



## The uniqueness of isolation of nutmeg essential oil from nutmeg seeds (*Myristica fragrans* Houtt.) and its effects on physical and chemical properties

Riyanto\*, Sri Umayah and Puthut Marhaendro

Department of Chemistry, Faculty of Mathematics and Natural Science, Islamic University of Indonesia,  
Jalan Kaliurang KM 14,5 Sleman Yogyakarta 55584, Indonesia

Received 22 April 2020; Revised 12 February 2021

Isolation of nutmeg essential oil from nutmeg seeds (*Myristica fragrans* Houtt.) using the steam distillation method is a unique technique because nutmeg essential oil contains heavy and light oil. Nutmeg essential oil is the main product produced by nutmeg (*Myristica fragrans*), isolated by the steam distillation method. The main component of nutmeg essential oil is myristicin and several other typical compounds of essential oils. This study discusses the results of steam distillation from nutmeg seeds, characterizing nutmeg essential oils, and analyzing their suitability for INS number 06-2388-2006. From the Gas Chromatography-Mass Spectrometry (GC/MS) results, the main content of nutmeg essential oil is myristicin (30.30%) and followed by  $\alpha$ -Pinene (12.01%). The physical characteristics of nutmeg essential oil are colourless. They have a distinctive scent of nutmeg oil, a density of 0.8822 g/mL, a refractive index of 1.4781, solubility in ethanol 90% obtained at a concentration ratio of 1:3, optical rotation (+) 24,3714°, and the remaining evaporation is 1.72%. It can be seen that the overall results of this nutmeg essential oil quality test are following the *Indonesian National Standard (INS) number* 06-2388-2006 (INS No. 06-2388-2006). From this research, it can be concluded that the steam distillation method for 24 hours with 12 hours without pressure and 12 hours last with pressure can be used as a reference method for the isolation of nutmeg essential oil.

**Keywords:** Chemical properties, Essential oils, *Myristica fragrans*, Nutmeg, Physical properties, Steam distillation.

**IPC code; Int. cl. (2015.01)**-A61K 36/00, A61K 131/00

### Introduction

Nutmeg (*Myristica fragrans*) is a native plant of Indonesia, which has been known as a species plant since the 18<sup>th</sup> century. Some regions in Indonesia are the biggest nutmeg producers, including the Maluku Islands, North Sulawesi, and Aceh<sup>1</sup>. Taxonomical classification of nutmeg plant is as follows, Kingdom: Plantae, Division: Tracheophyta, Division: Magnoliophyta, Class: Magnoliopsida, Order: Magnoliales, Family: Myristicaceae, Genus: *Myristica* Gronov (Nutmeg) and Species: *Myristica fragrans* Houtt<sup>2</sup>. The nutmeg plant has a characteristic form of regular tree branching, and the leaves are small to moderate; the fruit is round, with large seeds, abundant flowers, and a specific nutmeg scent<sup>1</sup>. Nutmeg plants have several conditions to be able to grow well, including 2000-3000 mm/year rainfall with 108-180 rainy days, temperature of 18-34 °C, fertile and loose soil, contains humus, good drainage, and soil pH between 5.5-6.5 with sandy clay soil structure. This plant grows well at an altitude between 0-700 meters above sea level<sup>3</sup>.

Almost all parts of nutmeg plants can be extracted and used for trade commodities, including the flesh of the fruit, flowers, and nutmeg seeds. Fruit flesh, flowers, and nutmeg seeds are widely used for the food and beverage industry, the pharmaceutical and drug industries as well as the cosmetics and perfume industries in the country. Nutmeg essential oil itself is a raw material for the pharmaceutical industry, soap making, perfume, and many others<sup>1</sup>.

Nutmeg essential oil is the main product produced from nutmeg plants by distillation techniques. Nutmeg essential oil is most often extracted from nutmeg seeds using steam distillation. The most useful is in the pharmaceutical industry and the food industry. This essential oil contains the main compounds of myristicin and several other compounds, such as  $\alpha$ -pinene, sabinene, gamma-terpinene, limonene, and others<sup>4</sup>. The requirement for myristicin levels as the main compound of nutmeg oil, according to the INS No.06-2388-2006 is at least 10%. Indonesian National Standards on nutmeg essential oils intended to maintain the quality of the production of essential oils. Some aspects that are focused on INS No. 06-2388-2006 include colourless or pale yellow colour

\*Correspondent author  
Email: riyanto@uii.ac.id

with a distinctive scent of nutmeg oil, density (20°C/20°C) 0.880-0.910 g/mL, refractive index (n<sub>D</sub>20) 1.470-1.497, solubility in ethanol 90% (1:3) clear, optical rotation (+) 8-25°, maximum remaining evaporation is 2% and myristicin levels of at least 10%<sup>5</sup>.

Nutmeg essential oil is used as a raw material for the pharmaceutical and drug industries. Nutmeg essential oil has good biological effectiveness. Some researchers have reported that nutmeg essential oil has effective biological properties. Myristicin is a significant component of nutmeg essential oil, which has several properties used as a treatment for a long time. This compound has functioned as an antimicrobial, antioxidant, hepatoprotective agent, anti-inflammatory, and analgesic. As is well known, this compound acts as a serotonin receptor antagonist and is also known as a hallucinogenic compound<sup>6-7</sup>. Besides, the typical compound of nutmeg essential oil is also proven to have a monoamine oxidase (MAO) inhibitor effect and has a neurotoxicity effect on neuroblastoma<sup>7</sup>. However, the use of myristicin as a medicinal compound is considered because excessive acute doses will trigger organ damage. Myristicin poisoning can cause several symptoms, such as delirium, seizures, nausea, palpitations, and blurred vision<sup>7</sup>.

