Identification of Trimyristine from Oil, Crystals, and Residue of Nutmeg (*Myristica fragrans*) in South Aceh (Indonesia) Using GC-MS

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Submission: November 14, 2021; Revision: April 6, 2022, April 21, 2022, May 11, 2022, May 18, 2022, May 24, 2022; Received: June 9, 2022; Published: May 31, 2023

ABSTRACT

South Aceh Regency is the largest nutmeg-producing area in Aceh Province, Indonesia, but its potential for oil production has not been fully optimized. Nutmeg (*Myristica fragrans*) oil is one of the essential oil groups obtained from the distillation of the nutmeg plant, including its peel, mace, and seeds. One of the essential compounds found in nutmeg oil is trimyristin. Therefore, this research aimed to identify and determine the content of trimyristin in the oil, crystals, and residue of nutmeg seeds from South Aceh. It was carried out in stages wherein nutmeg oil distillation was performed by a simple distillation method. Trimyristin was separated from the oil using the reflux method and from the residue by the maceration method. The compound was obtained from the isolation of nutmeg oil using chloroform as a solvent. The oil was characterized based on SNI No. 06-2388 of 2006, then trimyristin was isolated and identified by Gas Chromatography-Mass Spectrophotometry (GC-MS). The results showed that the characterization of nutmeg oil did not fulfill the SNI . The color and smell tests were not in accordance with those of nutmeg oil, the specific gravity was 0.909, the refractive index was 1.63, and the solubility in alcohol was cloudy. Trimyristin of 11.23% was only found in the residue, but it was not detected in the oil and crystals.

Keywords: Gas Chromatography-Mass Spectrophotometry (GC-MS); *Myristica fragrans*; trimyristin

INTRODUCTION

Aceh is one of the provinces in Indonesia known for its production of nutmeg plants, which are processed and cultivated to produce oil. Nutmeg oil produced in this province has a typical sharp aroma and a high yield of 11% (Hasmita, Mistar & Redha., 2019). Data on the distribution of nutmeg plants taken from the Central Statistics Agency in 2020 shows that Aceh has a total average production of 5,943 tons from 6 regencies/cities. These include South Aceh with 5,251 tons, as well as Southwest Aceh, Simeulu, Besar, Nagan Raya, and North Aceh with 292 tons, 280 tons, 41 tons, 41 tons, and 38 tons, respectively. Based on the data, one of the largest nutmeg-producing regions is South Aceh, with a plantation area of 16,941 hectares and a production yield of 5,943 tons (BPS Aceh, 2020). This high production yield means that South Aceh has great potential for commodity exports. However, this potential is still not fully maximized because most people only use nutmeg

DOI: http://doi.org/10.22146/agritech.70430
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

as a kitchen spice or candy, with only a few distilling it into oil (Mujiburrahmad, Marsudi & Usman, 2019).

Nutmeg oil is an essential oil obtained from distilling the peel, mace, as well as seeds of nutmeg plants. This is generally carried out using simple and steam distillation (Supriyantini et al., 2017). Angreni, Liunokas & Karwur, (2020) stated that the mace, seeds, and flesh of nutmeg plants produce essential oil of 15.30, 16, and 7%, respectively. Furthermore, nutmeg oil is characterized by its clear yellowish color and typical aroma (Hidayati et al., 2015). The essential oil derived from the seeds and mace is typically used in the pharmaceutical, perfume, cosmetic, and beverage industries (Kapelle, Syamsul & Yandra., 2014). Torry (2014) stated that the essential oil in the seeds also has bactericidal activity. One of the essential components found in nutmeg seeds is trimyristin, which is also present in crystals and residue.

Trimyristin, also known as myristin fat or glycerol trimyristate, is a derivative of an ester compound that is soluble in non-polar solvents such as alcohol, benzene, chloroform, and diethyl ether, but insoluble in water. According to previous reports, the content make-up about 40-75% of the total oil in nutmeg. Trimyristin is a white to yellowish-white crystalline solid with a melting point of 56-57 °C and a boiling point of 311 °C (Kapellee, et al., 2014). In recent years, this fat has been commonly produced from coconut, palm kernel, and babassu oil, but the amount produced is limited. Therefore, efforts have been made to isolate trimyristin from nutmeg oil. This isolation method produces more amount compared to other oil because the distillation process of nutmeg oil does not need fractionation which requires expensive components and processes (Idrus et al., 2014). M A'Mun (2013) stated that trimyristin is commonly used as a whitening agent, such as soap and lotion.

