Isolation of guaiene from patchouli (*Pogostemon cablin* Benth.) oil using vacuum fractionation distillation

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**Abstract.** Guaiene is one component of patchouli oil, which is used in the flavor and pharmaceutical industries. It can be isolated using vacuum fractionation distillation. This study aim to study the effect of isolation stages on the performance of the distillation process. The method used is the descriptive method with two-stage fractionation and one-stage fractionation as its treatment. In one-stage fractionation, patchouli oil is fractionated with 8 temperature ranges: 249–261, 261–273, 273–280, 280–286, 286–291, 291–302, 302–319, and 319–321 °C. At two-stage fractionation, fractionation was carried out to obtain the patchouli alcohol component as the highest component of patchouli oil, then guaiene was isolated from the remaining fractions with 5 temperature ranges: 249–261, 261–274, 274–281, 281–287 and 287–300 °C. Vacuum fractionation distillation was conducted using the BR 36–100 Spinning Bend Distillation System. The results showed that at one-stage fractionation produced 5 cut fractions, while the fractionation of two-stage fractions produced 2 cut fractions. One-stage fractionation takes longer time than two-stage fractionation (23.12 and 19.32 hours, respectively), with electricity consumption also being greater, 207.83 and 176.18 kWh, respectively.

1. Introduction

Patchouli oil is one of the main commodities of Indonesian essential oils, and Indonesia is the biggest producer of patchouli oil followed by Europe and India [1, 2]. The oil is mostly exported to America, Europe and other Asian countries. The oil is extracted from leaves mixed with stems of patchouli (*Pogostemon cablin* Benth) using steam distillation [3]. Patchouli oil is generally used in the perfume industry especially as fixative material or binder the main constituent of perfume so as not to evaporate easily. Lately several studies have shown that patchouli oil also has potential in the pharmaceutical industry (drugs) as an antibacterial, antifungal and anti-cancer ingredient [4], it can be used as an anti-repellant as well.

The main component of patchouli oil which greatly influences its quality is the patchouli alcohol content. In the world of trade, patchouli alcohol (PA) greatly determines prices in addition to other characteristics such as color and aroma. Indonesian patchouli oil has an average PA content of 32.2% [5], this is far above the content of patchouli oil PA produced by India, which is 23.2% [6] and China amounting to 29.21% [7]. Although the level of PA is higher but the selling price of Indonesian oil is below the oil price of the two countries. This is due to other oil quality determinants such as appearance
(color), aroma (burned smell and other odors) under Indian and Chinese oil. For this reason, efforts need to be made to increase the selling value and added value of patchouli oil. One of the efforts to increase patchouli added value is by making process engineering to produce downstream products of patchouli oil which is crystal patchouli. Crystal patchouli is produced through patchouli oil fractionation distillation and crystallization.

A research by Nurjanah et al. [8] produced crystalline patchouli by the process of fractionation distillation and crystallization. In the fractionation distillation, 3 fractions were produced based on the boiling point temperature of 230–283 °C (Cut 1), 283–290 °C (Cut 2), and 290–300 °C (Cut 3). The PA content of Cut 1 is 6.72–8.77%, Cut 2 is 53.26–57.91% and Cut 3 is 85.32–87.61%. The results showed that fractionation with PA > 80% could be produced in the Cut 3 fraction, so that the Cut 3 fraction was used as a raw material for making patchouli crystals. Cut 2 fraction is 9% while Cut 1 fraction is 56%. Cut 1 fraction contains 20.32% patchouli alcohol, 13.43% Δ-guaiene, 17.89% α-guaiene, and 6.67% α-patchoulene. The high amount of Δ-guaiene and α-guaiene content in the Cut 1 fraction has the potential to be isolated into a single component of guaiane.

Guaiane is one of the compounds that affects the aroma of patchouli oil. Guaiene compounds that are widely found in patchouli oil are α-guaiene and Δ-guaiene [9]. The Δ-guaiene compound has anti-inflammatory activity against PAF (Platelet Activating Factor), a phospholipid mediator produced by various cells at the time of being exposed to allergic diseases, inflammation, asthma and others [10].

