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Riyanto Riyanto * and Vika Nur Jinan

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Keywords: nutmeg oil; carbon dioxide; supercritical; fluid; extraction

*Article*

# Nutmeg Oil *(Myristica Fragrans)* Extraction Using $CO_2$ Supercritical Fluid Extraction (SCFE)

**Riyanto [1,*] and Vika Nur Jinan [1]**

[1] Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Islam Indonesia, Sleman, Yogyakarta, Indonesia, 55584

* Correspondence: riyanto@uii.ac.id; Tel.: +62274 895920/+62274 898582

**Abstract:** Demand for nutmeg essential oil in Indonesia and the world is increasing. Nutmeg essential oil is obtained from young nutmeg seeds extracted by water steam distillation (WSD). The weakness of the extraction process with WSD is the long distillation time (24 hours), wasteful of fuel, wasteful of manpower, produces polluting gases which damage the environment and requires a lot of water as a coolant. The purpose of this study was to compare the WSD and SCFE methods for extracting nutmeg essential oil. The research was conducted by extracting nutmeg essential oil using two methods are WSD and SCFE. The results showed that the chemical composition of the nutmeg essential oil produced by the two methods was almost similar. Nutmeg essential oil produced by both methods shows the same characterization. The yield between the two methods is almost the same, in the range of 10-12% using extraction time 15-60 minutes. The SCFE method has advantages over WSD are a faster extraction time, does not require heating materials and $CO_2$ gas can be reused. In conclusion, SCFE is an alternative method to increase the production of nutmeg oil.

**Keywords:** Nutmeg oil; extraction; carbon dioxide; supercritical, fluid, extraction.

## 1. Introduction

The nutmeg plant has fruit, seeds and flowers which can be extracted as very important commodities. Parts of the nutmeg plant such as flowers, fruit (flesh and seeds) are widely used in cosmetics, medicine, beverage, perfume, aromatherapy, and food industries. Nutmeg oil extracted from nutmeg seeds can be used as a raw material for making soap, medicine, perfume, and aromatherapy [1–3].

Nutmeg oil can be produced from nutmeg seeds through a distillation process. The method widely used for extracting nutmeg oil is Water Steam Distillation (WSD). Nutmeg oil is used by the pharmaceutical industry as a raw material for medicines, additives in food and drinks. Nutmeg oil contains chemicals such as myristicin, limonene, sabinene, alpha and gamma-terpinene [4,5]. Myristicin is a very important compound in nutmeg oil. The myristicin content in nutmeg oil *(Myristica fragrans)* according to the ISO 3215:1998 standard is at least 10%. Myristicin levels also determine the quality of nutmeg oil. Other parameters to determine the quality of nutmeg oil are colorless or pale yellow, has a characteristic aroma of nutmeg oil, refractive index of 1.470-1.497, density of 0.880-0.910 g/mL, optical rotation +8-25$^{o}$, solubility in ethanol 90% (1:3) clear and maximum remaining evaporation is 2% [6,7].

The pharmaceutical industry widely uses nutmeg oil as a precursor. Based on research results, it shows that nutmeg oil has biological activity. Myristicin is a compound in nutmeg oil that has long been used as medicine. This compound has analgesic, antimicrobial, anti-inflammatory, hepatoprotective and antioxidant effects. This compound is also known as a hallucinogenic compound, namely a compound that acts as a serotonin receptor antagonist [8,9]. This compound also acts as a neurotoxic agent in neuroblastoma, namely by inhibiting monoamine oxidase. Myristicin used in excessive doses can cause damage to human organs. Symptoms caused by poisoning with this compound are nausea, seizures, delirium, blurred vision, and palpitations [9].

Nutmeg oil refined by water steam distillation (WSD) meets SNI for export purposes. The weaknesses of the extraction process by water vapor distillation are low yield, long distillation time (24 hours), wasteful of fuel, wasteful of manpower, produces polluting gases which damage the environment and requires a lot of water as a coolant. Refining nutmeg oil by WSD yields little profit. Refining nutmeg oil by water steam distillation produces a yield of 9-10% [10]. Because of these problems, the researchers proposed the use of a new extraction method are the supercritical fluid extraction (SCFE) method using $CO_2$ gas.