$\alpha$ -pinene is an essential component of this nutmeg essential oil has a beneficial effect in the world of medicine, namely as a gastroprotective agent in the presence of its anti-ulcerogenic activity<sup>8</sup>. Several benefits of  $\alpha$ -pinene include anti-inflammatory potential, antibacterial and antifungal agents, cytogenetic, and anti-anxiety (anxiolytic-like)<sup>9-13</sup>. Besides, it reported that this compound exhibits oxidative and antiproliferative effects on hepatoma<sup>14</sup> and also has a synergistic antitumor effect with paclitaxel against Non-small-cell Lung Carcinoma<sup>15</sup>.

$\beta$ -pinene is a compound that has several beneficial functions, including antiviral, antibacterial, antifungal, and also antihypertensive<sup>13,16-18</sup>. Besides,  $\beta$ -pinene reported has a synergistic antitumor effect with paclitaxel against Non-small-cell lung carcinoma<sup>15</sup>.

Terpinene-4-ol has many benefits, such as aromatherapy to provide a calming effect, as a muscle relaxant agent, as an antimicrobial and antibiotic, as an antiparasitic, anticonvulsant, potential as a tumournecrosis<sup>19-26</sup>, plays a role in autophagic and apoptosis in human leukemic cells<sup>27,28</sup> also plays a role in therapeutic agents in gastrointestinal cancer<sup>29</sup>.

$\gamma$ -terpinene affects anti-inflammatory and microbiocidal<sup>30</sup>. According to research, it reported that

treatment with  $\gamma$ -terpinene alleviated inflammatory parameters such as oedema and pro-inflammatory cytokine production, as well as cell migration into the inflamed site<sup>31</sup>. This monoterpene promotes the prostaglandin E2/interleukin-10 axis, which inhibits the production of these pro-inflammatory cytokines<sup>30</sup>.

Limonene is the main compound that makes up citrus essential oils but found in nutmeg essential oils even in smaller amounts. This compound has many effects on the part of medicine and pharmacology, including anti-inflammatory and analgesic, antibiotic, antiviral, antioxidant<sup>16,32-35</sup>. Another essential function of this component of citrus oil is its role in the food and beverage industry and the perfume and cosmetics industry as aromatherapy, perfume, food, and beverage<sup>36,37</sup>.

The essential oil isolation was carried out using three distillation methods, namely water distillation or hydrodistillation, water and steam distillation, and steam distillation. Water distillation or hydrodistillation, the water is mixed with the starting material in the boiling container. Water and steam distillation are the plant material are suspended on a grille above the boiling water, and steam from a boiler is forced through the kettle. Steam distillation is the plant material is put in a kettle without water, and steam from a boiler is forced through. Nutmeg seeds have very hard properties so that the steam distillation method is very suitable for the isolation of nutmeg essential oil.

This paper presents the results of research on the isolation of nutmeg essential oil from nutmeg seeds using the steam distillation method is a unique technique because nutmeg essential oil contains heavy and light oil. The steam distillation method on the isolation of nutmeg essential oil is a unique technique that was done with two distillation steps. The first step is distillation for 12 hours without pressure for the isolation of light nutmeg essential oil. The second step is distillation for 12 hours with the pressure for isolation of heavy nutmeg essential oil. The quality of nutmeg essential oil is determined mainly by the distillation process, mixing the final product, and the quality of nutmeg essential oil.

## Materials and Methods

### Material

The raw materials used in this study were dried young nutmeg seeds (250 kg for one process). All chemicals used in this research from Merck.

### Preparation of sample

Young nutmeg seeds that have been dried (Fig. 1), were ground using a crusher to make powder.

### Steam distillation apparatus

The steam distillation device that has been used in this research consists of four main parts: a boiler, a kettle or a container, a condenser, and a separator. The steam distillation apparatus used in this study is shown in Fig. 2.

Boilers (Fig. 2a) function to produce steam, which is used for the distillation process. Boilers are made of iron-steel with a diameter of 105 cm and a height of 125 cm. The structural form of the boiler is a conical top cylinder. Boilers made of iron steel have a minimum thickness of 5 cm so that they can operate at a minimum pressure of 1 Bar. Boilers are equipped with pressure control devices, alarms in case of overpressure, and water volume control devices in the boiler.

The kettle or the starting material place (Fig. 2b) functions as a nutmeg powder. The material put in a kettle without water, and steam from a boiler is forced



Fig. 1 — Dried young nutmeg seeds.



Fig. 2 — Steam distillation apparatus: a) boiler, b) kettle as the starting material place, c) the condenser, and d) separator.

through. A Kettle is made of high-quality stainless steel, has a diameter and height of 80 and 140 cm, respectively. The top of the kettle made a cone so that steam (water and essential oils) more quickly to the condenser. This tool is equipped with a stimulus made of stainless steel with a distance of 8 cm from the bottom of the surface of the boiler material, temperature control, pipes crossed at the bottom of the boiler material. The steam pipe is equipped with pressure, a faucet for condensing vapours, and also for washing laundry water. The top of the kettle is equipped with a swan neck shaped device to flow steam from the kettle to the condenser. Gooseneck shape is slightly curved and sloping direction 15° of kettle ingredients directly to the condenser.

