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Effect of Distillation Time on Physicochemical Characteristics and Compound Identification of Nutmeg Oil Using GC–MS

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ABSTRACT

Introduction: Nutmeg oil (*Myristica fragrans* Houtt.) is a high-value essential oil containing various bioactive compounds, including myristicin, α-pinene, limonene, and γ-terpinene, which are widely used in the food, pharmaceutical, and cosmetic industries. The quality and quantity of nutmeg oil are strongly influenced by the distillation process, particularly the distillation duration, which determines the extraction efficiency of volatile and semi-volatile compounds. **Materials and Methods:** Distillation was carried out for 6, 12, and 24 hours. The obtained oils were characterized based on distillation rate, yield, color, odor, specific gravity, ethanol solubility, and evaporation residue according to the Indonesian National Standard (SNI 06-2388-2006). **Results:** GC–MS analysis identified 36 compounds in the nutmeg oil samples. The major constituents included α-pinene, α-terpinene, limonene, γ-terpinene, and myristicin. The highest oil yield (1.91%), specific gravity (0.9100), and evaporation residue (1.9837%) were obtained at 24 hours of distillation. Meanwhile, myristicin content increased significantly with longer distillation time, reaching 38.74% at 24 hours. Statistical analysis revealed that distillation time significantly affected distillation rate, yield, and evaporation residue ($p < 0.001$). Significant differences were also observed in specific gravity and ethanol solubility, as determined by the Kruskal–Wallis test ($p < 0.05$). **Conclusion:** Overall, the 12-hour distillation period was considered the optimum condition because it provided the most balanced composition between aromatic volatile compounds and bioactive constituents while maintaining acceptable physicochemical quality.

Keywords: Nutmeg oil, Steam distillation, Distillation time, GC-MS, *Myristica fragrans*.

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INTRODUCTION

Nutmeg oil, derived from the seeds of *Myristica fragrans* Houtt., is a high-value essential oil extensively utilized across the food, pharmaceutical, and cosmetic sectors. As a global leader in nutmeg production, Indonesia's key regions—including Maluku, North Sulawesi, Aceh, West Sumatra, and West Papua—account for approximately 89.95% of the national output, which was projected to reach 44,597 tons in 2023. The physicochemical properties and aromatic profile of nutmeg oil are heavily dependent on the extraction parameters, particularly the duration of distillation [1,2]. Since volatile compounds have unique boiling points and polarities, the duration of thermal exposure determines which constituents are recovered. Steam distillation is the preferred method for this study

due to its efficiency in maintaining oil integrity and minimizing the thermal breakdown of sensitive compounds [3–5].

Distillation time significantly influences both the oil yield and the specific chemical fractions obtained. Brief extraction periods (approximately 6 hours) typically recover low-boiling-point monoterpenes, while an intermediate duration of 12 hours facilitates the release of oxygenated monoterpenes and sesquiterpenes. Conversely, extended distillation (up to 24 hours) may maximize yields and extract heavier aromatic fractions, though it carries an inherent risk of thermal degradation.

Nutmeg extracts and essential oil (EO) are used in new drug developments in India, China, and other tropical countries due to the various pharmacological activities [6–8]. Sabinene (20.2%), terpinen-4-ol (12.1%), safrole (10.3%), α-pinene (9.7%), β-phellandrene (6.6%), and γ-terpinene (5.9%) were reported to be some of the main compounds of nutmeg EO, which is extracted by distillation. Nutmeg has been used in folk medicine for multiple purposes, such as an antimicrobial, antioxidant, psychostimulant, and antithrombotic. In Ayurveda, the seeds have been used to treat poor digestion, insomnia, and urinary incontinence. Currently, nutmeg is known for its use in treating various health disorders, such as bowel irregularities, rheumatoid arthritis, and muscle spasms. In some countries, like Zanzibar, nutmeg was chewed as an alternative to smoking marijuana. Some studies have highlighted the potential of nutmeg as an anticonvulsant, analgesic, and anti-inflammatory herb [9,10]. Lignans and neolignans have been reported from the plant with various pharmacological properties, including anticancer and antidepressant activities [8].

Chemical characterization is performed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify volatile markers based on molecular weight and fragmentation [11]. Furthermore, physical quality assessments—encompassing color, odor, specific gravity, ethanol solubility, and evaporation residue—are conducted in accordance with the Indonesian National Standard (SNI 06-2388-2006).