Separation of components can be done using the fractionation distillation method based on the boiling point of the component. In this process, there are important variables that affect the fraction or distillate produced, such as temperature, pressure, fractionation column, and reflux ratio [11, 12]. Determination of the distillation temperature used can be a boiling point of the component, so that the distillation temperature range can be determined according to the boiling point of the constituent material. Determination of the pressure used is able to influence the quality of the distillate produced, the oil should be fractionated in a vacuum, because at high pressure and temperature can result in decomposition of the oil produced. Determination of fractionation columns can also affect the success of the fractionation distillation process, a longer column will provide a larger path for contact between vapor and liquid in the column so that more effective separation occurs, while short columns will cause overflow or buildup of fluid in column so that the longest column is set as the column used for the fractionation distillation process [13]. This study aimed find out the effect of isolation stages on the performance of the distillation process. Isolation of guaiane was conducted into two-stage and one-stage fractionation distillation. Vacuum fractionation distillation was conducted using the BR 36–100 Spinning Bend Distillation System.

2. Methodology
Patchouli oil was obtained from distiller in Garut, West Java, Indonesia. Fractionation distillation process was conducted using B/R Distillation System Model 36–100 Instrument-Spinning Band (figure 1) which is integrated with the computer by the BR M 690 control program. The fractionation distillation process was carried out with two treatments, namely one stage and two stages of guaiane isolation.

Figure 1. B/R Distillation System Model 36–100 Instrument-Spinning Band.
2.1. Fractionation distillation
A 100 mL patchouli oil was poured into a boiling flask which connected with a 90 cm fractionation column. The heater under the boiling flask was turned on. The process conditions included vacuum pressure, boiling temperature, reflux ratio, equilibration time, initial heat, heat rate, condenser temperature, and maximum pot temperature were set in the computer. After the process conditions are adjusted according to the distillation plan, the vacuum pump was turned on and fractionation distillation process was began. The fractionation temperature is adjusted based on the boiling point temperature of the components in the material according to the results of GCMS analysis (Nurjanah et al., 2017): seychellene (250–251 °C), α-patchoulen (265–268 °C), Δ-guaiene (274–275 °C), α-guaiene (281–292 °C) and patchouli alcohol (287–288 °C). Fractionation distillation was conducted at pressure: 10 mmHg, initial heat 20%, maximum pot temperature: 300 °C and condenser temperature: 30 °C, while temperatures of each fraction were presented in table 1.

| Table 1. Temperature of fractionation distillation. |
|---------------------------------------------|
| Processing | Raw material | Fraction | Temperature |
| 2 stages | Patchouli oil | Fraction 1 | 230–283 °C |
| 1st stage | | Fraction 2 | 283–290 °C |
| | | Fraction 3 | 290–300 °C |
| 2nd stage | Fraction 1 of the 1st stage | Fraction 1 | 249–260 °C |
| | | Fraction 2 | 261–273 °C |
| | | Fraction 3 | 274–281 °C |
| | | Fraction 4 | 281–287 °C |
| | | Fraction 5 | 287–300 °C |
| 1 stage | Patchouli oil | Fraction 1 | 249–261 °C |
| | | Fraction 2 | 261–273 °C |
| | | Fraction 3 | 273–280 °C |
| | | Fraction 4 | 280–286 °C |
| | | Fraction 5 | 286–291 °C |
| | | Fraction 6 | 291–302 °C |
| | | Fraction 7 | 302–319 °C |
| | | Fraction 8 | 319–321 °C |

2.2. Fractionation distillation time
Distillation time is the sum of the heating up time and the time needed for formation of each fraction in hours.