The condenser (Fig. 2c) functions to convert all water vapour and oil vapour into the liquid phase. The tube makes of stainless series with a tube diameter of 80 cm, a tube height of 125 cm, a circular column pipe. This condenser has equipped with a cooling water inlet at the bottom of the condenser tube and cooling water outflow at the top of the tube. The condensation process more perfect, the condenser immersed in a drum containing circulating water.

A separator (Fig. 2d) is used to separate oil from condensed distilled water. To prevent evaporation and oil loss, the temperature of the oil in this separator was maintained at a temperature of 20-30 °C. The shape is a cylinder with two layers, with the same height of 50 cm and an outer layer diameter of 150 cm, while an inner layer diameter of 30 cm, this section equipped with an oil and water discharge channel system that opened and closed using a valve.

### The process of isolating nutmeg seed oil

As much as 251 kg of nutmeg has been pounded first with a crushing machine to become powdered nutmeg, put into a teapot, closed, and the gunshot is turned on. The distillation has been carried out for 24 hours which is divided into two processes, namely distilled steam without pressure and distilled steam using pressure.

Steam distillation without pressure is carried out in the first 12 hours, by processing the nutmeg powder which has been put into the kettle of ingredients; The distillation process has been carried out at a temperature of 100-110 °C, without pressure to remove oil from the pores of the distilled nutmeg powder. The gas exhaust valve is opened to remove the remaining air in the previous distillation process and indicate whether H<sub>2</sub>O water vapour has formed or is still

forming an atmosphere if H<sub>2</sub>O water vapour, the tap is closed. The steam trap has been opened until it is full so that water vapour enters the boiler material, water vapour, and oil vapour pass through the boiler material tube to the condenser. For the separator, two layers are formed, namely light phase nutmeg oil and water or hydrosol, then the oil is separated and collected into bottles.

Distillation using pressure has been carried out in the last 12 hours. Steam distillation without pressure in the previous 12 hours, with a temperature of 120-130 °C and a pressure of 1 bar, by opening the tap of the boiler material tube at an angle of 30° (the pressure control shows 1 bar) the vacuum process takes place. In the separator, two layers are formed, water or hydrosol, and heavy phase nutmeg oil, then the oil is separated and collected into bottles.

The last process is mixing or blending nutmeg oil. Both phases of nutmeg oil, light phase, and heavy phase are mixed until homogeneous, anhydrous sodium sulfate is added to bind water or hydrosol which is involved in mixing nutmeg oil.

#### Nutmeg essential oil quality test

Nutmeg essential oil quality testing has been carried out at the Central of Essential Oils Studies (CEOS) the Islamic University of Indonesia to determine the quality of the oil, the oil quality analysis was carried out based on the nutmeg oil standard INS No.06-2388-2006. Physical test: test conditions (colour and odour), density, refractive index (nD 20), solubility in ethanol 90% at 20 °C, optical rotation, residual evaporation, and myristicin compounds. Density was analyzed using a 5 mL pycnometer. The refractive index (nD 20) was analyzed using the Abbe refractometer. Analysis of optical rotation using a Polarimeter Polax-2L consisting of a standard tube of 20 cm and a sample tube of 10 cm.

GC/MS Shimadzu QP 2010 SE has been used for the determination of the myristicin content in nutmeg

oil. The solution is injected into a GC inlet injection temperature of 200 °C where it is evaporated and swept onto the chromatography column by a carrier gas. The flow-through column sample and the compound consisting of the nutmeg oil mixture were separated based on their significant interaction with the column layer column (stationary phase), column temperature of 60 °C, the flow of 0.75 mL/min, and carrier gas of helium (mobile phase). The last portion of the column passes through a heated transfer path and ends by entering the ion source where the compound elutes from the column becomes ions. The ionization ion (EI) of an electron has ionized the sample molecule resulting in the loss of one electron. Molecules with one electron missing are called molecular ions and are represented by M<sup>+</sup> (radical cations) when the resulting peaks of these ions are visible in the mass spectrum. This gives the molecular weight of the compound. This information is used to identify compounds of interest and help explain the structure of the unknown components of a mixture.

#### Results and Discussion

##### The effect of the steam distillation on physical properties of nutmeg essential oil

Table 1 shows the results that nutmeg seeds that were distilled for 24 hours were clear or not yellow and fresh-smelling typical of nutmeg oil. These results are under those required as a recommendation test in the INS No. 06-2388-2006. This condition indicates that the equipment used in the distillation process does not affect the organoleptic properties of nutmeg essential oils. The material made of stainless and also the process of steam distillation pressurized to avoid the occurrence of materials becoming mangosteen during distillation because the nutmeg material does not come in direct contact with the heat from the burning fire (distillation boiler separate from the steam boiler).

Table 1 — Result of nutmeg oil quality test

S. No.	Parameters	Standard INS No. 06-2388-2006	Result	Conclusion
1	Colour	Colourless or pale yellow	Colourless or pale yellow	Suitable
2	Odour	Typical nutmeg oil	Typical nutmeg oil	Suitable
3	Density (20 °C)	0.880-0.910	0.8822	Suitable
4	Refractive index (nD 20)	1.470-1.497	1.4781	Suitable
5	Solubility in ethanol 90% at 20 °C	1:3 colourless	1:3 colourless	Suitable
6	Rotation optical	(+) 8° - (+) 25°	(+) 24.37	Suitable
7	Residue evaporation (%)	Maximum 2.0	1.78	Suitable
8	Myristicin (%)	Minimum 10	30.30	Suitable

The specific density of nutmeg oil as a result of the research is 0.8822 g/mL. The specific density of the nutmeg's oil is between 0.880-0.910 g/mL. It is shown that variation of the value of specific density is very small between and within the ecotypes<sup>5</sup>. These results compared with the standard nutmeg seed oil in the INS No. 06-2388-2006 turned out to meet the criteria, and this shows that the process of nutmeg refining, especially the separation between water and oil has carried out optimally<sup>38</sup>. The results of the study of water content in nutmeg oil were minimized because one indicator that can use as a benchmark is the increase in the specific gravity of nutmeg seed oil, namely the high water content in the product. If this happens, it assumes that the process of separation between water and the essential oil fraction is less than perfect.