According to Hidayati et al., (2015), methods used to produce nutmeg oil include steam, water, and steam-water distillation. Steam distillation is the most common method, as carried out by Astuti, (2019) which characterized and isolated nutmeg oil and obtained a myristin content of 0.95%. Furthermore, Hasmita et al. (2019) used a rotary evaporator and found a similar content of 53.41% at a temperature of 150 °C for 1 h. Kapelle & Laratmase (2014) also isolated trimyristin from nutmeg seeds using steam-water distillation and obtained a content of 11%. This research used distillation as one of the separation methods based on boiling point with chloroform as a solvent to dissolve trimyristin.

Chloroform is used in the extraction process because it is a non-polar solvent that can completely dissolve trimyristin. Moreover, the identification of trimyristin from nutmeg seeds has been carried out extensively. Torry (2014) extracted nutmeg seeds using chloroform as a solvent and obtained white crystals containing 39.09 g or approximately 18.36% of trimyristin. According to Kapelle (2014), the extraction of nutmeg powder using chloroform yielded a slightly yellowish-white solid of 17 grams (11%). Hidayati (2016) also obtained a trimyristin yield of 5.2% from the extraction of nutmeg powder using chloroform. Therefore, the identification of trimyristin was carried out on 3 samples in this research, namely oil, crystals, and residue of nutmeg seeds using a simple distillation method with chloroform as a solvent. Gas Chromatography-Mass Spectrophotometry (GC-MS) was also used to determine the amount of trimyristin produced. This research aimed to identify and determine the amount of trimyristin in nutmeg seeds from South Aceh, including oil, crystals, and residue obtained from the extraction process.

MATERIALS AND METHODS

Materials

The materials used were oil, crystals, and residue of nutmeg (*Myristica fragrans*) seeds taken from Jamboe Papen Village, Meukek Sub-district, South Aceh Regency, as well as chloroform (smart-lab), anhydrous Na_2SO_4 (merk), and acetone (smart-lab).

Sampling Technique

Nutmeg seeds were taken using the purposive sampling method to the identify trimyristin compound. The samples were taken in one sub-district which represented other sub-districts in South Aceh. Sampling was carried out in Jamboe Papen Village, Meukek Sub-district, South Aceh Regency, in May 2021 at 02:00 PM with up to 20 kg. In addition, this research only took old nutmeg seeds from farmers.

Research Procedure

Several procedures were used in the form of sample preparation, the distillation of nutmeg seeds into oils, identification of trimyristin compounds, formation of crystals, and manufacture of nutmeg oil soap.

Sample Preparation

Nutmeg seeds collected were air-dried, cut into small pieces of about 0.5-1 cm and crushed using a blender, then the samples obtained were filtered using a sieve to obtain powder.

Distillation of Nutmeg Seeds into Oil

The distillation tool was carefully assembled and prepared, then 150 g of nutmeg powder was weighed

and placed into a round-bottom flask. Furthermore, water solvent was added until the powder was submerged in approximately 300 mL with a ratio of 1:2. The distillation process was carried out at the boiling point of water for 2 hours. After obtaining nutmeg oil, anhydrous Na_2SO_4 was added to clarify the oil then the filtrate and residue were separated using a Buchner funnel. The oil was further characterized for its physical properties according to SNI No. 06-2388 of 2006. The distillation process produced nutmeg oil and residue, which were then subjected to the separation of trimyristin. The residue was extracted through maceration, while the oil was extracted using the reflux method.

Formation of Trimyristin Crystals from Distilled Nutmeg Oil

Nutmeg oil separated during the distillation process was placed into a 250 mL round-bottom flask and mixed with chloroform solvent. The mixture was subjected to reflux with a water bath for 30 minutes at a temperature of 34 °C, then, it was cooled and filtered using filter paper. The next step was carried out using a rotary evaporator to separate the chloroform solvent from the residue. The residue obtained was placed into a 250 mL glass beaker and 100 mL of acetone was added, then, it was heated in a water bath. Afterward, it was cooled at room temperature for 1 hour and continued with further cooling using ice water at a temperature of 0 °C for 30 min until white crystals were formed in the solution. The white crystals were filtered using a Buchner funnel and the yield was calculated, while the trimyristin compound was identified with GC-MS.