There is insufficient research on the elution of nutmeg oil as a function of distillation time. Therefore, this study hypothesized that capturing nutmeg essential oil (EO) at sequential time points during distillation would yield fractions with unique compositions and bioactivities. Therefore, this study aimed to investigate the effect of different steam-distillation durations (6, 12, and 24 hours) on the physicochemical characteristics and chemical composition of nutmeg oil obtained from Banda nutmeg seeds. The findings are expected to provide scientific information on optimal distillation conditions for producing high-quality nutmeg essential oil that meets national quality standards.

MATERIALS AND METHODS

The instruments employed in this research included a steam-distillation apparatus, a pycnometer, an analytical balance, a thermometer, a water bath, volumetric pipettes, a separating funnel, a desiccator, an evaporation dish, and a Gas Chromatography–Mass Spectrometry (GC–MS) instrument. The materials used in this study included dried nutmeg seeds (*Myristica fragrans* Houtt.), distilled water, and 90% ethanol. The nutmeg seeds were obtained from Banda, Indonesia. All chemicals used were of analytical grade.

Preparation of Nutmeg Samples

Dried nutmeg seeds are cleaned of dirt, washed with clean water, dried at room temperature, and then ground in a blender until a coarse, homogeneous powder forms.

Steam Distillation Process

Approximately 1 kg of powdered nutmeg was introduced into the distillation flask. Steam distillation was conducted for 6, 12, and 24 hours. The distillate obtained from each treatment was collected, and the essential oil layer was separated from water using a separating funnel to obtain pure nutmeg oil.

Physicochemical Characterization

The characterization of the oil is carried out through tests of distillation rate, yield, color, odor, specific gravity, solubility in ethanol, and residue after evaporation. **The distillation rate** is determined by the volume of oil produced per unit time (mL/h). **The yield** is calculated as the ratio of the oil mass to the raw material mass. **Color** is observed visually by comparing the oil's color against a white background, while **odor** is assessed by sensory observation through the sense of smell. The specific gravity is measured with a pycnometer at 25 °C using the mass ratio of oil to water. **Solubility in ethanol** is determined by adding 90% ethanol dropwise to 1 mL of oil until a clear solution forms. **The residue of evaporation** is determined by weighing 5 g of oil in an evaporation dish, then heating

it over a water bath for 4 hours until a constant weight is achieved; the results are expressed as a percentage of the initial weight.

GC–MS Analysis

The oil's chemical composition was analyzed using a GC-MS instrument under standard operating conditions. The identified compounds are compared with the library system (NIST) to determine the name and structure of the compounds based on the match of molecular weight and fragmentation pattern.

Statistical Analysis

The test results data were analyzed using one-way analysis of variance (One-Way ANOVA) at a 5% significance level using SPSS software version 16.0. Before conducting the ANOVA test, the data were tested for normality using the Shapiro–Wilk method and for homogeneity of variances using the Bartlett test. Data is considered normally distributed and homogeneous if the probability value is greater than the significance level ($p>0.05$). Hypothesis testing is conducted under the condition that the null hypothesis (H_0) is accepted and the alternative hypothesis (H_1) is rejected if $p>0.05$, which means there is no significant difference between the distillation time treatments. Conversely, if $p<0.05$, then H_1 is accepted and H_0 is rejected, which means there is a significant difference between the distillation time treatments on the characteristics of nutmeg seed oil.

RESULTS AND DISCUSSION

The objective of this research was to evaluate how different distillation durations impact the chemical makeup and physical properties of nutmeg oil (*Myristica fragrans* Houtt.) sourced from Banda. Using the steam distillation method on 11 kg of dried seeds, oil samples were collected at 6, 12, and 24 hours. Various parameters—including yield, distillation speed, physical appearance, solubility, and chemical profile via GC-MS—were rigorously monitored. Findings suggest a direct correlation between prolonged distillation and the resulting oil's volume and integrity. Total oil recovery climbed from 100 mL at the 6-hour mark to a peak of 210 mL after 24 hours. Conversely, the extraction velocity slowed from 16.67 mL/hour to 8.75 mL/hour (Table 1). This deceleration suggests that high-volatility compounds are primarily released during the early phases of the process. Such observations support established steam distillation principles, in which the transport of volatile oils is determined by the volume of steam interacting with the raw material. As the process continued, the remaining compounds required more energy and longer extraction time due to their lower volatility and higher molecular weight.