In this study, nutmeg seeds weighing 251 kg, oil volume obtained 29.2 kg. The total yield obtained was 11.63%. The data above shows, the more extended the refining process, the higher the yield of nutmeg. The increase in the yield of nutmeg oil with increasing distillation time is caused by several factors, including the process of vapour pressure penetration in the nutmeg. The yield of nutmeg oil depending on the size of the chopped nutmeg, the higher the size of the chopped nutmeg, then the penetration of steam into the nutmeg is longer to press out nutmeg seed oil so that nutmeg oil will increase its yield with a longer distillation process<sup>5</sup>. Another factor is the amount of material in the material boiler, the more the amount of material in the material boiler, the longer the steam it takes to enter the content, especially in the middle of the pile of nutmeg material so that the increase in yield will be along with the length of the distillation process. Nutmeg seeds used with a yield of 11.63% were old, young mixed nutmeg seeds, when they were not wet and not dry. This yield is better than the yield reported by Suprihatin *et al.*<sup>39</sup> that is as much as 6.72%. Some information obtained through the industry of essential oils (nutmeg oil) states that the yield of essential oils (nutmeg oil) can reach 10-12% if distilled with the steam water distillation method and a distillation time of 20-22 hours. But in addition to the distillation time, several factors affect the yield of nutmeg oil, including the age of nutmeg, nutmeg, since it was picked and then distilled, the process of chopping the nutmeg before refining, and the area of origin of the nutmeg plant. According to Cinthia *et al.*<sup>40</sup>, the amount of essential oil found in the seed varies with origin, soil, and climate.

Table 1 shows the refractive index of the nutmeg seed oil results of the research. The refractive index value is a comparison between the speed of light in the air and the speed of light in substances at a specific temperature. The nutmeg refractive index test results were 1.4781. The results of the study, when compared with standard nutmeg, turned out to meet the requirements<sup>38</sup>. One parameter that can be used as a measure or guideline to determine the purity of nutmeg oil is to test the refractive index of nutmeg oil and compare it to the requirements of the nutmeg oil standard. If the refractive index of nutmeg oil does not meet the application needs, it is suspected that the nutmeg oil in the distillation process is less than optimal, or the presence of a non-nutmeg component is mixed. The refractive index in nutmeg oil is greatly influenced by the number of compounds both heavy and light fractions in nutmeg oil. If a lot of heavy fraction compounds are contained in nutmeg oil, then the more difficult the light is refracted in the nutmeg oil, the refractive index will be higher. The results of the analysis of physicochemical properties in the separation of nutmeg oil components showed that the weight fraction had higher viscosity, specific gravity, and refractive index values than the mild fraction<sup>1,38</sup>.

Table 1 shows the results of the solubility test in alcohol nutmeg oil. The nutmeg seed oil results of the study after the solubility test in alcohol turns out at a ratio of 1 part nutmeg oil, and after adding three parts of alcohol, 90% give a bright appearance. Nutmeg oil is sensitive to light and temperature, soluble in alcohol but not in water<sup>5,38</sup>. One indicator of essential oils (nutmeg oil) is not pure or mixed with mineral oil, namely the testing of the solubility in alcohol will give a turbid colour. The results showed that there were no other components that polluted nutmeg oil. These results meet the requirements of INS No. 06-2388-2006. The solubility value of nutmeg oil in ethanol is an indication of the ability of nutmeg oil to dissolve completely in the alcohol. The higher the amount of concentrated ethanol that is needed to dissolve nutmeg oil, the more difficult it is to dissolve in ethanol. Generally, essential oils (nutmeg oil) containing oxygenated terpene compounds (terpene-o) are more soluble than those containing terpenes. Two compounds will dissolve entirely in specific proportions and concentrations if the polarity is the same.

Optical rotation is the response of molecular structures to the path of a single wave of light. The magnitude of the optical rotation depends on the type

and concentration of the compound. The length of the way the light travels through the compound and the measurement temperature. Optical rotation analysis used to determine the purity of refined nutmeg oil, the results of the research are (+) 24.3714°. The optical rotation has a positive (+) value, meaning that the rotation of the plane of polarization is clockwise or to the right (dextrorotary)<sup>5</sup>. Optical rotation is an important analysis in essential oils to detecting adulteration of essential oils. The optical rotation is a summation of the optical rotations of the chemical compounds in the essential oil. These results compared with the quality requirements of nutmeg seed oil INS No. 06-2388-2006 turned out to meet the criteria, and this shows in the 24-hour distillation process, meaning that the content of light oil and heavy oil by the compounds inside was extracted well during the refining process.