2.3. Energy requirement
Energy requirements are calculated using equation:

\[ W = P \times t \]  

Where: \( W \) = Energy consumption (kWh)  
\( P \) = Power/electricity (kW)  
\( t \) = Operation time (h)
Power is calculated using formula:

\[ P = V \times I \times CF \]  

(2)

Where:

- \( P \) = Power/electricity (kW)
- \( V \) = Voltage (V)
- \( I \) = Current (A)
- \( CF \) = Correction factor = 0.84

2.4. Gas chromatography-mass spectroscopy (gcms) analysis

The analysis of patchouli oil and fraction distillation products were performed on Agilent 6890 with a Agilent 19091S-433 Capillary Column (length: 30.0 m, diameter: 250.00 µm, and 0.25µm film thickness). Initial oven temperature was 100 °C, and final temperature was 280 °C, with run time: 30 min. The carrier gas was helium with a flow rate of 402.4 mL/min, initial temperature was 280 °C, and the pressure was 10.48 psi. The components were identified using The National Institute of Standards and Technology (NIST 3.0) and WILEY 275 mass spectral libraries, and by comparison of the mass spectra with published data.

3. Results and discussions

3.1. Composition of raw materials

Raw material used in 1 stage isolation of guaiene is patchouli oil. Meanwhile, raw material used in 2 stages isolation of guaiene is patchouli oil in the 1st stage and fraction 1 resulting from fractionation in the 2nd stage. The composition of the two raw materials both patchouli oil and fraction 1 were determined using GCMS. GC chromatograms are presented in figure 2. GCMS results on both raw materials are presented in table 2 and 3. The results showed that patchouli oil had high patchouli alcohol content (30.59%), with low \( \alpha \)-guaiene content (17.34%) and \( \Delta \)-guaiene (18.4%). Meanwhile, fraction 1 contains very small patchouli alcohol (6.05%) with high content of \( \alpha \)-guaiene and \( \Delta \)-guaiene (22.10% and 24.06% respectively).

Table 2. Composition of patchouli oil as raw material of 1 stage isolation.

| No  | Retention time (minute) | Component                  | Percentage (%) |
|-----|-------------------------|----------------------------|----------------|
| 1   | 2.429                   | \( \beta \)-Pinene         | 0.21           |
| 2   | 7.917                   | \( \beta \)-Patchoulene     | 2.88           |
| 3   | 8.088                   | \( \beta \)-Elemene         | 1.08           |
| 4   | 8.678                   | Caryophyllene              | 3.4            |
| 5   | 9.063                   | \( \alpha \)-Guaiene        | 17.34          |
| 6   | 9.208                   | Seychellene                | 9.21           |
| 7   | 9.464                   | \( \alpha \)-Patchoulene    | 5.92           |
| 8   | 9.524                   | \( \alpha \)-Patchoulene    | 3.51           |
| 9   | 9.866                   | Ledene                     | 0.51           |
| 10  | 10.131                  | \( \beta \)-Chamigrene      | 0.58           |
| 11  | 10.336                  | \( \alpha \)-Selinene       | 3.71           |
| 12  | 10.507                  | \( \alpha \)-Bulnesene/\( \Delta \) guaiene | 18.4 |
| 13  | 11.679                  | 2-Acetyl-4,47-trimethylbicyclo [4.3.0]nonene | 0.68 |
| 14  | 11.978                  | (+)-endo-7-Oxabicyclo [2.2.1]hept-5-ene-2-carboxylic acid | 0.38 |
| 15  | 12.166                  | Caryophyllene oxide        | 0.39           |
| 16  | 13.081                  | \( \beta \)-Guadiene        | 0.43           |
| 17  | 13.841                  | Patchouli alcohol          | 30.59          |
| 18  | 15.081                  | Kaemferol                  | 0.78           |
|     | Total                   |                            | 100            |
Figure 2. Chromatogram of patchouli oil (a) and fraction 1 (b).

