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## Research Article

### Comparative Studies on Physicochemical properties and GC Analysis of Fatty Oil of the Two Varieties of the *Myristica fragrans* Houtt (Nutmeg) Seed

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#### Abstract

Fatty oil obtained by solvent extraction method using light pet-ether (40°-60°C) of the fruits oil of *Myristica fragrans* Houtt (Nutmeg) collected from India and Sri Lanka the oil was analyzed by GC. Total seven fatty acids were identified from the fatty oil of Indian nutmeg. The major constituents were oleic acid (37.73%), arachidic acid (26.97%), palmitic acid (21.97%). On the other hand total six fatty acids were identified from the fatty oil of Sri Lankan nutmeg. The major constituents were myristic acid (84.60%), palmitic acid (7.38%). Physico-chemical characteristics, such as iodine value, acid value, color, Solubility, moisture, ash, crude fiber, protein of the nutmeg were also determined.

#### 1. INTRODUCTION

*M. fragrans* Houtt. (Family- Myristicaceae) is a native of Moluccas, indigenous to India, Indonesia and Sri Lanka now cultivated in many tropical countries of both hemispheres. In India, it is grown in Madras state (Nilgiris, Coimbatore, Salem, Ramanathapuram, Tirunelveli, Kanyakumari and Madurai districts). Few trees are found in various localities in Kerala, Assam and other States. It is used as a remedy for stomachache, rheumatism and vomiting in pregnancy<sup>1-4</sup>.

The name 'Myristica' is derived from the Greek word 'Myron', a sweet liquid distilled from the plant<sup>5</sup>. Direct somatic embryogenesis was achieved from intact and fragmented zygotic embryos<sup>6</sup>. Glycosidically bound compounds have been extensively studied in grapes and wines, fruits and aromatic plants<sup>7-13</sup>. Nutmeg contains 25–50% lipids as fixed oil comprising mainly of myristic, petroselinic and palmitic acids<sup>14</sup>.

However many researches have been carried out on nutmeg (*M. fragrans* Houtt), but no systematic research on comparative studies has been reported on the fatty oil of nutmeg in India and Sri Lanka. Some disagreement about the presence of its constituents was observed. Therefore, present work was undertaken to carry out a complete investigation of the fatty oil of nutmeg of two varieties (Indian & Sri Lankan) including its physical & chemical properties along with GC analysis.

#### 2. MATERIALS AND METHODS

The fresh Indian nutmeg is available in the local markets of Dhaka city (collected from chalk bazaar). In Bangladesh a lot of Indian nutmeg is imported in bulk quantities from Kerala, India. The Sri Lankan nutmeg is also collected from local markets (chalk bazaar). The collected samples were washed clearly by water to remove

dust materials. Then they were dried. Finally the dried nutmeg was ground by Fritsch mortar grinder, Germany for one hour. Then the powder was sieved prior to the extraction process. The particle diameters obtained were 0.25 and 0.50mm.

#### 2.1 Physico-chemical Studies

Physico-chemical characteristics of seed such as iodine value (pet-ether extract), moisture, ash, color, Solubility, crude fiber, protein were determined by following the standard procedures<sup>15-16</sup>, and the results were shown in table-1.

##### 2.1.1 Isolation of Fatty Acids and Preparation of Methyl Ester

The neutral lipids were extracted from the air-dry powdered fruit successively with light petroleum ether (40-60°C), n-hexane and chloroform in a Soxhlet apparatus each for 22h, respectively. Pet-ether, hexane and chloroform extracts were mixed to equal weight (2mg) to analyze the fatty acid in the fruit. The sample was kept in a nitrogen atmosphere in a refrigerator. Fruit sample was analyzed according to the method reported by Griffin<sup>17</sup> for esterification and fatty acid methyl ester (FAME) extraction.

##### 2.1.2 Preparation of Standard Fatty Acid Methyl Ester (FAME)

Eleven standard free fatty acids (C<sub>6</sub>, Caprylic acid; C<sub>8</sub>, Lauric acid; C<sub>14.0</sub>, myristic acid; C<sub>16.0</sub>, palmitic acid; C<sub>16.1</sub>, palmitoleic acid; C<sub>18.2</sub>, linoleic acid; C<sub>18.1</sub>, oleic acid; C<sub>18.0</sub>, stearic; C<sub>20.4</sub>, arachidonic acid; C<sub>22.0</sub>, behenic acid; and C<sub>24.0</sub>, lignoceric acid) were individually weighed. About 5mg of each was taken in a reaction tube and BF<sub>3</sub>CH<sub>2</sub>OH reagent (5ml) was added to it. The mixture was boiled for 5 min. Hexane (5 ml) was added to it and boiled for further 1 min. After cooling the tube a solution of saturated salt was added and vortexed. Then the upper layer containing methyl esters was transferred to a vial with anhydrous sodium sulfate at the bottom. Then the ester was filtered through syringe filter and transferred to a small vial (2ml). The solvent was concentrated by blowing nitrogen gas and stored in a refrigerator before analysis by GC.

