Separation of Oleoresin from Nutmeg Using Ultrasound Assisted Extraction and Hexane as Solvent

Victoria Kristina Ananingsih, Bernadeta Soedarini, Emerentiana Karina
Department of Food Technology, Soegijapranata Catholic University, Semarang, Indonesia

Email: kristina@unika.ac.id

Abstract. Indonesia has many herbs and spices that function as ingredients for making herbal medicine or for cooking. Nutmeg (Myristica fragrans) is an Indonesia’s origin plant that has many health benefits. It produces specific flavors come from its bioactive compounds, namely oleoresin. Oleoresin can be extracted from nutmeg to get the concentrated compounds; hence it can be applied easily as the ingredient of food and beverage products. Ultrasonic assisted extraction (UAE) is applied in this research since it has higher yield compared to other extraction methods. This research is aimed to optimize the process condition of ultrasonic assisted extraction of nutmeg to get the good qualities of oleoresin. Solvent used in this research was hexane with extraction temperatures of 39, 45, 52 °C, and extraction times of 30, 45 and 60 minutes. Ratio between nutmeg and solvent were 5:100, 10:100 and 15:100. Frequency used was 45 kHz. The results showed that the optimum process condition to separate oleoresin from nutmeg were at extraction temperature of 52 °C, extraction time of 60 minutes and ratio between nutmeg and hexane of 5:100. This optimized process condition produced yield 62% of extracted oleoresin. Antioxidant activity and phenol content of this optimized oleoresin were 44.78% and 0.11 ppm respectively.

Keywords: nutmeg, oleoresin, ultrasound, hexane

1. Background

Nutmeg (Myristica fragrans) is an original plant from Indonesia that grows well in tropical climates at altitudes below 700 meters above sea level. When the nutmeg is cut into two parts, the nutmeg seeds appear wrapped in a red layer called mace. Nutmeg seeds can be used to give flavor to food products due to the presence of oleoresin and essential oils contained in them. As a country of origin of nutmeg, Indonesia supplies nearly 75% of the world’s nutmeg and mace seed needs. The popularity of nutmeg on the global market began in the 16th century since Ternate, Tidore, Ambon and the Banda Islands became the main pathway for foreign countries to enter Indonesia. In 2006, Indonesia exported 347,919 kg of nutmeg with a total export value of 1,861,232 USD. Then in 2017, Indonesia exported 11,505,972 kg of nutmeg with a total export value of 50,138,286 USD (Directorate General of Plantations, 2017).

Nutmeg consists of three parts namely flesh, mace, and nutmeg seeds. Nutmeg meat can be processed as a pickled fruit product. Whereas mace and nutmeg seeds are known to have high oleoresin content, but oleoresin has easily oxidized properties. Oleoresin extract from nutmeg has a high amount of demand for domestic and export needs so it requires more handling to maintain its quality. Therefore it need a method to produce optimal amount of oleoresin. The most commonly used method to obtain oleoresin...
is the extraction method. Ultrasound extraction has high mass transfer efficiency, less use of organic solvents, and higher extraction rates than the Soxhlet method. \cite{1} At present ultrasound, extraction has begun to be applied in industries such as edible oil extraction. \cite{2} Ultrasound extraction is more effectively applied for extraction at low temperatures which would be advantageous for extracting heat-sensitive compounds. \cite{3}

This study aims to determine the effect of the ratio between the solvent and the ingredients, temperature, and extraction time on the effectiveness of the nutmeg oleoresin extraction process. In addition, this study also aims to determine the total phenolic content and antioxidant activity in nutmeg oleoresin extract.

2. Methods

2.1. Materials

The materials used in this study were nutmeg, n-hexane, Folin-Ciocalteau compound, sodium carbonate solution (7.5%), DPPH solution in ethanol 0.1 mM, gallic acid, KOH 0.1 N solution, phenolphthalein indicator, a solution of 0.5 N HCl, and distilled water.

2.2. Response Surface Methodology (RSM)

This method is used to analyze problems where several independent variables influence the response variable and create a model or experimental design to optimize the response. The relationship between the response of Y and the independent variable X is as follows:

\[ Y = b_0 + \sum_{i}^{k} b_i x_i + \sum_{i}^{k} b_{ii} x_i^2 + \sum_{i}^{k} b_{i} x_i^2 \]

Explanation:
Y = response variable
X\(_i\) = independent variable / factor (i = 1,2, ... k)
b = coefficient

The full experimental design in this study can be seen as follows:

a) Independent Variable
- Temperature, connoted as X\(_1\) with points: 390C, 450C, and 520C.
- Time, connoted as X\(_2\) with points: 30 minutes, 45 minutes and 60 minutes.
- Ratio, connoted as X\(_3\) with points: 5:100, 10:100, and 15:100.