The high content of patchouli alcohol on patchouli oil shows that patchouli oil used in this study meets the SNI 06–2385–2006 standard on patchouli oil which is a minimum of 30%. While the patchouli alcohol content in fraction 1 is very low (6.05%), it due to the higher boiling point of patchouli alcohol than guaiene. Patchouli alcohol is a heavy fraction that will evaporate above the guaiene boiling point. Therefore, patchouli alcohol will extracted at fraction 2 or 3.
Table 3. Composition of fraction 1 as raw material of 2\textsuperscript{nd} stage of 2 stages isolation.

| No | Retention time (minute) | Component                                                                 | Percentage (%) |
|----|-------------------------|--------------------------------------------------------------------------|----------------|
| 1  | 7.909                   | β-Patchoulenene                                                          | 3.55           |
| 2  | 8.079                   | Beta-elemene                                                             | 1.87           |
| 3  | 8.490                   | 2H-[1,2']Bipyridinyl-3'-carboxylic acid, 3,4,5,6-tetrahydro- (4-methoxyphen... | 1.37           |
| 4  | 8.678                   | Caryophillene                                                           | 3.83           |
| 5  | 9.071                   | α-Guaiene                                                               | 22.10          |
| 6  | 9.216                   | Seychellene                                                             | 13.66          |
| 7  | 9.464                   | α-Patchoulenene                                                          | 14.97          |
| 8  | 9.858                   | α-Ylangene                                                              | 0.73           |
| 9  | 10.123                  | Aromadendrene                                                           | 0.83           |
| 10 | 10.328                  | α-Selinene                                                              | 4.83           |
| 11 | 10.516                  | α-Bulnesene/A guaiene                                                   | 24.06          |
| 12 | 11.054                  | Aromadendrene                                                           | 0.69           |
| 13 | 11.961                  | 1-Pentene, 5-(2,2-dimethylcyclopropyl)- 2-methyl-4 methylene- ........... | 0.65           |
| 14 | 12.149                  | Caryophyllene oxide                                                     | 0.78           |
| 15 | 13.773                  | Patchouli alcohol                                                       | 6.05           |
|    | Total                   |                                                                         | 99.97          |

3.2. Yield of fractionation distillation

The yield shows how much product is produced from the distillation fractionation process. The results are presented in figure 3 and 4, table 4 and 5. In the one-stage isolation of guaiene, fractionation distillation is carried out at 8 temperature ranges (table 1). However, only 5 fractions were produced, i.e. fractions of cut 1, cut 2, cut 3, cut 4 and cut 5. Fractions cut 6, cut 7 and cut 8 did not come out because all the material had been boiled, it might due to the boiling points of the components that were close together so that the material had evaporated all in the previous fraction. Whereas, in the two-stage guaiene isolation process, the resulting fraction is only 2 fractions of the 5 given temperature range. The fractions formed are fractions cut 1 and cut 2. Both processes resulted in highest yield of fraction cut 1 (43% of 1 stage and 62% of 2 stages).

Figure 3. Fractions of 2 stages guaiene isolation. Figure 4. Fractions of 1 stages guaiene isolation.
Table 4. Yield of fraction in 2 stages guaiene isolation.

| Temperature | Fraction | Percentage (%) |
|-------------|----------|----------------|
| 249–261 °C  | Cut 1    | 62             |
| 261–273 °C  | Cut 2    | 28             |
|             | Residue  | 10             |
| Total       |          | 100            |

Table 5. Yield of fraction in 1 stage guaiene isolation.

| Temperature | Fraction | Percentage (%) |
|-------------|----------|----------------|
| 249–261 °C  | Cut 1    | 43             |
| 261–273 °C  | Cut 2    | 18             |
| 273–280 °C  | Cut 3    | 4              |
| 280–286 °C  | Cut 4    | 6              |
| 286–291 °C  | Cut 5    | 6              |
|             | Residue  | 23             |
| Total       |          | 100            |

3.3. Fractionation distillation time and energy consumption

The results on the fractionation distillation time and energy consumption are presented in table 6. The 1 stage guaiene isolation required longer time (23.12 h) compared to the time required in 2 stage guaiene isolation (19.23 h). This is because at the 1 stage process, patchouli alcohol content as component with high boiling point (287–288 °C) is much higher (30.59%) in compared to the content of patchouli alcohol in 1 stage guaiene isolation (6.05%). Therefore, the high content of patchouli alcohol causes isolation of 1 stage requires more time.

