

# Microwave-Assisted Extraction of Trimyristin from Nutmeg using Ethanol: A Greener Approach for the Undergraduate Organic Chemistry Laboratory

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**Abstract** The extraction of trimyristin from nutmeg, a species of the genus *Myristica fragrans*, is a typical experiment executed in the organic chemistry teaching laboratory. In this vein, trimyristin was extracted from nutmeg using the microwave-assisted extraction (MEA) method with 95% ethanol and diethyl ether. This MEA procedure promised to be more practical than pre-existing protocols utilized in the undergraduate teaching laboratory. This study established ethanol, a greener solvent than diethyl ether, as more efficient with up to 20% isolated crude yield for extracting this triglyceride from nutmeg using MEA. The recrystallized trimyristin was characterized by melting point, IR, <sup>1</sup>H-, and <sup>13</sup>C-NMR spectroscopy.

**Keywords:** microwave-assisted extraction, trimyristin, ethanol, undergraduate organic chemistry laboratory

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## 1. Introduction

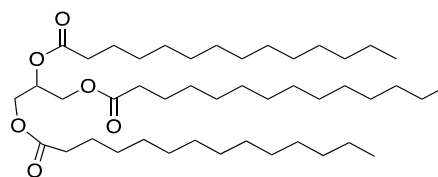
Microwave-assisted extraction (MAE) of natural products from plant materials is a relatively new technique that combines microwave technology and traditional solvent extraction [1]. This method has increased usage in the pharmaceutical and food industries over decades [2,3,4,5].

Compared to several extraction methods, such as ultrasound, pulsed electric field extraction, Soxhlet extraction [6], supercritical fluid extraction, and conventional extraction (reflux), MAE has proven to be a valuable technique applicable to academic laboratories. This is due to its low-energy utilization, lessened extraction time, and improved isolated yield [2,3,4].

The efficacy of MAE is rooted in the fact that under microwave treatment, the polar water molecules and any other vaporizable components present in the presumably dried plant material can interact directly with the constantly changing electromagnetic waves. This caused a temperature and internal pressure rise in the plant cells, triggering them to rupture. The organic solvent can quickly diffuse into the plant cells and remove soluble compounds. [7,8] Such a rapid process increased the number of compounds extracted from the plant material.

Nutmeg belongs to a species of the genus *Myristica fragrans*. Several studies have reported its chemical components and their biological implications [9]. It is a rich source of essential oils [9] and fats. Isolated

compounds include myristicin, terpinolene, limonene, and trimyristin (1) [10] (Figure 1). Trimyristin is the primarily saturated fat isolated from nutmeg seeds [10]. It is a triglyceride of myristic acid, a common emulsifier and surfactant used in soap and oleochemical industries.



Trimyristin (1)

**Figure 1.** The structure of trimyristin

A common practice for undergraduate organic chemistry laboratories is to use diethyl ether as the solvent of choice when extracting trimyristin from nutmeg [10]. This solvent is highly flammable and has the potential to form peroxides due to auto-oxidation [11]. These peroxides' built-up over extended periods is hazardous as they can create explosives. Thus, the recommendation for suitable disposal of excessive diethyl ether by Federal and local agencies.

Although volatile and flammable, ethanol is less likely to form dangerous peroxides on extended storage; hence, using this solvent with microwave-assisted extraction is a more environmentally friendlier approach to extracting natural products in the organic chemistry teaching laboratory. Considering this, we resolved to

expose organic undergraduate students to an eco-friendlier procedure for isolating trimyristin from nutmeg using the MAE method. The isolated product was purified via recrystallization and characterized using melting point and NMR spectroscopy.

## 2. Chemistry

### 2.1. Experimental Section

An Anton Paar Monowave 450 MW reactor with an autosampler was used to extract trimyristin from grounded nutmeg purchased from a local grocery. The melting point of recrystallized trimyristin, determined as 53-54°C (lit. 55 - 56°C) [10], was obtained on a Thermo Scientific MEL-TEMP melting point device and is uncorrected. The pure trimyristin was characterized by IR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectral data. A Perkin Elmer Spectrum BX FT-IR system and a Bruker 300 MHz FT-NMR spectrometer with TMS were used as internal standards. Diethyl ether, 95% ethanol, and acetone were purchased from Sigma Aldrich and were used without further purification.

#### 2.1.1. Procedure A: Microwave Extraction of Trimyristin from Nutmeg

A 30 mL microwave vial containing grounded nutmeg (4.000 g) and solvent (10 mL) was sealed and placed into a microwave reactor. The vials' contents were set to heat as fast as possible to either 86°C or 125°C at 850 W. These temperatures were held for 15 minutes and cooled to 55°C [12]. It was then filtered by gravity. The filtrate was concentrated under a vacuum to yield the crude trimyristin as a yellow/orange solid. This experiment was repeated twice, and the average percentage yield of the crude trimyristin was recorded. The average results for the crude trimyristin were as follows:

<b>Ethanol</b>	(86°C, 0.812 g, 20.3%)
	(125°C, 0.819 g, 20.4%)
<b>Diethyl ether</b>	(86°C, 0.496 g, 12.4%)

#### 2.1.2. Procedure B: Conventional Extraction of Trimyristin from Nutmeg Using Ethanol

To a 100 mL RBF containing ground nutmeg (4.000 g) was added ethanol (10 mL). The mixture was refluxed for

60 minutes and then cooled to room temperature. It was then filtered by gravity, and the filtrate was concentrated under a vacuum to yield the crude trimyristin as a yellow/orange solid (0.808 g, 20.2% yield).