#### The effect of the steam distillation on chemical properties of nutmeg essential oil

Fig. 3 show the chromatogram from nutmeg essential oil is the mixture of light and heavy nutmeg essential oil. The results of research on the content of myristicin using GC/MS amounted to 30.30%, and these results are under those required as a recommendation test in INS No. 06-2388-2006. Compounds in nutmeg essential oil are shown in Table 2. The results of GCMS analysis of nutmeg essential oil found 25 components of nutmeg oil. From the test results, the 5 largest components were obtained in the components are myristicin (30.30%),  $\alpha$ -pinene (12.01%), (-)-4-terpineol (9.75%), (-)- $\beta$ -pinene (9.65%), and  $\gamma$ -terpinene (6.63%). Hasmita *et al.*<sup>38</sup> have reported the 5 largest components were obtained in nutmeg essential are  $\alpha$ -alpha terpinene 13.71%,  $\beta$ -pinene 18.74%, delta limonene 9.12%, trans

terpineol 10.08%, and myristicin obtained at 14.84%. This difference in chemical composition is due to differences in raw material, distillation dan separation techniques. Myristicin is an important chemical compound in nutmeg oil with a minimum composition of 10%. Myristicin is the main aromatic component in determining the quality and distinctive aroma of

Table 2 — Compounds in nutmeg essential oil the mixture light and heavy nutmeg essential oil from Fig. 3

S. No.	Name of compounds	Retention time (min)	Area (%)
1	Myristicin	12.719	30.30
2	$\alpha$ -Pinene	4.313	12.01
3	(-)-4-Terpineol	7.813	9.75
4	(-)- $\beta$ -Pinene	4.884	9.65
5	$\gamma$ -Terpinene	5.977	6.63
6	(-)-Limonene	5.558	4.99
7	$\beta$ -Phellandrene	4.800	4.61
8	(+)-2-Carene	5.380	4.41
9	Safrole	9.449	2.76
10	$\alpha$ -Terpinolene	6.419	1.99
11	$\alpha$ -Thujene	4.191	1.94
12	$\alpha$ -Terpineol	7.989	1.83
13	$\beta$ -Myrcene	4.955	1.82
14	Eugenol	10.404	0.92
15	(E)-Isoeugenol	11.702	0.90
16	$\alpha$ -Phellandrene	5.212	0.89
17	$\beta$ -Asarone	12.979	0.86
18	4-Pentylanisole	9.575	0.77
19	Neryl acetate	10.626	0.74
20	$\alpha$ -Copaene	10.730	0.64
21	$\delta$ -3-Carene	5.302	0.41
22	p-Cymene	5.487	0.39
23	Methyleugenol	10.978	0.39
24	Camphene	4.514	0.21
25	$\alpha$ -Terpinyl acetate	10.254	0.19
	Total		100

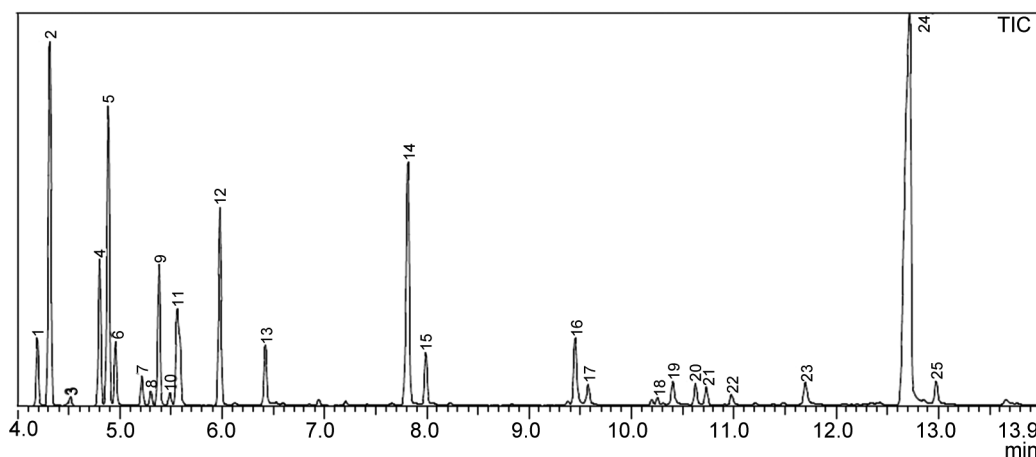


Fig. 3 — Chromatogram of essential oil from is the mixture of light and heavy nutmeg essential oil.

nutmeg oil so that the myristine component is used as a determinant indicator of nutmeg oil quality<sup>5</sup>.

The isolation technique using steam distillation greatly affects the chemical properties of the nutmeg essential oil. Steam distillation was carried out for 24 hours with the first 12 hours without pressure, which resulted in light nutmeg essential oil. Steam distillation at 12 hours of the second year under pressure produced the heavy nutmeg essential oil. The quality of nutmeg essential oil is largely determined by the heavy nutmeg essential oil because it contains myristicin (30.30%) and safrole (2.76%). Myristicin levels obtained in the bottom product or residue

are much higher than the per cent components of myristicin in the top product or distillate<sup>38</sup>.

Nutmeg essential oil that meets INS No. 06-2388-2006 is a mixture of light and heavy essential oil. Fig. 4 shows the condition of the separator and nutmeg essential oil. Nutmeg essential oil, the mixture of light, and heavy nutmeg essential oil show in Table 3. According to Sudrajat *et al.*<sup>41</sup>, myristicin content in the bottom product (residue) is slightly increase compare to the initial myristicin content at nutmeg oil as raw material. This is due to during distillation, there are still many other components which more volatile compound evaporated to distillate product.