Formation of Trimyristin Crystal from the Residue of Distilled Nutmeg Seeds

About 50 g of the distilled nutmeg seeds residue and 100 mL of chloroform were added to a 250 mL glass beaker, followed by an extraction process using the maceration method for 3 days. The solution formed was filtered every 24 h using a Buchner funnel and fresh chloroform was added back in the amount of 100 mL. The filtrate was evaporated using a rotary evaporator, then followed by a recrystallization process by adding 25 mL of 95% ethanol. The result was filtered to identify the content of trimyristin using GC-MS.

Organoleptic Tests

Organoleptic tests were carried out to characterize the physical properties of nutmeg oil in accordance with SNI No. 06-2388 of 2006. In this research, the tests were performed by 15 panelists who were given samples of nutmeg oil and a questionnaire in the form of choices on the color and smell of the oil.

RESULTS AND DISCUSSION

Characterization of Nutmeg Oil

To test the characteristics of nutmeg oil, the first step taken was to distill the oil using simple distillation. This is an essential method of separating two compounds based on the boiling point of the solvent used. After the distillation process, trimyristin was separated from the oil through the reflux method and the residue using the maceration method to obtain white crystals. The oil, residue, and white crystals were identified using GC-MS, while the solvent used to separate trimyristin was chloroform. Furthermore, the residue obtained from the distillation was separated for trimyristin using the maceration method. The use of chloroform was based on the polarity of trimyristin which was identified to be non-polar, thereby requiring a non-polar solvent.

The distillation process was carried out by grinding nutmeg seeds into powder and dissolving in chloroform. A simple distillation method was performed for 2 hours using the boiling point of chloroform at 61°C, and it was ensured that the temperature did not exceed this point. This is because trimyristin is usually formed below the boiling point of the solvent.

Table 1. Characterization of nutmeg oil

No.	Characterization	Chloroform solvent	SNI No. 06-2388 of 2006
1	Organoleptic tests a. Color b. Smell	 Clear white Typical of nutmeg oil	 Clear white Typical of nutmeg oil
2	Specific gravity	0,909	0,880-0,910
3	Refractive index	1,63366	1,470-1,497
4	Solubility in ethanol (1:3) mL	Cloudy	Clear

When the temperature exceeded the boiling point of the solvent, the trimyristin also evaporated along with the solvent. Additionally, when the boiling point of the solvent approached the boiling point of trimyristin, the amount produced was small, and the formed trimyristin crystals.

After obtaining nutmeg oil from the distillation process, its physical and chemical properties were characterized based on SNI No. 06-2388 of 2006 in the form of organoleptic tests comprising color and smell, specific gravity, refractive index, and solubility in ethanol (1:3) mL. The purpose of the characteristic test was to determine the changes during the analysis process as well as the content of trimyristin in the powder, and the results are shown in Table 1.

Table 1 shows that the physical properties test results of South Aceh nutmeg oil did not fulfill SNI No. 06-2388 of 2006 as indicated by the refractive index and solubility in 90% ethanol. Organoleptic tests were conducted by 15 panelists who examined if the color and smell of the oil were in accordance with SNI No. 06-2388 of 2006. Based on the results, nutmeg oil was clear with a typical aroma, and a specific gravity of 0.909, which still fell within the Indonesian National Standard. According to Idrus et al. (2014) and Kaseke et al. (2014), specific gravity is a combination of the molecular weight of the compounds making up nutmeg oil at a specific volume. Furthermore, the refractive index obtained from the physical properties test during the oil distillation using chloroform solvent was 1.6336. Hidayati et al. (2015) stated that the refractive index is influenced by the light fraction components in the form of monoterpene hydrocarbons as well as the heavy components including oxygenated monoterpenes and sesquiterpenes. When the heavy fraction components are abundant, the density and specific gravity increase. The refractive index obtained from nutmeg oil exceeded SNI No. 06-2388 of 2006 because it contained heavy fraction components that increased the oil density, causing the light passing through the oil to bend toward the normal line (Hidayati et al., 2015). Additionally, the high refractive index was due to suboptimal distillation processes leading to the formation of compounds that were not part of nutmeg oil components.