#### 2.1.3. Procedure C: Extraction of Trimyristin from Nutmeg Using Diethyl Ether at Room Temperature

This experiment was adopted from the procedure provided by Frank and coworkers [6]. Diethyl ether (10 mL) was added to a 100 mL round bottom flask containing grounded nutmeg (4.000 g). This mixture was stirred for 15 minutes, and the resulting mixture was filtered into a preweight 100 mL RBF. An additional 2-3 mL of diethyl ether was used to transfer the nutmeg extract quantitatively. The solvent was evaporated on a rotator evaporator to give the crude trimyristin as a yellow solid (0.690 g, 17.3 %).

#### 2.1.4. Recrystallization of Crude Trimyristin

Crude trimyristin (0.921 g) was recrystallized by transferring the solid to a 50 mL beaker. Then a 50 mL Erlenmeyer flask containing 10 mL of acetone was heated in a steam bath and immediately transferred to the beaker. The mixture was reheated in a steam bath until most of the solids dissolved. It was quickly filtered while hot by gravity to remove any insoluble particles remaining in the flask. The filtrate was cool to room temperature and eventually put in an ice bath at ~0°C to give the crystalline trimyristin. The crystals were collected by vacuum filtration and washed with 2 mL of ice-cold acetone to yield pure trimyristin as a white powder (0.051 g, 6% yield), Mpt. 53-54°C.

## 3. Spectral Data for Trimyristin

IR cm<sup>-1</sup>: 1738.

<sup>1</sup>H - NMR (300 MHz, CDCl<sub>3</sub>): δ 5.27 (m, 1H), 4.30 (dd, J = 4.2, 11.7 Hz, 2H), 4.15 (dd, J = 6.0, 11.7 Hz, 2H), 2.31 (m, 6H), 1.63 (m, 6H), 1.12-1.40 (m, 60 H), 0.89 (t, J = 6.6 Hz, 9H).

<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): δ 173.5, 173.1, 69.1, 62.3, 34.5, 34.3, 32.2, 29.9, 29.7, 29.6, 29.4, 29.3, 25.1, 22.9, 14.3.

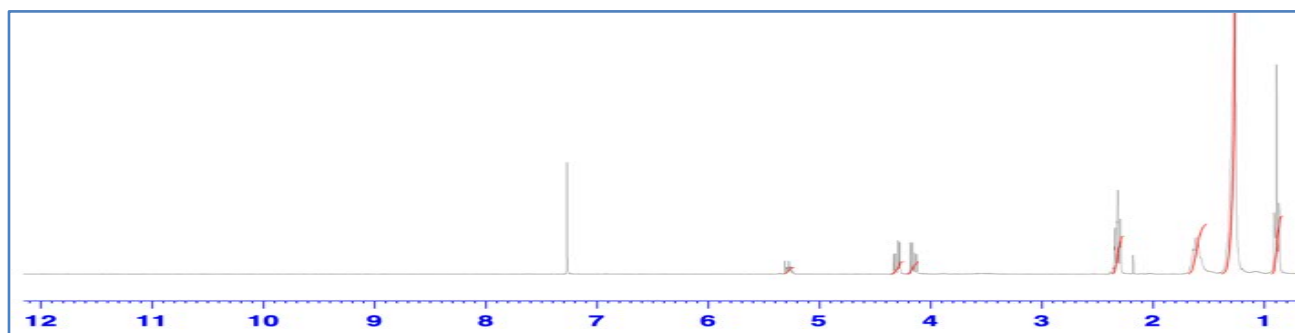


Figure 2. <sup>1</sup>H-NMR spectra of recrystallized trimyristin

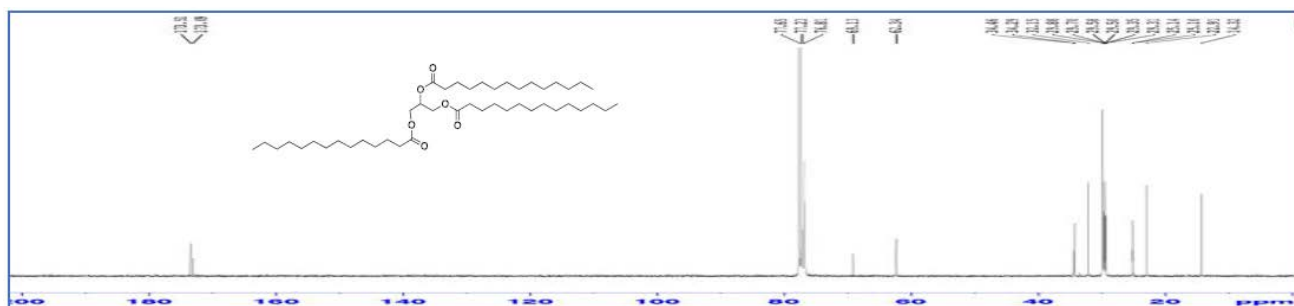


Figure 3.  $^{13}\text{C}$ -NMR spectra of recrystallized trimyristin

Table 1. Comparison of the percentage of crude trimyristin extracted from ~4.000 g of nutmeg under MW, conventional heating, and R.T. using ethanol and diethyl ether as solvents