b) Response Variable
- Percentage of yield
- Total phenolic
- Percentage of antioxidant activity

c) First order model: factorial design 2\(^k\), each factor having a low level (code: -1), middle level (code: 0), high level (code: +1), and a level on the axial axis (code: -α and +α). The α value for the CCD design is:

\[ \alpha = (2^k)^{1/4} \]
Explanation:

- \( \alpha \): axial axis
- \( k \): number of factors or independent variables

In this study, there are 3 (three) independent variables, then an \( \alpha \) value of 1.682 is obtained. The magnitude of the variables with codes - \( \alpha \) and + \( \alpha \) can be calculated by the equation:

\[
- \alpha = (0) - 1.682 \cdot [(0) - (-1)] \\
+ \alpha = (0) + 1.682 \cdot [(0) - (-1)]
\]

With this equation, we obtain 3 (three) independent variables with 5 (five) level variables.

2.3. Oleoresin Extraction Process

The oleoresin extraction process begins by drying the nutmeg seeds using an oven at 60°C for 1 hour. Then nutmeg crushed using a blender. Furthermore, nutmeg seed powder is put into an Erlenmeyer tube and the n-hexane solvent is added, and then tightly closed with aluminum foil. After the Erlenmeyer tube is inserted in an ultrasonic bath that has been set in temperature and time. The extraction temperature range used is 39°C, 45°C, and 52°C. The extraction time range used is 30 minutes, 45 minutes, and 60 minutes. The ratio of the sample to the solvent used is 5:100, 10:100, and 15:100. Then the extract is filtered with a filter cloth. After that, the extract is evaporated with a rotary vacuum evaporator for 4-6 minutes at 40°C. With a speed of 50 rpm. Furthermore, the extract was put into a vial bottle and stored in a freezer so that it can be used for further analysis.[5]

2.4. Nutmeg Oleoresin Quality Testing

Tests carried out to measure the extraction efficiency of nutmeg oleoresin or rendemen[6], testing of antioxidant activity[7], and total phenolic testing[8].

3. Results and Discussion

3.1. Effectiveness of Oleoresin Extraction

The results of the optimization analysis of the yield of nutmeg oleoresin extraction can be seen in Figure 1, 2 and which is a fitted surface graph depicting the relationships between variables in three coordinate segments so as to form a 3D (three-dimensional) curve, namely (1) time and ratio variables (2) time and temperature (3) temperature and ratio, to the yield of nutmeg oleoresin yield. Based on the graph, the red area shows the optimal area. In this study, the optimal point of extraction temperature was 41.27°C, extraction time was 55.30 minutes, and the ratio of material was 11.79 grams to the yield of nutmeg oleoresin.
Figure 1. Optimization Analysis Results of Nutmeg Oleoresin Extraction Results Based on Time and Ratio

Figure 2. Optimization Analysis Results of Nutmeg Oleoresin Extraction Results Based on

Figure 3. Analysis Results of Optimization Results of Nutmeg Oleoresin Yield Based on Temperature and Ratio
Figure 4. Results of Nutmeg Oleoresin Yield by Temperature, Time, and Ratio

Based on Figure 4, it can be concluded that the yield of nutmeg oleoresin obtained is fluctuating. Some points show that the higher the extraction temperature, the higher the yield produced and the other points show that the higher the extraction temperature, the lower the yield. This also occurs at the time of extraction, some points indicate that the longer the extraction time, the higher the yield produced and some other points indicate that the shorter the extraction time, the lower the yield produced. Whereas in the ratio of materials used, most points indicate that the less material used, the higher the yield produced.

Table 1. Results of Regression Analysis of Nutmeg Oleoresin Yield

| Parameter       | Regression Analysis Results | Coefficient | P Value |
|-----------------|----------------------------|-------------|---------|
| A constant      |                            | 109,514     | 0.202   |
| Time (X2)       |                            | -1,185      | 0.263   |
| Ratio (X3)      |                            | 0,214       | 0.942   |
| X1.X2           |                            | -0,170      | 0.027   |
| X1.X3           |                            | 0,046       | 0.010   |
| R²              |                            | 91.27%      |         |

It can be seen in Table 1 that the interaction between temperature and time variables has a significant effect on the yield of nutmeg oleoresin because the p value is 0.027 or smaller than α (0.05). In addition, the interaction between temperature and ratio variables also had a significant effect on the yield of nutmeg oleoresin because the p value was 0.010 or smaller than α (0.05). Based on the results of the regression analysis, an equation is obtained:

\[ Y = 109.514 - 1.185X_2 + 0.214X_1 - 0.170X_1.X_2 + 0.046X_1.X_3 \]

Explanation:
Y = yield
X₁ = temperature
X₂ = time
X₃ = ratio
3.2. Antioxidant Activity

The graph above is a fitted surface graph that illustrates the relationship between variables in three coordinate segments so as to form a 3D (three-dimensional) curve that is the temperature and time variable of the antioxidant activity of nutmeg oleoresin. In this study, the optimal extraction temperature was obtained at 43.82°C, the extraction time was 51.92 minutes and the ratio of 1.59: 100 to the antioxidant activity of nutmeg oleoresin.