Extraction of nutmeg oil using WSD has many disadvantages such as long extraction time, uses a lot of fuel, is a source of air pollution and high costs. SCFE is a new extraction method that is more efficient and lower cost. This method does not produce air pollution gases and is environmentally friendly. SCFE requires little solvent, resulting in high yields and shorter extraction times. SCFE is very suitable for the extraction of materials that cannot withstand high temperatures and are sensitive to heat. $CO_2$ gas used in SCFE does not destroy ozone, is not flammable, safe for human health and environmental, reusable, inert, has high solubility, non-flammable, non-corrosive and is non-toxic. This gas is easy to apply and controlled with temperature changes. $CO_2$ gas works in a supercritical state which is influenced by temperature and pressure. Supercritical fluid is a fluid with a temperature higher than the critical temperature and a pressure higher than the critical pressure [11].

SCFE is an extraction method based on the solubility of chemicals with supercritical $CO_2$ liquid. This method can be used for the extraction of active ingredients from plants and animals. The $CO_2$ gas that has been extracted is released and reused. This method is known as supercritical fluid extraction. SCFE can use carbon dioxide gas, sulfur hexafluoride, heptane, ammonia, ethane, and nitrous oxide. $CO_2$ gas is often chosen for the SCFE process because it has several advantages such as being colorless, low critical temperature, chemically inert, easy to obtain in a pure state and cheap. $CO_2$ gas is widely used to extract natural materials.

$CO_2$ gas as an extractant is in a critical state at a temperature of 31.1 $^oC$ and a pressure of 7.38 MPa. Cheap operational conditions, suitable for materials that are sensitive to heat and high boiling points. Natural ingredients that are often extracted with $CO_2$ gas include essential oils, spices, flavorings, and vitamins. $CO_2$ gas is very stable, non-toxic, and does not cause products to oxidize. Extraction with $CO_2$ gas does not contain nitrates, dangerous heavy metals and is free from dangerous residual solvents. $CO_2$ gas is easily recovered in its pure state by changing the temperature and pressure. $CO_2$ gas can be reused for the next process, so supercritical $CO_2$ extraction is more efficient.

SCFE with $CO_2$ gas is an environmentally friendly extraction model [12–14]. Several researchers have used the SCFE method for the extraction of essential oils such as extraction of turmeric [15], cumin *(Carum carvi L.)* [16], sucupira branca seeds [17], algerian rosemary [18], lippia thymoides and Schauer *(Verbenaceae)* [19], Leptocarpha rivularis [20], dry ginger [21], candeia wood *(Eremanthus erythropappus)* [22], clove leaves *(Syzygium aromaticum)* [23], rosehip seeds [24], Santolina chamaecyparissus [25], perovskia atriplicifolia benth [26] and chamaecyparis obtuse [27].

Therefore, the SCFE method needs to be applied for the extraction of food, biological, pharmaceutical, and cosmetic ingredients. Materials containing aromatic compounds are very suitable for extraction using this method. The SCFE method can maintain aroma and increase product purity. This method is widely used to extract various types of seeds such as fennel, cumin, and celery. This method also has the potential to extract essential oils from various types of plants. The method can be used to extract essential oils such as jasmine, rose, cinnamon, ginger, chrysanthemum, and various types of spices. One of the essential oils with a lot of market demand is nutmeg oil. Meanwhile, nutmeg oil is obtained by water steam distillation (WSD). This method is widely used by nutmeg oil entrepreneurs. This method has many disadvantages, namely long extraction time (24 hours), requires a lot of fuel, complicated product separation and produces polluting gases. Therefore, it is necessary to carry out comparative research on nutmeg oil extraction using the WSD and SCFE methods. Nutmeg plants and seeds are shown in Figure 1.