Entry	Solvent	Extraction Method	Temp. / $^{\circ}\text{C}$	Time/min.	% yield of crude trimyristin
1	Diethyl ether	Conv.	R.T.	15	17.3%*
2	Diethyl ether	MW	86	15	12.4%*
3	Ethanol	MW	86	15	20.3%*
4	Ethanol	MW	125	15	20.4%
5	Ethanol	Reflux	78	60	20.2%

\* The average % yield of crude trimyristin was determined in triplicates.

## 4. Results and Discussion

### 4.1. MW versus Conventional Extraction of Trimyristin

Initially, the extraction of trimyristin was carried out by undergraduate students in the laboratory at R.T. They obtained a crude yield of 17.3% (Entry 1). This data was higher than expected when nutmeg was extracted with diethyl ether under microwave conditions at 86  $^{\circ}\text{C}$ . The latter produced a 12.4% crude yield. (Entry 2). This slight decrease in yield resulted from excessive loss of the volatile diethyl ether under the MAE condition. This was readily observed from the reduction in the volume of the mixture once the microwave vials were removed from the MW reactor.

Comparing the yield from the extraction of nutmeg with diethyl ether at R.T. (Entry 1) to the MAE extraction with ethanol at 86  $^{\circ}\text{C}$  (Entry 3), the latter condition was slightly more effective with a 3% increase in the crude yield. Also, refluxing ethanol in the extraction marginally affected the yield of trimyristin after 60 minutes. (Entry 5)

To increase the yield from the MAE method, the temperature was elevated to 125  $^{\circ}\text{C}$  (Entry 4). Under this condition, the isolated yield of trimyristin hardly improved (~ 0.5 %). Thus, the MAE condition using ethanol at 86  $^{\circ}\text{C}$  and 15 minutes was the most suitable for extracting trimyristin from grounded nutmeg.

It is worth noting that both  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR analyses of the crude ethanol extract were consistent with the presence of trimyristin, essential oil, such as myristicin, and unsaturated fats with alkene functionalities.

Given that the MAE method using ethanol allowed the students to complete the experiment in a shorter time than under refluxing conditions, the use of microwave technology in the extraction of trimyristin from nutmeg offers a more suitable and eco-friendly procedure for isolating this natural product in the undergraduate organic chemistry laboratory.

### 4.2. Spectroscopic Analysis of Trimyristin

Analysis of the IR spectrum of the recrystallized trimyristin was consistent with the literature [13]. The carbonyl irradiation at 1738  $\text{cm}^{-1}$  indicated the presence of the ester  $\text{C}=\text{O}$  functional groups. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of recrystallized trimyristin were identical to those reported in the literature [10]. The  $^1\text{H}$  NMR spectrum for trimyristin helps students to reinforce what they encountered in the nuclear magnetic spectroscopic analysis lecture earlier in the semester to resolve the structure of this natural product. (Figure 2) The downfield signals resonating at 4.15, 4.30, and 5.37 ppm indicated the two methylenes ( $-\text{CH}_2-\text{O}-$ ) and the methine ( $-\text{CH}-\text{O}-$ ) moiety of the glycerol group of trimyristin, respectively. The more upfield signals at 2.31 ppm acknowledged the six methylene protons, which were alpha to the carbonyl functionalities of the myristate groups. The  $^{13}\text{C}$ -NMR spectrum confirmed the presence of the carbonyl carbons of the ester linkages at 173.5 ppm (2C) and 173.1 ppm (1C) and the three carbons on the glycerol backbone at 69.1 ppm (C), and 62.3 ppm (2 C) in the molecule. (Figure 3)

## 5. Conclusion

This article compared five separate protocols for extracting trimyristin from nutmeg. These extractions used diethyl ether and ethanol under R.T., conventional, and microwave heating conditions. It established ethanol as a greener and more eco-friendly solvent than diethyl ether for the MW-assisted extraction of trimyristin from nutmeg. Thus, demonstrating a practical application for the undergraduate organic chemistry teaching laboratory.

The simplicity and expediency of the MW extraction method provide students with a fun way to explore laboratory techniques in chemistry, hopefully igniting their interest in organic chemistry.

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## Statement of Competing Interests

The Authors have no competing interests.

## List of Abbreviations

Conv.- Conventional  
 FTIR- Fourier Transform Infrared Spectroscopy  
 R.T.- Room Temperature  
 MAE. – Microwave-assisted extraction  
 MW. - Microwave  
 Mpt.-Melting point  
 NMR- Nuclear Magnetic Resonance Spectroscopy  
 TMS – Tetramethylsilane

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