Nutmeg essential oil is different from other essential oils that have chemical components with a specific gravity above and below 1. Essential oils with the specific gravity below one are called light oils, and the specific gravity above one is called heavy oils. Heavy oils in nutmeg essential oils are myristicin and safrole. Light oils such as  $\alpha$ -Pinene, (-)-4-Terpineol, (-)- $\beta$ -Pinene,  $\gamma$ -Terpinene, (-)-Limonene,  $\beta$ -Phellandrene, (+)-2-Carene and  $\alpha$ -Terpinolene. The structure of chemicals compounds of nutmeg essential oil is shown in Fig. 5.

The quality of nutmeg essential oil is determined by myristicin levels, which is at least 10%. The determinant quality factor of nutmeg oil is determined by the content of myristicin because it gives a distinctive aroma to nutmeg oil<sup>42</sup>. Nutmeg essential

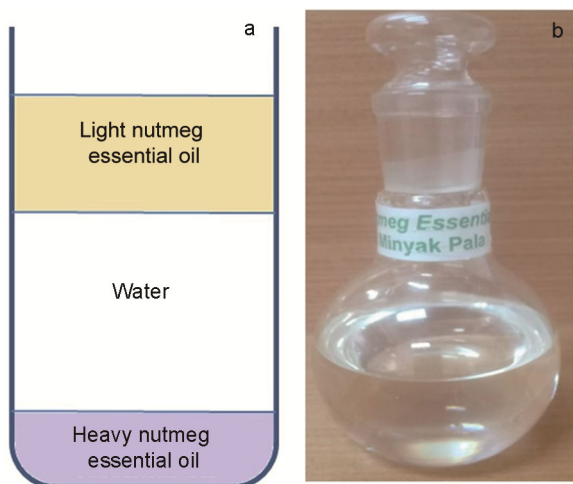


Fig. 4 — a) Result of the steam distillation technique in the separator and b) mixture of light and heavy nutmeg essential oil.

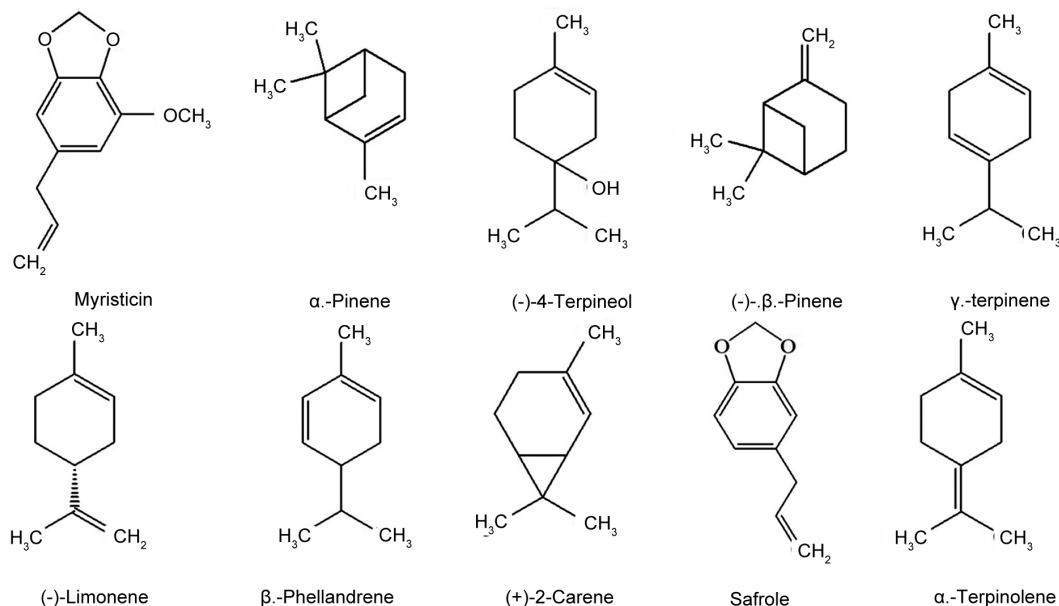


Fig. 5 — Structure molecule of main chemicals compounds of nutmeg essential oil.

Table 3 — Main compounds in nutmeg essential oil the mixture light and heavy nutmeg essential oil

S. No.	Name of compounds	Density (g/mL)	Area (%)	Light and heavy nutmeg essential oil
1	Myristicin	1.142	30.30	Heavy
2	$\alpha$ -Pinene	0.858	12.01	Light
3	(-)-4-Terpineol	0.929	9.75	Light
4	(-)- $\beta$ -Pinene	0.872	9.65	Light
5	$\gamma$ -Terpinene	0.838	6.63	Light
6	(-)-Limonene	0.842	4.99	Light
7	$\beta$ -Phellandrene	0.846	4.61	Light
8	(+)-2-Carene	0.862	4.41	Light
9	Safrole	1.064	2.76	Heavy
10	$\alpha$ -Terpinolene	0.837	1.99	Light

oil is a combination of light and heavy oils. Heavy oil with a specific gravity above one so that it is located in the separator above water, light oil is underwater. Heavy oil can be produced at high pressure, so distillation with steam is very suitable.