In this paper, the research results of extracting nutmeg oil are presented using a new method is SCFE. Comparative data on the yield, physical and chemical properties of nutmeg oil between the two methods are WSD and SCFE, are presented in the results of this study. SCFE can be used as a solution to increase the productivity of agricultural products, especially nutmeg oil.

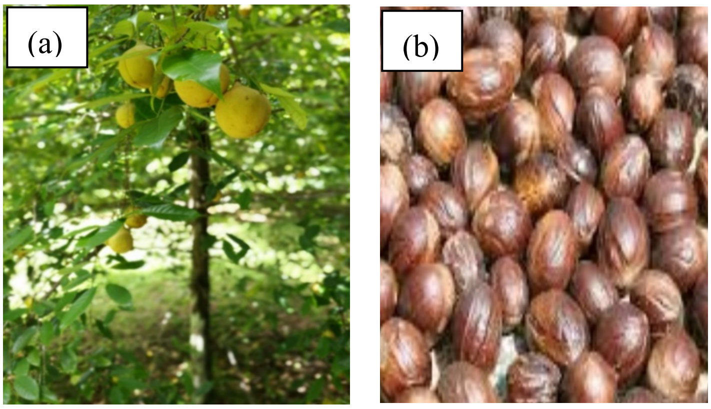


**Figure 1.** Examples of nutmeg plants (a) and nutmeg seeds (b).

## 2. Materials and Methods

### *2.1. Equipment and materials*

The equipment was used a set of WSD, a set of SCFE, Gas Chromatography-Mass Spectrometry (GC-MS) from the Shimadzu QP 2010 SE type, Abbe refractometer, Polarimeter Polax-2L and a pycnometer. The research material is young nutmeg seeds obtained from Bogor, West Java, Indonesia, pure $CO_2$ gas.

### *2.2. Preparation of raw materials for nutmeg seeds*

Dry young nutmeg seeds are mashed using a blender. Young nutmeg powder weighed as much as 5 kg. Young nutmeg powder is divided into two pieces, each as much as 2.5 kg. The two raw materials were extracted using the WSD and SCFE methods.

### *2.3. The process of isolating nutmeg seed oil using WSD*

As much as 2.5 kg of young nutmeg powder is included in the sample container. The extraction process was carried out for 24 hours through two stages was steam distillation without pressure and distillation with pressure. Steam distillation without pressure was carried out in the first stage is 12 hours at 100-110 $^{o}$C, without pressure. The second stage of distillation under pressure was carried out for 12 hours at a temperature of 120-130 $^{o}$C using a pressure of 1 bar. After 24 hours of collecting nutmeg oil in the separator, three layers were formed, water, heavy fraction of nutmeg oil (below), water (in the middle) and light fraction of nutmeg oil (above). The nutmeg oil is separated, mixed with the heavy and light fractions, and collected in a brown bottle. The WSD equipment used in this study is shown in Figure 2a.

### *2.4. The process of isolating nutmeg seed oil using SCFE method*

As much as 2.5 kg of young nutmeg powder was put into the extraction tube and then closed tightly and tightly. $CO_2$ gas pipe installed and connected to the extractor. The $CO_2$ gas cylinder valve that connects to the extractor is opened. $CO_2$ gas was introduced at a pressure of 120 bar with a temperature of 35 $^{o}$C and an extraction time of 60 minutes. After 60 minutes the valve towards the separator is opened to release $CO_2$ gas. The faucet towards the separator is opened slowly until the pressure in the extraction tube runs out or 0 bar then wait a few minutes to extract the nutmeg oil extract. Nutmeg oil is collected in a brown bottle. The SCFE part is shown in Figure 2b and the SCFE scheme is shown in Figure 3.


(a) (b)

**Figure 2.** Extraction of nutmeg oil using water steam distillation with part A. boiler B. place of raw materials C. condenser D. separator (a) and component parts of SCFE (b).

