100 100

Note: V1= variety A, V2= variety B, V3= variety C, P1= whole leaves, P2= chopped leaves

In general, the percentage of eugenol from chopped leaf was relatively higher (69.94%) compared to whole leaf (68.29%). On the other side, mean percentage of caryophyllene in chopped leaf was relatively lower (24.90%) compared to whole leaf (26.19%). The result indicates a pattern where chopping treatment increase eugenol composition in the oil yielded. [10]Karwur and Semangun (2014) reported that oil products from whole material will result in higher eugenol percentage (80.88%) compared to grinded material (70.90%). However, in other plant part that is flower, whole material yielded in higher eugenol concentration compared to chopped clove flower. For that matter, further research in plant parts used for distillation need to be conducted.

In an addition of material treatment factor, plant variety was also indicated to affect the chemical compound in clove leaf oil. Variety A produced the highest eugenol yield (72.29%) compared to variety B and C. Meanwhile, the mean percentage of caryophyllene in variety C was the highest (27.45%) compared to variety A and B. This result indicated that genetic factor affects the chemical compound in clove leaf oil. Tresnawati et al. (2011) in [4] reported that the difference in chemical constituent could be affected by environment factors as well as genetic factor.

Eugenol is one of the most important compounds found in clove leaf oil [8]. Higher concentration eugenol in clove oil will increase its quality [11]. Eugenol percentage value in this research was lower than the requirement of Indonesian National Standard (SNI) 06-2387-2006 [12] that is 78%.

Aside from eugenol, caryophyllene is also an important compound in clove leaf oil. The oils produced in this research were having higher percentage value of caryophyllene (22.46% - 28.56%). This value was higher than the requirement of SNI 06-2387-2006 [12] that require it to be higher than 17%. Essential oil from whole leaf of variety C clove had the highest caryophyllene concentration. [4] reported
that the highest caryophyllene concentration was found in variety of Sikotok (28.8%) and the lowest was found in Siputih variety (13.44%). This difference might be affected by the phytochemistry character from clove leaf that dominated by terpenoid compound.

3.3. **Physico-Chemical Properties**

Plant variety showed significant difference in specific gravity value (P=0.003). The highest SG (specific gravity) value showed by variety A with value of 1.036. There was no significant difference in the resulted oil color (yellowish clear) and its aroma (typical aroma of clove oil). In general, refraction index value of whole leaf oil (1.533 – 1.534) was not significantly different with chopped leaf oil (1.533 – 1.535).

Clove leaf oil from variety A was according to SNI 06-2387-2006 standard for its color, aroma, SG, refraction index, and ethanol miscibility for all chopping treatment factor (Table 2).

| Test            | Standard       | Factor | Result                        | Comparison to standard |
|-----------------|----------------|--------|-------------------------------|------------------------|
| Color           | Yellow – dark brown | VIP1   | Yellowish clear               | According to standard  |
|                 |                | V2P1   | Yellowish clear               | According to standard  |
|                 |                | V3P1   | Yellowish clear               | According to standard  |
|                 |                | V1P2   | Yellowish clear               | According to standard  |
|                 |                | V2P2   | Yellowish clear               | According to standard  |
|                 |                | V3P2   | Yellowish clear               | According to standard  |
| Aroma           | Typical clove oil aroma | VIP1   | Typical clove oil aroma       | According to standard  |
|                 |                | V2P1   | Typical clove oil aroma       | According to standard  |
|                 |                | V3P1   | Typical clove oil aroma       | According to standard  |
|                 |                | V1P2   | Typical clove oil aroma       | According to standard  |
|                 |                | V2P2   | Typical clove oil aroma       | According to standard  |
|                 |                | V3P2   | Typical clove oil aroma       | According to standard  |
| Specific gravity 20° | 1.025 to 1.049 | VIP1   | 1.040                         | According to standard  |
|                 |                | V2P1   | 1.022                         | Not according to standard|
|                 |                | V3P1   | 1.027                         | According to standard  |
|                 |                | V1P2   | 1.033                         | According to standard  |
|                 |                | V2P2   | 1.015                         | Not according to standard|
|                 |                | V3P2   | 1.023                         | Not according to standard|
| Refraction index (nD) 20° | 1.528 to 1.535 | VIP1   | 1.534                         | According to standard  |
|                 |                | V2P1   | 1.533                         | According to standard  |
|                 |                | V3P1   | 1.533                         | According to standard  |
|                 |                | V1P2   | 1.535                         | According to standard  |
|                 |                | V2P2   | 1.533                         | According to standard  |
|                 |                | V3P2   | 1.534                         | According to standard  |
| Miscibility in 70% ethanol | 1 : 2 clear, subsequently clear | VIP1   | 1 : 1 clear                   | According to standard  |
|                 |                | V2P1   | 1 : 1 – 1 : 2 clear           | According to standard  |
|                 |                | V3P1   | 1 : 2 clear                   | According to standard  |
According to standard

|               | V1P2 | V2P2 | V3P2 |
|---------------|------|------|------|
| 1 : 1 – 1 : 2 clear |      |      |      |
| According to standard |      |      |      |

Eugenol (%) Minimum 78%

|               | VIP1 | V2P1 | V3P1 | V1P2 | V2P2 | V3P2 |
|---------------|------|------|------|------|------|------|
| Not according to standard | 72.29 | 66.49 | 66.10 | 71.71 | 68.97 | 69.15 |

Beta Caryophyllene Maximum 17%

|               | VIP1 | V2P1 | V3P1 | V1P2 | V2P2 | V3P2 |
|---------------|------|------|------|------|------|------|
| Not according to standard | 22.46 | 27.56 | 28.56 | 22.60 | 25.76 | 26.34 |

Note: V1= variety A, V2= variety B, V3= variety C, P1= whole leaves, P2= chopped leaves

The color of clove leaf oil resulted in this research might be affected by the amount of relatively similar compounds (4-7 compounds) and dominated by eugenol and caryophyllene. Eugenol concentration between variety might affect the SG value of clove leaf oil. It was reported that higher concentration of heavy fraction in the oil will also increase its SG value. Chopping treatment factor did not show any significant difference, but SG value showed relatively low value in chopped leaf (1.015 – 1.033) compared to whole leaf (1.022 – 1.041). In the time of distillation of the material, steam penetration to smaller material size was easier because its tissue is more open so the amount of hot steam contacted with oil will be higher. That condition will result in heavy fractions of oil that easier and faster to evaporate [13].

Refraction index value increased after the chopping treatment. Smaller material size by chopping will accelerate oil evaporation so heavy fractions contained in oil will cause in higher molecule density. Molecule density in the oil will make it harder for light to pass through the oil [14]. The result from refraction index value did not give significant difference.

Better ethanol miscibility was caused by higher oxygenated hydrocarbon compound in variety A (72.96%) for whole leaf with its main component eugenol and caryophyllene oxide. Higher concentration of oxygenated hydrocarbon compound in the oil means higher ethanol miscibility of the oil, with the result that the ethanol needed to dilute the oil will be reduced [8]. Miscibility value in ethanol in this research was according to the Indonesian National Standard (SNI) 06-3954-2006 [12] that requires the ethanol miscibility in range of 1:1 – 1:2.

4. Conclusion

Variety C clove with chopped treatment showed the highest yield of essential oil. The main components of clove essential oil were eugenol and caryophyllene. Between the three analyzed varieties, variety A in both chopping factor was fulfilled the color, aroma, specific gravity, refraction index and ethanol miscibility parameter of the standard.
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