The solubility of nutmeg oil in ethanol indicated that the oil and alcohol had the same polarity. Based on the results, the solubility was cloudy and did not fulfill SNI No. 06-2388 of 2006 as it did not dissolve completely in alcohol. This was due to other components involved in the distillation process that caused the oil to be impure and mixed with other compounds.

The content of nutmeg oil from South Aceh and the yield of trimyristin crystals produced are shown in Table 2. The % nutmeg oil yield was 8%, while the % yield of trimyristin crystals from the oil and residue was 0.13%, and 8.55%, respectively. According to Hidayati et al. (2015), several factors influence the yield value such as time, sample size, and distillation temperature. Besides, nutmeg oil yield produced is higher compared to Papua nutmeg oil at 3.11% (M A'Mun, 2013) and lower than that of Banda at 12.5% (Idrus et al., 2014).

Nutmeg oil is one type of essential oil found in nutmeg seeds but the low quality of the seeds causes the prospect of oil trading to be low. The low and high quality of the oil depends on the genetic properties of nutmeg plant and the fertility of the planting medium.

The characterization of nutmeg oil has not yet complied with SNI No. 06-2388 of 2006 due to the incomplete distillation process, time, temperature, sample size, and age of the seeds. This research used the same distillation time of 2 hours at a temperature of ± 100°C and the higher the temperature used, the higher the evaporated oil. The low content of nutmeg oil produced reduced the number of trimyristin crystals obtained. Additionally, the acetone used to bind trimyristin crystals was the technical type, not pure. This is because pure acetone is very rare and requires official permission in the buying and selling process as it is one of the chemicals used in making narcotics.

The yield produced includes nutmeg oil of 8%, as well as the crystal yield from the oil and residue of 0.13%, and 8.55% respectively. These data indicated that most trimyristin crystals were obtained from the residue.

Table 2. Nutmeg oil yield and trimyristin crystals (oil and residue)

No	Sample	Initial sample weight (g)	The volume of nutmeg oil produced (mL)	% Nutmeg oil yield	% Yield of trimyristin crystals from oil	% Yield of trimyristin crystals from residue
1	Nutmeg powder (chloroform solvent)	150	12	8	0.13	8.55

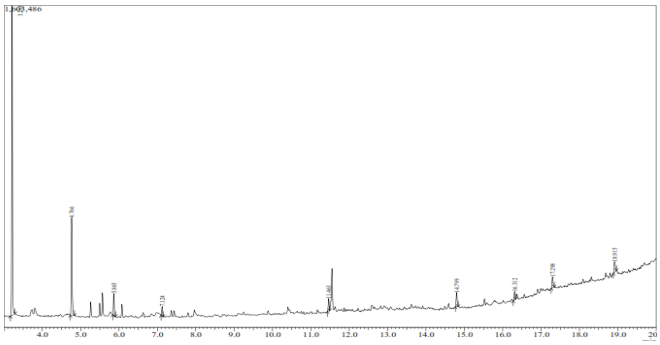

Figure 1. Chromatogram of nutmeg (*Myristica fragrans* Houtt) oil using GC-MS

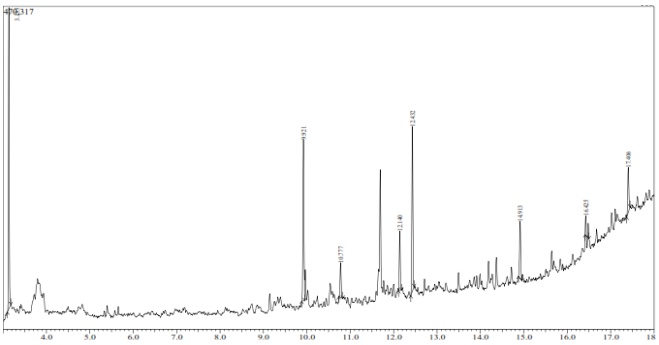

Figure 2. Chromatogram of trimyristin crystals in the chloroform extract of nutmeg oil using GC-MS

Identification of Trimyristin Compounds in Nutmeg Oil, Crystals, and Residue

The identification results of trimyristin in the oil, crystals, and residue of nutmeg seeds are shown in Figures 1, 2, and 3. GC-MS analysis was performed using the SHIMADZU GC-MS-QP2010, while separation was carried out using a Rxi-5ms capillary column with a helium carrier gas, injection volume of 1.00 µL, oven temperature of 70°C, and injection temperature of 280°C. GC-MS was aimed at identifying compounds in nutmeg seeds, such as trimyristin.