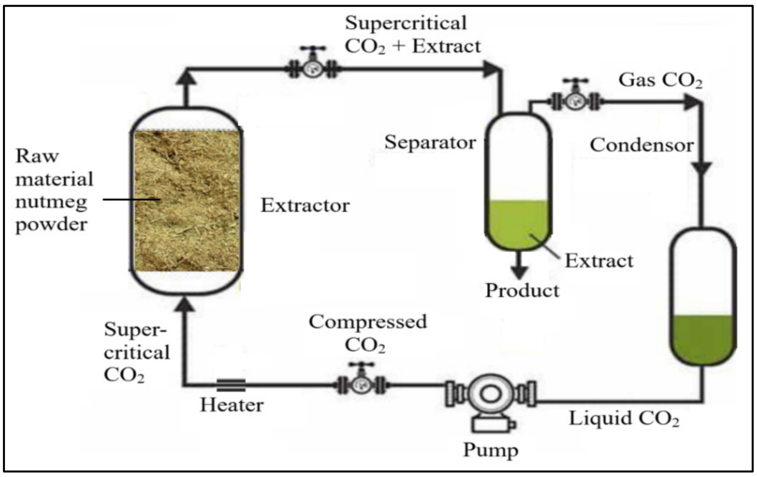


**Figure 3.** Procedure scheme for extraction of nutmeg oil using SCFE.

*2.5. Characterization of nutmeg oil*

Nutmeg oil extracted using the WSD and SCFE methods is characterized based on ISO 3215:1998 oil of nutmeg. The parameters analysed include colour and odor, venom index, density, optical rotation, solubility in ethanol, evaporation residue and myristicin compound content. The refractive index was analysed with an Abbe refractometer, the density was determined with a pycnometer, the optical rotation was analysed with a Polax-2L polarimeter. Analysis of myristicin and other compounds using GC-MS Shimadzu QP 2010 SE.

## 3. Results

*3.1. Physical appearance of Nutmeg oil*

The nutmeg oil produced by both methods is put in a bottle. The results of nutmeg oil are shown in Figure 4. The colour produced from the WSD method is clearer (Figure 4a) than the SCFE method. The SCFE method causes all pattern chemicals to be extracted in $CO_2$. Based on the ISO 3215:1998 standard nutmeg oil has a colourless or pale-yellow colour [28]. Nutmeg oil with both methods produces colour and odor according to quality standards. The yellow colour of nutmeg oil extracted with SCFE is caused by non-essential compounds such as fat being extracted [14].

Carbon dioxide gas in a supercritical state can be used to separate fragrances, pigments, antioxidants, flavourings, essential oils, and fatty acids. Because of this, this gas is widely used for

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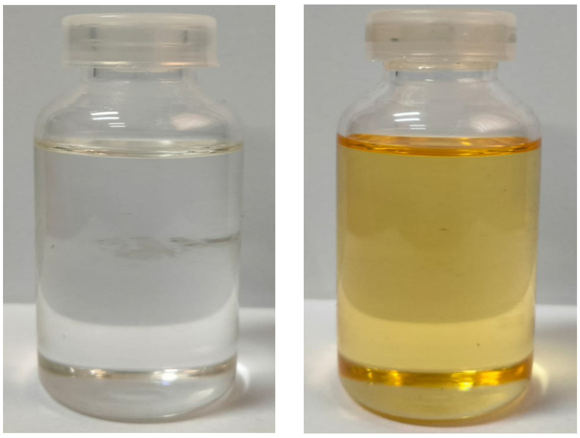
extraction of animal and plant samples. The extraction results are used as ingredients for making soap, food, perfume, cigarettes, drinks [29]. The essential oil extracted using SCFE is yellow because carotenoid compounds are also extracted. This compound causes food to have a reddish yellow colour. Essential oils with a reddish yellow colour are preferred by consumers. This compound has antioxidant and anticancer properties thereby improving human health [30,31].