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## 2.2 Gas Chromatography (GC)

The fatty acid methyl ester were identified by Gas Chromatographic method using a capillary glass column (60m x 0.25m ID), coated with 100% cyanopropyl polysiloxane open tubular (WCOT0 fused silica (SP2340, Sipco, Bellefonte, Pennsylvania, USA) and flame ionization detector in GC-14A series (Shimadzu Co, Tokyo, Japan). Helium gas used as the carrier gas was at a flow rate of 3.5mL/min. Air and hydrogen flow rates were 350 and 25mL/min, respectively. The detector and injector temperatures were selected as 240°C and 235°C, respectively. The oven temperature was set to 135°C for 14

min and heated to 230°C with a heating rate of 3°C/min and maintained at this temperature for 6 min. Each fatty acid methyl ester (FAME) in extracts was identified by comparing retention times (LIPID STANDARD, Sigma Chemical Co, St Louis, Mo, USA).

## 3. RESULTS AND DISCUSSION

The result of the Physico-chemical properties of *M. fragrans* (nutmeg) fatty oil of India and Sri Lanka appeared in Table -1.

**Table 1:** Comparative studies on Physico-chemical properties of fatty oil of nutmeg from India and Sri Lanka.

Physical Properties		Indian Nutmeg	Sri Lankan Nutmeg
Oil yield 95 kg/100g		32.46%	33.45%
Organoleptic Evaluation	Taste	Spicy bitter taste	Spicy bitter taste
	Odor	Spicy	Spicy
	Color	Orange yellow	Orange yellow
	Appearance at room temperature (30°C)	Homogeneous, opaque liquid, lighter than water	Homogeneous, opaque liquid, lighter than water
Ash		2.99%	2.49%
Moisture		14.41%	14.11%
Crude fiber		9.07%	9.41%
Protein		7.25%	7.12%
Solubility in	Alcohol	Soluble	Soluble
	Distilled water	Insoluble	Insoluble
	Chloroform	Soluble	Soluble
	CCl <sub>4</sub>	Soluble	Soluble
	Pet-ether	Soluble	Soluble
	Diethyle ether	Soluble	Soluble
n-Hexane		Insoluble	Insoluble
<b>Chemical properties</b>			
Acid value		18.68	17.50
Saponification value		179.03	175.87
Iodine value		52.12	49.32
Peroxide value		49.0	48.82

The slight variation of this oil content and the composition of the fatty oil depend on several factors such genotype, stage of maturity, cultivation peculiarities, soil composition and climate differences in various geographical locations. Fluctuation of the oil composition can import change in the organoleptic properties of the plant belonging to the botanical spices and variety. So far we aware till now no systemic investigation on the *M. fragrans* (Nutmeg) have

not been investigated in Bangladesh by using modern analytical techniques.

GC analyzed results which include the active principles with their retention time; molecular formula; molecular weight and composition of the fatty oil of *M. fragrans* (Nutmeg) of two varieties were presented in table-2.

**Table 2:** Chemical constituents of the fatty oil of nutmeg from India and Sri Lanka

No.	Name of fatty acid	Retention time		Area		Relatives %	
		Indian nutmeg	Sri Lankan nutmeg	Indian nutmeg	Sri Lankan nutmeg	Indian nutmeg	Sri Lankan nutmeg
1	Myristic	10.29	10.31	193	172249	1.96	84.60
2	Palmitic	13.33	13.33	2156	15029	21.97	7.38
3	Oleic	15.83	15.83	3703	11639	37.73	5.71
4	Stearic	16.18	16.17	690	1245	7.03	0.61
5	Linoleic	15.76	15.75	97	1735	0.98	0.85
6	Lauric	-	7.16	-	1702	-	0.83
7	Arachidic	18.81	-	2647	-	26.97	-
8	Behenic	21.24	-	326	-	3.32	-

Total seven fatty acids were identified from the fatty oil of Indian nutmeg. The major constituents were oleic acid (37.73%), arachidic acid (26.97%), palmitic acid (21.97%)

On the other hand total six fatty acids were identified from the fatty oil of Sri Lankan nutmeg. The major constituents were myristic acid (84.60%), palmitic acid (7.38%).

Results show that fatty oil from both of two countries oils are a complex mixture of numerous, many of which are found in trace amounts. It is worth monitoring that there is a great variation in the chemical composition of these two region oil of *M. fragrans*

(Nutmeg). This confirms that the reported variation in oil is due to geographic divergence and ecological conditions.

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