### Conclusion

This study suggests that the isolation of nutmeg essential oil from nutmeg seeds (*Myristica fragrans* Houtt.) using the steam distillation method. Nutmeg essential oil was isolated using the steam distillation method for 24 hours (first 12 hours without pressure and last 12 hours with pressure). The total yield obtained was 11.63%. Nutmeg essential oil was content of myristicin amounted to 30.30%. Nutmeg essential oil produced from this research has quality test is following the INS No. 06-2388-2006. In future, the effect of steam pressure on the quality and quantity (yield) of essential oils can be undertaken as the steam pressure in the isolation method by steam distillation greatly affects the quality and amount of essential oil produced.

### Acknowledgement

This research was supported by the Ministry of Research and Technology of the Republic of Indonesia through "Penelitian Terapan Unggulan Perguruan Tinggi" Research Grant 2020 for financial support.

### Conflict of interest

The authors declare that there is no conflict of interest.

### References

- Simanjorang T M, Irham, Waluyati L R and Mulyo J H, Comparative and competitive advantages of nutmeg farming in two regions in Maluku Province, Indonesia, *Biodiversitas*, 2020, **21**(3), 1165-1173.
- Ali M A, Hamiduddin, Zaigham M and Ikram M, Phytopharmacological potential of Jaiphal (*Myristica fragrans* Houtt): A spice of medicinal importance and its utilization in Unani Medicine, *Int J Green Pharm*, 2018, **12**(1), 26-36.
- Naeem N, Rehman R, Mushtaq A and Ghania J B, Nutmeg: A review on uses and biological properties, *Int J Biol Chem Sci*, 2016, **9**, 107-110.
- Saputro M, Andarwulan N and Faridah D, Physical characterization and essential oil properties of West Sumatra mace and nutmeg seed (*Myristica fragrans* Houtt) at different ages at harvest, *J Pharmacogn Phytochem*, 2016, **5**(6), 371-376.
- Marzuki I, Joeffie B, Aziz S A, Agusta H and Surahman M, Physico-chemical characterization of Maluku nutmeg oil, *Int J Sci Eng*, 2014, **7**, 61-64.
- Morita T, Jinno K, Kawagishi H and Sugiyama K, Hepatoprotective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/D-Galactosamine-induced liver injury, *J Agric Food Chem*, 2003, **51**(6), 1560-1565.
- Dawidowicz A L and Dybowski M P, Simple and rapid determination of myristicin in human serum, *Forensic Toxicol*, 2013, **31**(1), 119-123.
- Pinheiro M A, Magalhães R M, Torres D M, Cavalcante R C, Mota F S, *et al.*, Gastroprotective effect of alpha-pinene and its correlation with the antiulcerogenic activity of essential oils obtained from *Hyptis* species, *Pharmacogn Mag*, 2015, **11**(41), 123-130.
- Kim D S, Lee H J, Jeon Y D, Han Y H, Kee J Y, *et al.*, Alpha-pinene exhibits anti-inflammatory activity through the suppression of MAPKS and the NF-KB pathway in mouse peritoneal macrophages, *Am J Chin Med*, 2015, **43**(4), 731-742.
- Saki K, Bahmani M and Kopaei M R, The effect of most important medicinal plants on two important psychiatric disorders (anxiety and depression)- A review, *Asian Pac J Trop Med*, 2014, **7**(1), S34-S32.
- Satou T, Kasuya H, Maeda K and Koike K, Daily inhalation of  $\alpha$ -pinene in mice: Effects on behavior and organ accumulation, *Phytother Res*, 2014, **28**(9), 1284-1287.
- Turkez H and Aydin E, *In vitro* assessment of cytogenetic and oxidative effects of  $\alpha$ -pinene, *Toxicol Ind Health*, 2013, **32**(1), 168-176.
- Rivas da Silva A C, Lopes P M, Barros de Azevedo M M, Costa D C, Alviano C S, *et al.*, Biological activities of  $\alpha$ -pinene and  $\beta$ -pinene enantiomers, *Molecules*, 2012, **17**(6), 6305-6316.
- Chen W Q, Xu B, Mao J W, Wei F X, Li M, *et al.*, Inhibitory effects of  $\alpha$ -pinene on hepatoma carcinoma cell proliferation, *Asian Pac J Cancer Prev*, 2014, **15**(7), 3293-3297.
- Zhang Z, Guo S, Liu X and Gao X, Synergistic antitumor effect of  $\alpha$ -pinene and  $\beta$ -pinene with paclitaxel against Non-Small-Cell Lung Carcinoma (NSCLC), *Drug. Res*, 2015, **65**, 214-218.
- Astani A and Schnitzler P, Antiviral activity of monoterpenes beta-pinene and limonene against herpes simplex virus *in vitro*, *Iran J Microbiol*, 2014, **6**(3), 149-155.
- Bahmani M, Kopaei M R, Hassanzadazar H and Taherikalani M, Pseudomonas phytotherapy: A review on most important Iranian medicinal plants effective on *Pseudomonas aeruginosa*, *Iran J Microbiol*, 2016, **8**(5), 347-350.
- Moreira I J, Menezes P P, Serafini M R, Araújo A A, Quintans-Júnior L J, *et al.*, Characterization and