(a) (b)

**Figure 4.** Physical appearance of nutmeg oil, where nutmeg oil is extracted with WSD (a) and SCFE (b).

*3.2. Analysis of nutmeg oil content with GC-MS*

Nutmeg oil produced by both methods was analysed using GC-MS. The chromatogram of the analysis results is shown in Figure 5. The chromatograms of Figure 5a and 5b show the chromatograms of the analysis results of nutmeg oil extracted with WSD and SCFE. Nutmeg oil extracted with WSD and SCFE showed 25 peaks or 25 compounds. The names of the compounds and the percent composition are shown in Table 2. The composition of the main compounds in nutmeg oil are Myristicin, α.-Pinene, (-)-4-Terpineol, (-)-β.-Pinene, γ.-Terpinene, (-)-Limonene and Sabinene. There is a significant difference between the two methods, the sabinene compound. The sabinene compound with a composition of 29.94% (Table 1) was only produced using SCFE. This phenomenon is because the compound has a high polarity, so it dissolves easily in $CO_2$ gas [16]. $CO_2$ also can extract volatile compounds [13].

Table 1 shows that the main compounds in nutmeg oil using the WSD method are Myristicin (30.30%), α.-Pinene (12.01%), (-)-4-Terpineol (9.75%), (-)-β.-Pinene (9.65%), γ.-Terpinene (6.63%) and (-)-Limonene (4.99%). While the main compounds in nutmeg oil using the SCFE method are sabinene (29.94%), α.-Pinene (18.01), Myristicin (16.22%), β.-Pinene (15.73%), limonene (5.91%) and Safrol (1.96%). The chemical composition of the extraction results with SCFE is the same as the results of the study by Singh et al. (2005) that nutmeg oil has a composition of sabinene (20.22%) [32].

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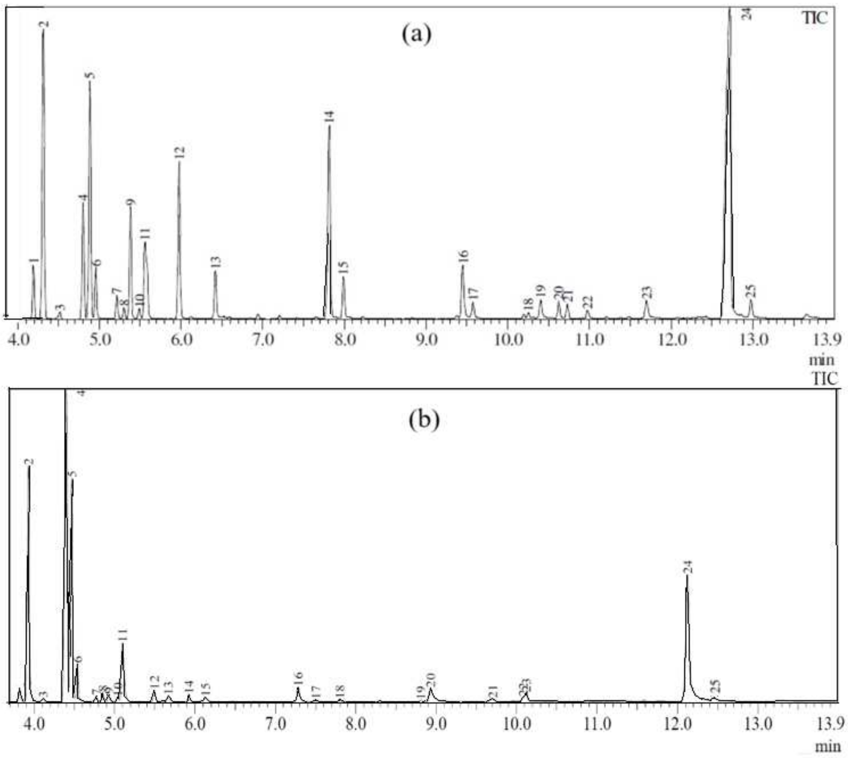