Table 1. Distillation Rate Determination Data

Oil Yield (mL)	Distillation Time (Hours)	Distillation Rate (mL/hr)
100 mL	6 Hours	16.67 mL/hour
155 mL	12 Hours	12.92 mL/hour
210 mL	24 Hours	8.75 mL/hour

The extraction efficiency, reflected in the oil yield, demonstrated a positive correlation with steam exposure time, increasing from 0.91% to 1.91% (Table 2). While prolonged distillation enhances recovery, it poses a potential threat to the oil's integrity by thermally degrading sensitive aromatic constituents. Organoleptic Properties revealed a consistent, clear yellow (Table 3**)** hue across all samples, fulfilling the requirements of the SNI 06-2388-2006 standard; this suggests that the terpenoid compounds remained shielded from oxidation throughout the extraction. Furthermore, the persistence of a potent, distinct nutmeg fragrance confirms that key volatile markers, specifically α-pinene and myristicin, remained stable under the thermal conditions of the process.

Table 2. Yield Result Data

Nutmeg Oil Sample	Yield (%)
6 Hours	0.91%
12 Hours	1.41%
24 Hours	1.91%

Table 3. Color Test Data

Nutmeg Oil Sample	Color	SNI Standard 06-2388-2006
6 Hours	Clear Yellow	Colorless to Pale Yellow
12 Hours	Clear Yellow	Colorless to Pale Yellow
24 Hours	Clear Yellow	Colorless to Pale Yellow

Specific gravity values in Table 4 increased from 0.8863 at the 6-hour mark to 0.9100 after 24 hours, aligning consistently with the SNI (0.880–0.910) quality specifications. This upward trend suggests that extended extraction periods facilitate the recovery of heavier molecular fractions, particularly phenylpropanoids. Furthermore, the oil demonstrated excellent solubility in ethanol at a 1:5 ratio in Table 5 across all treatments, confirming a high degree of purity and the absence of non-polar impurities. Finally, the evaporation residue in Table 6 was maintained below the 2% threshold, indicating minimal presence of non-volatile materials such as waxes or resins.

Table 4. Spesific Gravity Data

Nutmeg Oil Sample	Specific Gravity	SNI Standard 06-2388-2006
6 Hours	0.8863	0.880-0.910
12 Hours	0.9030	0.880-0.910
24 Hours	0.9100	0.880-0.910

Table 5. Solubility Data in Ethanol

Nutmeg Oil Sample	Solubility in Ethanol	SNI Standard 06-2388-2006
6 Hours	1:5 Clear	1:3 Clear
12 Hours	1:5 Clear	1:3 Clear
24 Hours	1:5 Clear	1:3 Clear

Table 6. Evaporation Residual Data

Nutmeg Oil Sample	Residual Evaporation	SNI Standard 06-2388-2006
6 Hours	1.8860%	Maximum 2.0%
12 Hours	1.9397%	Maximum 2.0%
24 Hours	1.9837%	Maximum 2.0%

A total of 36 chemical constituents were detected via GC-MS analysis, dominated by α-pinene, α-terpinene, limonene, γ-terpinene, and myristicin. Due to its high volatility, α -pinene peaked early at 8.60% during the 6-hour interval before declining to 5.00% by the 24-hour mark. Conversely, myristicin levels rose significantly from 28.33% to 38.74%, as its lower vapor pressure necessitates longer distillation to achieve full recovery. Meanwhile, limonene and γ-terpinene exhibited optimal concentrations (7.17% and 6.72%, respectively) at the 12-hour stage, representing an ideal balance between their evaporative properties and heat resistance.

Table 7. Nutmeg Oil Component Data

No	Compound Name	Molecular Formula	R. Time	Nutmeg Oil Area %		
				6 Hours	12 Hours	24 Hours
1	α-Pinene	$C_{10}H_{16}$	7,055	8.60%	7.83%	5.00%
2	α-Terpinene	$C_{10}H_{16}$	9,823	2.73%	5.51%	5.06%
3	Limonene	$C_{10}H_{16}$	10,291	6.51%	7.17%	5.99%
4	γ-Terpinene	$C_{10}H_{16}$	11,296	4.77%	6.72%	6.66%
5	Myristicin	$C_{11}H_{12}O_3$	27,084	28.33%	30.36%	38.74%

As a hallmark constituent of nutmeg, myristicin possesses vital pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory effects. While the 24-hour distillation peak confirmed maximum efficiency for phenylpropanoid extraction, the 12-hour mark was identified as the ideal operational window. This duration effectively maintains the equilibrium between fragrant volatiles—such as α-pinene and limonene—and the bioactive myristicin, resulting in an essential oil that optimizes both sensory appeal and medicinal efficacy. The

experimental data were further validated through statistical evaluation. Normality testing using the Shapiro-Wilk method indicated that distillation rate, yield, and evaporation residue followed a normal distribution ($p > 0.05$). Conversely, specific gravity and ethanol solubility deviated from a normal distribution ($p < 0.05$), necessitating specific considerations for further comparative analysis.