Figure 1 is a chromatogram of nutmeg oil from identification using GC-MS. The chromatogram had several high peaks which were used to identify compounds in nutmeg oil, and the results were shown in Table 3.

Kapelle & Laratmase (2014) stated that trimyristin was commonly known as myristin fat, trimyristate glycerol, or tritetradekanoate glycerol. This compound is soluble in alcohol, benzene, chloroform, and diethyl ether but insoluble in water. In other words, trimyristin is a derivative of myristin in the form of fat.

Table 3 shows that nutmeg oil distilled using chloroform solvent contained 9 types of chemical compounds but neither trimyristin nor myristicin was found. This is because the boiling point temperature during the extraction process exceeded the boiling point of chloroform solvent, causing the trimyristin to evaporate along with the solvent.

Trimyristin crystals from the distilled nutmeg oil produced a trimyristin content of 0% with 8 identified compounds, as shown in Table 4. The compounds with the highest chromatogram peaks were methoxy, phenyl, and oxime at a retention time of 3.135 with a % area of 28.74%, followed by myristicin with values of 21.76 and 9.921 respectively. The chromatogram of trimyristin crystals from the distilled nutmeg oil is presented in Figure 2.

The residue from oil distillation was also identified using GC-MS and it was found to contain 11.23% trimyristin at a retention time of 32.499. Trimyristin was present in the residue (dregs) from the distillation process of nutmeg seeds at a retention time of 32.499 and a % area of 11.23%, as shown in Figure 3.

The residue (dregs) produced from the distillation of nutmeg seeds had a trimyristin content of 11.23% with a retention time of 32.499 and a % area of 11.23%. The identification of compounds from the residue yielded 15 compounds shown in Table 5. The results showed that the compound with

Table 3. Data on the identification of trimyristin compounds found in nutmeg oil distilled using chloroform as a solvent

No	Retention time	%Area	Compound name
1	3.213	55.55	Methoxy, phenyl-, oxime
2	4.766	22.53	Cyclotrisiloxane, hexamethyl-
3	5.865	4.42	Silane, dimethyl(dimethyl(but-2-enyloxy)silane
4	7.124	2.09	Arsenous acid, tris(trimethylsilyl) ester
5	11.463	2.98	Pogostol
6	14.799	3.52	Hexadecanoic acid, ethyl ester
7	16.312	2.12	Ethyl oleate
8	17.298	2.97	Hexadecanoic acid, 2-hydroxy-1,3-propane
9	18.915	3.81	1H-Indene, 2,3-dihydro-1-methyl- (CAS)

Table 4. Data on the identification of compounds found in trimyristin crystals from nutmeg oil distilled using chloroform as a solvent

No	Retention time	%Area	Compound name
1	3.135	28.74	Methoxy, phenyl-, oxime
2	9.921	21.76	Myristicin
3	10.777	4.54	Phenol, 2,6-dimethoxy-4-(2-propenyl)
4	12.140	7.29	Naphthalene, 1,2,3,4-tetrahydro-1-phenylBenzene,
5	12.432	21.06	1,1'-(1,2-cyclobutanediyl) bis-,
6	14.913	7.99	Hexadecanoic acid, ethyl ester (CAS) Ethyl
7	16.425	2.18	(E)-9-Octadecenoic acid ethyl ester
8	17.406	6.44	Hexadecanoic acid, 2-hydroxy

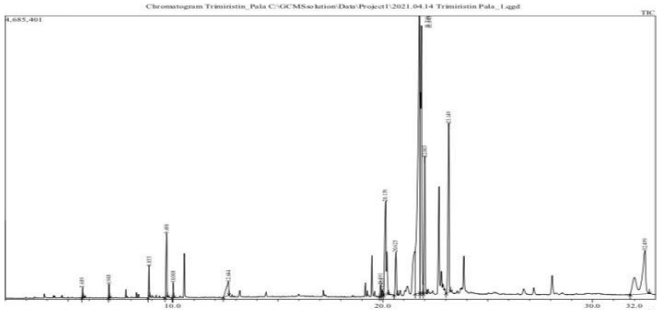

Figure 3. Chromatogram of trimyristin crystals in chloroform extract of residue from nutmeg (*Myristica fragrans Houtt*) seeds using GC-MS QP2010plus with a Hp-5MS column (Rxi-1MS)