**Figure 5.** Chromatogram of nutmeg oil analysis by GC-MS extraction using (a) WSD and (b) SCFE methods.

**Table 1.** Comparison of chemical composition of nutmeg oil between WSD and SCFE methods.

| No. | Name of compounds | WSD | | SCFE | |
|---|---|---|---|---|---|
| | | Retention Time (minutes) | Area (%) | Retention Time (minutes) | Area (%) |
| 1 | Myristicin | 12.719 | 30.30 | 12.127 | 16.22 |
| 2 | α.-Pinene | 4.313 | 12.01 | 3.927 | 18.01 |
| 3 | (-)-4-Terpineol | 7.813 | 9.75 | 7.284 | 1.41 |
| 4 | (-)-β.-Pinene | 4.884 | 9.65 | 4.466 | 15.73 |
| 5 | γ.-Terpinene | 5.977 | 6.63 | 5.494 | 0.89 |
| 6 | (-)-Limonene | 5.558 | 4.99 | 5.096 | 5.91 |
| 7 | β.-Phellandrene | 4.800 | 4.61 | - | - |
| 8 | (+)-2-Carene | 5.380 | 4.41 | 4.853 | 0.67 |
| 9 | Safrole | 9.449 | 2.76 | 8.928 | 1.96 |
| 10 | α.-Terpinolene | 6.419 | 1.99 | 5.923 | 0.63 |
| 11 | α.-Thujene | 4.191 | 1.94 | 3.819 | 0.93 |
| 12 | α.-Terpineol | 7.989 | 1.83 | 7.500 | 0.17 |
| 13 | β.-Myrcene | 4.955 | 1.82 | 4.537 | 2.75 |
| 14 | Eugenol | 10.404 | 0.92 | - | - |
| 15 | (E)-Isoeugenol | 11.702 | 0.90 | - | - |
| 16 | α.-Phellandrene | 5.212 | 0.89 | 4.770 | 0.44 |
| 17 | β.-Asarone | 12.979 | 0.86 | 12.454 | 0.35 |
| 18 | 4-Pentylanisole | 9.575 | 0.77 | - | - |
| 19 | Neryl acetate | 10.626 | 0.74 | - | - |
| 20 | α.-Copaene | 10.730 | 0.64 | 9.701 | 0.30 |
| 21 | δ-3-Carene | 5.302 | 0.41 | 4.853 | 0.67 |
| 22 | p-Cymene | 5.487 | 0.39 | 5.039 | 0.36 |
| 23 | Methyleugenol | 10.978 | 0.39 | - | - |
| 24 | Camphene | 4.514 | 0.21 | 4.112 | 0.30 |

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| | | | | | |
|---|---|---|---|---|---|
| 25 | α.-Terpinyl acetate | 10.254 | 0.19 | - | - |
| 26 | Sabinene | - | - | 4.399 | 29.94 |
| 27 | α.-Thujene | - | - | 3.819 | 0.93 |
| 28 | α.-Cubebene | - | - | 10.119 | 0.78 |
| 29 | Trans Sabinene Hydrate | - | - | 5.667 | 0.60 |
| 30 | α. Terpinene | - | - | 4.925 | 0.52 |
| 31 | α.-Copaene | - | - | 9.701 | 0.30 |
| 32 | Geranyl Acetate | - | - | 10.080 | 0.26 |
| 33 | Trans-Sabinene Hydrate Acetate | - | - | 7.799 | 0.21 |
| 34 | Borneol, Acetate | - | - | 8.805 | 0.14 |

The WSD and SCFE methods have different compositions of nutmeg oil due to different extraction principles. The WSD method uses a principle on the solubility of essential oils in water and volatility, while the SCFE method is based on the solubility in liquid $CO_2$ [23]. Polar compounds will easily dissolve in water, while non-polar compounds will easily dissolve in $CO_2$. The polarity of a compound is largely determined by the structure of the compound. The compounds in nutmeg oil with both WSD and SCFE methods are shown in Figure 6.