- antihypertensive effect of the complex of (-)- $\beta$ - pinene in  $\beta$ -cyclodextrin, *Curr Pharm Biotechnol*, 2016, **17**(9), 837-845.
- 19 Vakilian K, Atarha M, Bekhradi R and Chaman R, Healing advantages of lavender essential oil during episiotomy recovery: A clinical trial, *Complement, Ther Clin Pract*, 2011, **17**(1), 50-53.
- 20 Hammer K A, Carson C F and Riley T V, Effects of *Melaleuca alternifolia* (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility, *Antimicrob Agents Chemother*, 2012, **56**(2), 909-915.
- 21 Tighe S, Gao Y Y and Tseng S C G, Terpinene-4-ol is the most active ingredient of tea tree oil to kill demodex mites, *Transl Vis Sci Technol*, 2013, **2**(2), 1-8.
- 22 Nóbrega F F, Salvadori M G, Masson C J, Mello C F, Nascimento T S, *et al.*, Monoterpenoid terpinene-4-ol exhibits anticonvulsant activity in behavioral and electrophysiological studies, *Oxid Med Cell Longev*, 2014, **2014**, 1-9.
- 23 Greay S J, Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinene-4-ol, *Cancer Chemother Pharmacol*, 2010, **65**(5), 877-888.
- 24 De Sousa D P, Nóbrega F F, De Moraes L C and De Almeida R N, Evaluation of the anticonvulsant activity of terpinen-4-ol, *J Biosci*, 2014, **64**(1-2), 1-5.
- 25 Loughlin R, Gilmore B F, McCarron P A and Tunney M M, Comparison of the cidal activity of tea tree oil and terpinene-4-ol against clinical bacterial skin isolates and human fibroblast cells, *Lett Appl Microbiol*, 2008, **46**(4), 428-433.
- 26 Maia-Joca R P, Joca H C, Ribeiro F J, Do Nascimento R V, Silva-Alves K S, *et al.*, Investigation of terpinene-4-ol effects on vascular smooth muscle relaxation, *Life Sci*, 2014, **11**(1-2), 52-58.
- 27 Patompong K and Ratana B, Terpinene-4-ol induces autophagic and apoptotic cell death in human leukemic HL-60 cells, *Asian Pac J Cancer Prev*, 2013, **14**(12), 7537-7542.
- 28 Patompong K and Ratana B, Induction of intrinsic and extrinsic apoptosis pathways in the human leukemic MOLT-4 cell line by terpinen-4-ol, *Asian Pac J Cancer Prev*, 2012, **13**(7), 3073-3076.
- 29 Saphira S, Pleban S, Kazanov D, Tirosh P and Arber N, Terpinene-4-ol: A novel and promising therapeutic agent for human gastrointestinal cancers, *Plus One*, 2016, **11**(6), 1-13.
- 30 Ramalho T R, Oliveira M T, Lima A L, Bezerra-Santos C R and Piuvezam M R, Gamma-terpinene modulation of LPS-stimulated macrophages is dependent on the PGE2/IL-10 Axis, *Planta Med*, 2016, **82**, 1341-1345.
- 31 Ramalho T R, Oliveira M T, Lima A L, Bezerra-Santos C R and Piuvezam M R, Gamma-terpinene modulates acute inflammatory response in mice, *Planta Med*, 2015, **81**, 1248-1254.
- 32 Shao P, Zhang H, Niu B and Jiang L, Antibacterial activities of R-(+)-Limonene emulsion stabilized by *Ulva fasciata* polysaccharide for fruit preservation, *Int J Biol Macromol*, 2018, **111**, 1273-1280.
- 33 Khodabakhsh P, Shafaroodi H and Asgarpanah J, Analgesic and anti-inflammatory activities of *Citrus aurantium* L. blossoms essential oil (neroli): involvement of the nitric oxide/cyclicguanosine monophosphate pathway, *J Nat Med*, 2015, **69**(3), 324-331.
- 34 Shah B and Mehta A, *In vitro* evaluation of antioxidant activity of D-Limonene, *Asian J Pharm Pharmacol*, 2018, **4**(6), 883-887.
- 35 Roberto D, Micucci P, Sebastian T, Graciela F and Anesini C, Antioxidant activity of limonene on normal murine lymphocytes: Relation to H<sub>2</sub>O<sub>2</sub> modulation and cell proliferation, *Basic Clin Pharmacol Toxicol*, 2010, **106**(1), 38-44.
- 36 Miller J A, Thompson P A, Hakim I A, Lopez A M, Thomson C A, *et al.*, Safety and feasibility of topical application of limonene as a massage oil to the breast, *J Cancer Ther*, 2012, **3**(5A), 1-12.
- 37 Dosoky N S and Setzer W N, Biological activities and safety of citrus spp. essential oils, *Int J Mol Sci*, 2018, **19**(7), 1-25.
- 38 Hasmita I, Redha F and Junaidy R, Enhancement of quality of nutmeg oil using rotary vacuum evaporator, IOP Conf. Series: Journal of Physics: Conf. Series 1232, 012039, 2019.
- 39 Suprihatin S, Ketaren S, Ngudiwaluyo and Friyadi A, Isolation of myristicin from nutmeg oil (*myristica fragrans*) by steam distillation method, *J Teknol Ind Pertan*, 2017, **17**(1), 23-28.
- 40 Cinthia B S, Leonel T P, Ariovaldo B and Adelamar F N, Extraction of essential oil and lipids from nutmeg by liquid carbon dioxide, *J Supercrit Fluids*, 1999, **15**, 253-259.
- 41 Sudradjat S E, Timotius K H, Mun'im A and Anwar E, The isolation of myristicin from nutmeg oil by sequences distillation, *J Young Pharm*, 2018, **10**(1), 20-23.
- 42 Tan K P, Khoo H E and Azrina A, Comparison of antioxidant components and antioxidant capacity in different parts of nutmeg (*Myristica fragrans*), *Int Food Res J*, 2013, **20**(3), 1049-1052.