The longer the extraction time, the more extracts are produced. The longer time needed for the extraction process can make the contact between the sample with the solvent becomes longer so that the diffusion process can run optimally. The higher the temperature used in the extraction process will help the process of diffusion of solvents into the tissue material so that it can increase the activity of the solvent in hydrolyzing substances in the cell material and the extraction time can run faster. However, using too high a temperature and too long a time can damage the structure of the substance of the desired extract. The longer the extraction time and the more volume of the solvent used can produce a more concentrated extract. Also the longer the extraction time can increase the specific gravity of the extract because the extract also evaporates along with the solvent. The high yield in extraction results can also be influenced by the presence of residues that carry over into the extract. Another factor that influences yield yields is the ratio of the ingredients to the solvent. In this study, the highest yield was found in the ratio of ingredients to solvents of 1.59: 100, followed by a comparison of materials with solvents of 10: 100. The more the amount of solvent added to the sample, the greater the ability of the solvent to extract the oil contained in nutmeg seeds. However, solvents have a maximum limit on the ability to extract oil in nutmeg seeds.
Figure 6. Test Result of Nutmeg Oleoresin Antioxidant Activity

Based on Figure 6 it can be concluded that the results of testing the antioxidant activity of nutmeg oleoresin extract obtained are fluctuating. Some points indicate that the higher the extraction temperature, the higher the yield produced and some other points indicate that the higher the extraction temperature, the higher the antioxidant activity. This also occurs at the time of extraction and the ratio of material used, some points indicate that the longer the extraction time and the more material used, the higher the antioxidant activity and some other points indicate that the shorter the extraction time and the less material used, the lower its antioxidant activity.

3.3. Total Phenolic
The graph below is a fitted surface graph that illustrates the relationship between variables in three coordinate segments so as to form a 3D (three-dimensional) curve that is the time variable and the ratio of total phenolic nutmeg oleoresin. Based on the graph, the red area shows the optimal area.

Overall, the highest total phenolic content was obtained at a temperature of 36.28°C for 35 minutes at a ratio of a material to a solvent of 9.76: 100. The longer the extraction time, the higher the extract produced. In addition, the use of temperatures that are not too high can prevent extraction damage due to heat, but the extraction time required is getting longer. Therefore, the higher the yield produced, the more antioxidants (phenols) contained in it so as to increase the total phenolic number.

Figure 7. Analysis Results of Optimization of Total Test of Phenolic Oleoresin in Nutmeg Seeds Based on Time and Ratio
Based on Figure 8 it can be concluded that the total phenolic test results of nutmeg oleoresin extract obtained are fluctuating. Some points indicate that the higher the extraction temperature, the higher the polyphenols produced and the other points indicate that the higher the extraction temperature will reduce the polyphenol content in oleoresin extract. This also occurs in the extraction time variable and the ratio of the material used, some points indicate that the longer the extraction time and the many ingredients used, the higher the polyphenol content in oleoresin extracts. While some other points show that the longer the extraction time and the many ingredients used will reduce the levels of polyphenols in nutmeg oleoresin extract.

![Figure 8. Analysis Results of Optimization of Total Test of Phenolic Oleoresin in Nutmeg Seeds](image)

4. Conclusions

The combination of treatments to obtain the optimum percentage yield is at extraction temperature 41.27°C, extraction time for 55.30 minutes, and the ratio of 11.79:100. The combination of treatments to obtain antioxidant activity with optimum percentage is at extraction temperature of 43.82°C, extraction time for 51.92 minutes, and the ratio of 7.55:100. The combination of treatments to obtain total phenolic with optimum amount is at extraction temperature of 43.82°C, extraction time for 51.92 minutes, and the ratio of 7.55:100.

Based from the parameters tested, the yield is the response to the independent variables temperature, time, and ratio show that the yield has a mathematical model: \( Y = 109.514 - 1.185X_2 + 0.214X_1 - 0.170X_1X_3 + 0.046X_1X_2 \), with an \( R^2 \) value of 0.9127. Based on the mathematical model determined, the optimum value of the independent variables from the results of the yield analysis will produce an antioxidant percentage of 94.71%. Based on the mathematical model determined, the optimum value of the independent variables from the total phenolic analysis yield results the percentage of 30.11%.

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