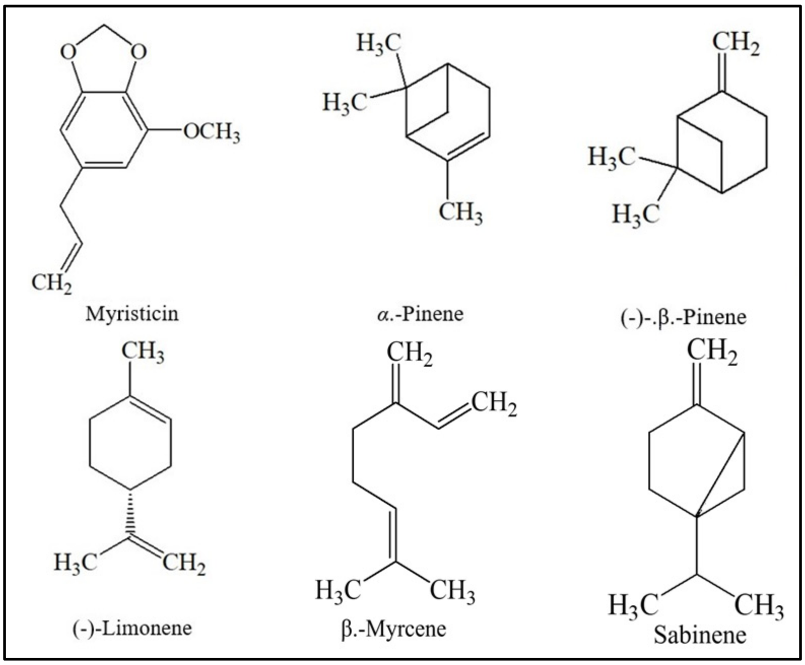


**Figure 6.** The main compounds contained in nutmeg oil were extracted using the WSD and SCFE methods.

*3.3. Characterization of nutmeg oil*

The quality of nutmeg oil is largely determined by the results of physical characterization. Physical characterization includes density, refractive index, solubility in ethanol, optical rotation, and residue evaporation. Table 2 shows a comparison of the characterization results of nutmeg oil between the WSD and SCFE methods. Both methods produce nutmeg oil in accordance with the ISO 3215:1998 oil of nutmeg. A very important export requirement for nutmeg oil is the content of myristicin. The myristicin content of the WSD and SCFE methods had significant differences, namely 30.30% and 16.22%, respectively. The minimum requirement for myristicin content is 10%, so that both methods produce nutmeg oil according to standards. Myristicin is the main compound in nutmeg oil [7].

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**Table 2.** Comparison of the characterization of nutmeg oil between the WSD and SCFE methods.

| No. | Parameters | ISO 3215:1998 Oil of nutmeg | Nutmeg oil | |
|---|---|---|---|---|
| | | | WSD | SCFE |
| 1 | Colour | Colourless or pale yellow | Colourless or pale yellow | Colourless or pale yellow |
| 2 | Odor | Typical Nutmeg oil | Typical Nutmeg oil | Typical Nutmeg oil |
| 3 | Density (20 $^0$C) | 0.880-0.910 | 0.8822 | 0.885 |
| 4 | Refractive index (nD 20) | 1.470-1.497 | 1.4781 | 1.484 |
| 5 | Solubility in ethanol 90% at 20 $^0$C | 1:3 colourless | 1:3 colourless | 1:3 colourless |
| 6 | Rotation Optical | (+) $8^0$ - (+) $25^0$ | (+) 24.37$^o$ | (+) 24,32$^o$ |
| 7 | Residue Evaporation (%) | Maximum 2.0 | 1.78 | 1.89 |
| 8 | Myristicin (%) | Minimum 10 | 30.30 | 16.22 |

**Table 3.** Comparison of yield and extraction time between WSD and SCFE methods.

| No. | Parameters | Nutmeg oil | |
|---|---|---|---|
| | | WSD | SCFE |
| 1 | Yield | 10-11% | 10-12% (essential oil ≥ 90%) and 15% (essential oil 80-90%) |
| 2 | Extraction time | 24 h | 15-60 minutes |