Table 6. Fractionation distillation and energy consumption.

| Fractionation distillation | Time (h) | Energy consumption (kWh) |
|----------------------------|----------|--------------------------|
| Two stages                 | 19.23    | 176.18                   |
| One stage                  | 23.12    | 207.83                   |

Energy consumption is directly related to the duration of fractionation according to equation (1). So the longer the fractionation time the higher the energy needs. From table 6 it can be seen that fractionation of one stage requires energy 176.18 kWh while fractionation of one stage requires energy of 207.83 kWh.

3.4. Composition of each fraction

The component of each fractions are presented in tables 7 and 8. The 2 stages of isolation produced 2 fractions, namely fraction cut 1 and cut 2, while 1 stage isolation produced 5 fractions namely fractions cut 1, cut 2, cut 3, cut 4, and cut 5. In the 2 stage isolation, fraction cut 1 contained α-guaiene of 31.05% and Δ-guaiene of 10.73% and did not contain patchouli alcohol. Whereas, the fraction cut 2 contained α-guaiene at 4.19%, Δ-guaiene at 54.52% and containing patchouli alcohol at 1.76%. In accordance with the theory that the Δ-guaiene has higher boiling point, then in the fraction cut 2 the levels of the Δ-guaiene are higher. The results showed that in the 2-step fractionation conditions could not produce guaiene with high purity.

In the 1 stage guaiene isolation, fraction cut 1 contained guaiene of 32.6%, fraction cut 2 contained guaiene of 2.16% and fraction cut 3, cut 4 and cut 5 did not contain α-guaiene. Whereas, the fraction cut 2 contained Δ-guaiene of 64.85% and the fraction cut 5 contained patchouli alcohol of 91.09%. The results of this study indicated that 1 stage isolation can produce higher purity of α-guaiene as well as Δ-guaiene in compared with 2-stage guaiene isolation.
| No | Retention time (min) | Component | Fraction cut 1 (%) | Fraction cut 2 (%) |
|----|----------------------|-----------|--------------------|--------------------|
| 1  | 6.985                | α-Terpinene | 0.28               |                    |
| 2  | 7.669                | 2-(1-Cyclohexenyl)-7-methyl-2,4,6-octatriene | 0.81               |                    |
| 3  | 7.9                  | β-Patchoulen | 5.16               |                    |
| 4  | 8.071                | β-Elemene | 2.75               |                    |
| 5  | 8.481                | 4-Fluorobenzoic acid, propargyl ester | 2.04               |                    |
| 6  | 8.678                | Caryophillene | 5.31              |                    |
| 7  | 9.097                | α-Guaiene | 31.05              | 4.19               |
| 8  | 9.174                | Seychellen | 15.7              | 5.84               |
| 9  | 9.481                | α-Patchoulen | 11.72             | 9.9                |
| 10 | 9.541                | Germacrene | 5.49              | 0.48               |
| 11 | 10.114               | δ-Gurjunene | 0.53              |                    |
| 12 | 10.140               | β-Guaiadiene |                      | 2.06               |
| 13 | 10.234               | Aromadendrene | 0.38            | 3.76               |
| 14 | 10.319               | α-Selinene | 2.52              | 11.06              |
| 15 | 10.499               | Δ-guaiene/α-Bulnesene | 10.73       | 54.52              |
| 16 | 11.661               | 2-Allyl-3,4-dimethoxybenzaldehyde 5,6-Dimethylphenanthrene | 2.53             |                    |
| 17 | 11.824               | β-Selinene |                      | 0.76               |
| 18 | 12.149               | Caryophyllene oxide | 0.27          | 3.14               |
| 19 | 13.764               | Patchouli alcohol |                      | 1.76               |
| 20 | 24.347               | Hexanedioic acid, bis (2-ethylhexyl) ester | 5.26             |                    |
|    |                      | Total | 100              | 100                |
Table 8. Component of 1 stage fractionation distillation.