Table 8. Normality Test Data

	Kolmogorov – Smirnov Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Refining Rate	.207	9	.200*	.877	9	.147
Yield	.119	9	.200*	.863	9	.104
Spesific Gravity	.273	9	.053	.802	9	.021
Ethanol Solubility	.414	9	<.001	.617	9	<.001
Remaining Evaporation	.196	9	.200*	.863	9	.104

*This is a lower of the true significance

To assess the equality of variances among the normally distributed variables, Levene's test was employed. The results confirmed homogeneity of variance ($p > 0.05$), satisfying the assumptions for a one-way Analysis of Variance (ANOVA).

Table 9. Homogenity Test Data

		Levene Statistic	Df1	Df2	Sig.
Refining Rate	Based On Mean	2,158	2	6	.197
	Based On Median	2,126	2	6	.200
	Based ON Median and With Adjusted df	2,126	2	2,619	.283
	Based On trimmed Mean	2,156	2	6	.197
Yield	Based On Mean	.414	2	6	.679
	Based On Median	.414	2	6	.679
	Based ON Median and With Adjusted df	.414	2	4,765	.683
	Based On trimmed Mean	.414	2	6	.679
Remaining Evaporation	Based On Mean	3,455	2	6	.100
	Based On Median	1,010	2	6	.419
	Based ON Median and With Adjusted df	1,010	2	3,991	.442
	Based On trimmed Mean	3,212	2	6	.113

Statistical evaluation via ANOVA revealed that distillation duration exerts a highly significant impact on the distillation velocity ($F = 276.151$; $p < 0.001$), oil yield ($F = 775.862$; $p < 0.001$), and evaporation residue ($F = 344.341$; $p < 0.001$). These high F-values and minimal p-values confirm that variations in processing time are a primary factor in determining the physical and quantitative outcomes of the extraction.

Table 10. One-Way ANOVA Test Data

		Sum of Square	Df1	Mean Square	F	Sig.
Refining Rate	Between Group	94,100	2	47,050	276,151	<001
	Within Group	1,022	6	.170		
	Total	95,122	8			
Yield	Between Group	1,500	2	.750	775,862	<001
	Within Group	.006	6	.001		
	Total	1,506	8			
Remaining Evaporation	Between Group	1,302	2	.651	344,341	<001
	Within Group	.011	6	.002		
	Total	1,313	8			

Concurrently, the Kruskal-Wallis analysis indicated that variations in distillation time significantly impacted specific gravity ($p = 0.027$) and ethanol solubility ($p = 0.018$). Further examination revealed that the 24-hour distillation interval achieved the highest mean ranks across both variables, underscoring the substantial influence of extended processing time on these physical properties. Empirically, extending the distillation period shows a positive correlation with yield, density, and the concentration of high-molecular-weight compounds. Nevertheless, a 12-hour distillation window represents the optimal equilibrium between quantity and quality. At this specific interval, the nutmeg oil maintains a harmonious ratio of monoterpene hydrocarbons to phenylpropanoids, maximizing both its sensory characteristics and biological potency. Given that all physical properties align with the SNI 06-2388-2006 quality standards, the extraction methodology employed in this research is confirmed to be effective. Consequently, it serves as a viable model for industrial essential oil production within the food, cosmetic, and medicinal sectors.

CONCLUSIONS

This research demonstrates that distillation duration is a primary determinant of both the physical properties and the chemical profile of nutmeg (*Myristica fragrans*) essential oil. All experimental intervals yielded oil characterized by a clear yellow hue and the distinctive fragrance required by the SNI 06-2388-2006 standard, confirming superior organoleptic integrity. The progression from a 6-hour to a 24-hour process resulted in a linear increase in yield, specific gravity, and evaporation residue. These findings underscore that longer extraction times effectively facilitate the recovery of heavy molecular fractions, thereby defining the final physical grade of the oil. Dynamic shifts in chemical constituents were further elucidated through GC-MS analysis. While monoterpenes like α-pinene, limonene, and γ-terpinene remained present throughout the process, the concentration of the bioactive phenylpropanoid, myristicin, intensified significantly over time, peaking at 38.74% after 24 hours. In contrast, shorter extraction windows favored the recovery of highly volatile components. In conclusion, the 12-hour distillation period is proposed as the optimal industrial standard; it strikes a critical balance between aromatic volatiles and therapeutic bioactive compounds, ensuring full compliance with national quality benchmarks.

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