Table 5. Data on the identification of compounds found in trimyristin crystals from the residue of nutmeg seeds distilled using chloroform solvent

No.	Retention time	%Area	Compound name
1	5.689	0.26	1-4-terpineol
2	5.948	0.33	Safrole
3	8.855	0.88	Eugenol
4	9.698	2.89	Myristicin
5	10.008	0.44	Elemicin, benzene, 1,2,3-trimethoxy-5-(2-propenyl)
6	12.644	1.84	Myristic acid, tetradecanoic acid
7	19.891	0.48	Isoeugenol
8	19.973	0.28	Coumarin, 3-benzoyl-4-phenyl
9	20.139	9.12	Dehydrodiisoeugenol
10	20.625	2.63	Benzoic acid, 6-formyl-2-3-dimethoxy-, ether
11	21.746	31.39	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS)
12	21.843	20.77	Phosphinic amide
13	22.003	7.57	Methoxyeugenol, 4-Allyl-2,6-dimethoxyphenol
14	23.149	9.89	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenol
15	32.499	11.23	Trimyristin

The results of the MS data in the form of a spectrum gave an ion peak with a molecular weight of m/e = 722 followed by fragmentation peaks with a molecular weight of 495, 339, 285, 211, and 57.

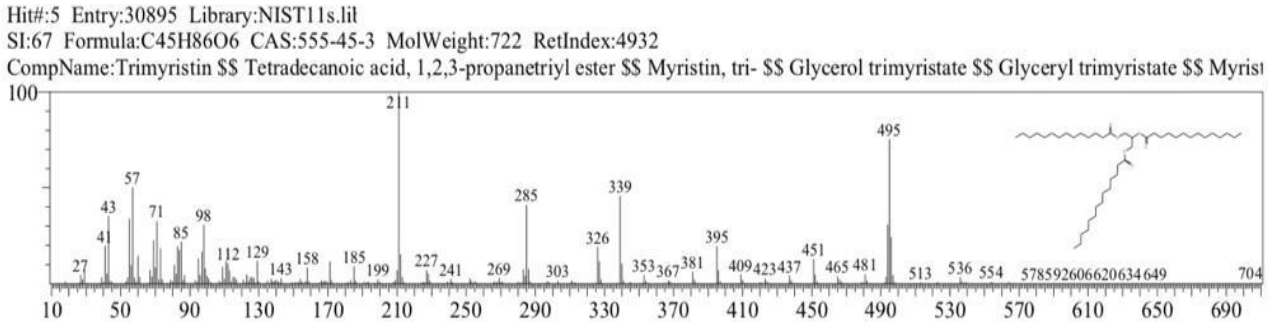

Figure 4. Trimyristin spectrum from the Mass Spectrophotometry (MS). Formula C45H86O6, SI : 67%. MolWeight : 722

the highest peak was Phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS).

The transesterification reaction in trimyristin with alcohol produces methyl ester, also known as myristic acid which is a slightly fatty white crystal soluble in alcohol or ether. The solubility properties can be used to crystallize it from hydrolyzed trimyristin. The transesterification reaction process of trimyristin with alcohol forms myristic acid, which can be used to produce soap (Kapelle & Laratmase, 2014).

Identification results of the oil, crystals, and residue of nutmeg seeds showed that trimyristin was only present in the residue. This was because the extraction process of trimyristin from crystals and oil used a reflux method, while the residue used a maceration method with chloroform as the solvent. Trimyristin is a non-polar compound that generally binds with non-polar solvents such as chloroform. However, during extraction with reflux, trimyristin in nutmeg oil and crystals was not identified because the heating temperature exceeded the boiling point of the solvent, causing the trimyristin to evaporate along with the solvent.

CONCLUSION

Based on the results, trimyristin was not detected in crystals from nutmeg oil distillation but was found in the residue (dregs). The content in the residue was 11.23%, while the oil and crystals contained 0%.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest from any party in writing this research.

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