## 4. Discussion

The physical properties of nutmeg oil are indicated by color. The color is related to the chemical content in nutmeg oil. Nutmeg oil extraction with WSD takes 24 hours for one process. Nutmeg oil contains a light fraction that can be extracted with WSD without pressure. The light fraction was produced at an extraction time of 12 hours. Heavy fractions were extracted by WSD using pressure. The light fraction is above the water while the heavy fraction is below the water. After being separated from the water, the two fractions are then mixed [10]. The color of nutmeg oil extracted using WSD is colorless (clear), while nutmeg oil extracted using SCFE is yellow. The color difference is due to the different compounds extracted using the two methods. The WSD method is for extracting volatile compounds, while SCFE extracts compounds that are soluble in liquid $CO_2$. The non-volatile compounds in nutmeg are also extracted by $CO_2$ gas. Non-volatile compounds such as oil, fat and lignin are extracted by $CO_2$ gas, giving rise to a yellow color.

The WSD and SCFE extraction methods produce nutmeg oil with very significantly different chemical compound compositions. The significant difference is that extraction with WSD contains the main component myristicin (30.30%) while SCFE contains the largest component sabinene (29.94%). This difference is caused by the properties of the two compounds. Myristicin has volatile properties and tends to be polar, while sabinene has volatile and non-polar properties. $CO_2$ gas has non-polar properties so sabinene has better solubility compared to myristicin. Comparison of the results of nutmeg oil characteristics with both methods shows almost the same results and meets the requirements of ISO 3215:1998. Both extraction methods produce nutmeg that meets ISO 3215:1998 including color, odor, density, refractive index, solubility in ethanol, rotation optical residue evaporator and myristicin content (minimum 10%).

The advantages of the SCFE method compared to the WSD method are the yield and extraction time. The yield with the SCFE method is 12%, while the WSD method produces a maximum yield of 11% (Table 2). The main advantage is that the extraction time is faster, a maximum of 1 hour, while the WSD is 24 hours. The advantage of the SCFE method is that it does not require fuel that produces $CO_2$ gas. $CO_2$ gas used for the extraction process in SCFE can be reused by releasing it into $CO_2$ in the gas phase. This process makes the SCFE method very efficient and environmentally friendly [33]. The weakness of the SCFE method is to produce nutmeg oil which requires refining, because it contains non-volatile chemical compounds. Non-essential chemical compounds such as lignin, oil and fat are also extracted in nutmeg oil [34]. Tabel 3 shows a comparison of yield and extraction time between WSD and SCFE methods. The extraction method with SCFE produces a higher yield than WSD. This method also requires a shorter extraction time. The SCFE extraction method is preferred because of its high efficiency, shorter extraction time and high selectivity [30,31].

The SCFE method in this research has not optimized the powder material parameters, pressure, and temperature. Further research that can be carried out is the optimization of various parameters that influence the quality and quantity of nutmeg oil. Important parameters that influence the extraction method with SCFE are $CO_2$ gas flow rate, pressure, temperature, and extraction time [35]. The use of co-solvents and the particle size of the raw materials also influence the extraction results [36].

## 5. Conclusions

The results of the analysis with GC-MS showed that the WSD and SCFE methods produced nutmeg oil with almost the same chemical compound content. The results of the characterization of nutmeg oil with the two methods also meet the quality standards of ISO 3215:1998 oil of nutmeg. The advantages of the SCFE method compared to the WSD method are faster extraction times, no need for heating, does not produce pollutant gases and are environmentally friendly. The SCFE method has the disadvantage that it requires further processing to separate volatile and non-volatile chemical compounds. The recommendation from the results of this study is that further research is needed to optimize SCFE performance, separate non-essential compounds and economic calculations, especially nutmeg oil.

**Author Contributions:** Conceptualization, R.; Methodology, Riyanto.; Formal analysis, V.N.J.; Data curation, V.N.J.; Writing-review & editing, Riyanto.; Funding acquisition, Riyanto. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Due to limitations such as privacy or ethical concerns, data are only available upon request. The corresponding author can provide the data described in this study upon request. Due to corporate limitations, the data is not publicly accessible.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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