| No | Retention time (min) | Component                                      | Fraction       |
|----|----------------------|------------------------------------------------|----------------|
| 1  | 7.002                | Δ-Elemene                                      | 0.2            |
| 2  | 7.686                | 2-(2-AcetylpHENYL)cyclopentanone               | 0.54           |
| 3  | 7.917                | β-Patchouline                                   | 5.58           |
| 4  | 8.088                | β-Elemene                                      | 2.58           |
| 5  | 8.498                | 4-Fluorobenzoic acid, propargyl ester          | 1.72           |
| 6  | 8.687                | Caryophyllene                                  | 6.96           |
| 7  | 9.105                | α-Guaiene                                      | 32.6           |
| 8  | 9.242                | Seychellen                                     | 15.78          |
| 9  | 9.482                | α-Patchouline                                   | 12.27          |
| 10 | 9.550                | β-Guaiene                                      | 6.28           |
| 11 | 9.695                | Caryophyllene                                  | 0.61           |
| 12 | 9.952                | Ledene                                         | 2.03           |
| 13 | 10.131               | β-Selinene                                     | 0.58           |
| 14 | 10.242               | Aromadendrene                                   | 0.25           |
| 15 | 10.336               | α-Selinene                                     | 2.67           |
| 16 | 10.362               | 4-(4-Isobutylphenyl)-2-pentene                 | 0.36           |
| 17 | 10.507               | α-Bulnesene/ Δ-Guaiene                          | 10.68          |
| 18 | 11.063               | Aromadendrene                                   | 0.13           |
| 19 | 11.072               | 2.4-Quinolinediol                               | 0.61           |
| 20 | 11.149               | α-Panasinsen                                    | 1.45           |
| 21 | 11.208               | Ledene                                         | 0.6            |
| 22 | 11.362               | 2,2,5,7-tetramethyl-3,4-dihydrornaphthalen-1(2H)-one | 0.69 |
| 23 | 11.619               | Elemol                                         | 0.19           |
| 24 | 11.670               | 4H-Inden-4-one, 1,2,3,5,6,7-hexahy dro-1,1,2,3,3-pentamethyl | 1.89 |
| 25 | 11.687               | β-Selinene                                     | 0.71           |
| 26 | 11.901               | Acetone (1R)-(+)camphor azine                  | 3.17           |
| 27 | 12.080               | Caryophyllene oxide                            | 0.17           |
| 28 | 12.260               | 5-Methoxy-1-(2-methylpropenyl)indica           | 2.74           |
| 29 | 12.268               | 2.6-Diethenyl-4-tert-butylphenol               | 0.73           |
| 30 | 12.380               | 2.5,7-tetramethyl-3,4-dihydrornaphthalen-1(2H)-one | 0.72 |
| 31 | 12.576               | Spathulenol                                    | 0.18           |
| 32 | 12.747               | Spiro [4.4]nonan-2-one                         | 0.43           |
| 33 | 12.867               | Longifolonaldehyde                             | 2.84           |
| 34 | 12.892               | Isoaromadendrene epoxide                       | 2.68           |
| 35 | 12.935               | Alloaromadendrene oxide                        | 1.32           |
| 36 | 13.089               | Veridiflorol                                   | 2.14           |
| 37 | 13.098               | α-Caryophyllene alcohol                        | 1.19           |
| 38 | 13.277               | Gamma-Gurjunene                                | 0.81           |
| 39 | 13.790               | Patchouli alcohol                              | 0.39           |
| 40 | 16.286               | Gamma-Cadinene                                 | 0.17           |

Total 99.99 | 100 | 100 | 100 | 100
4. Conclusion
Stage of fractionation distillation affected $\alpha$-guaiene and $\beta$-guaiene production, fraction distillation time and energy consumption. Isolation of guaiene in two stages needed less energy (176.18 kWh) and process time (19.23 h), with higher content of $\alpha$-guaiene (30.40%) than one stage fractionation distillation. One stage fractionation distillation produce higher $\beta$-guaiene (64.85%) than two stages fractionation distillation.

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