

## Fatty acids and leaf amino acids in *Myristica fragrans* Houtt. and related taxa

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### ABSTRACT

*Myristica fragrans* Houtt. produces two spices, nutmeg and mace. Fat content, fatty acid and amino acid profiles were evaluated in *M. fragrans*, *M. andamanica*, *M. malabarica*, *M. magnifica*, *M. amygdalina*, *M. beddomeii*, *M. prainii* and *Knema andamanica*. Fat content in nutmeg ranged from 28 to 38% and in mace, it ranged from 23 to 26%. Yield of nut/plant does not correlate with nut and mace fat recovery. While myristic acid dominate in the nut of *M. beddomeii* and *M. prainii*, mace of *Myristica fragrans* contains high palmitic acid. Total amino acid content was highest in *M. prainii*. Among the amino acids, phenylalanine dominates in all *Myristica* species and *Knema andamanica* has high alanine and threonine. Fatty acids of nutmeg and mace and amino acids of leaf were evaluated to establish the variability among the species.

**Key words:** *Myristica*, amino acids, fatty acids, nutmeg, mace.

### INTRODUCTION

Nutmeg is a unique spice as it produces two economically important spices namely nutmeg and mace. *M. fragrans* Houtt. is native of the Eastern Moluccas. It belongs to the *Myristicaceae* family with about 18 genera and 300 species. The few species in the family are *M. andamanica*, *M. malabarica*, *M. magnified*, *M. amygdalina*, *M. beddomeii*, *M. prainii*, *Knema andamanica* and *Myristica attenuata* (Gamble, 2; Hooker, 5; Parkinson, 7; Anon, 1). It was introduced in India for quite a long time and is grown mainly in Kerala, Tamil Nadu, Karnataka and Maharashtra. Fruit is ovoid, subglobose or pyriform and 1½-2 inch long. The pericarp is yellow with the arillus red and lacinate (Gamble, 2; Hooker, 5). Dried nutmeg and mace of *M. fragrans* are used as spices. The essential oil (also called volatile oil) and the oleoresin are the major products of interest from the spice. Nutmeg and mace are stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuaries and forms a constituent of pharmaceutical preparations. Higher doses have a narcotic effect. Delirium and epileptic convulsions are found to occur. Maya *et al.* (6) has reported variability in the concentration of essential oil constituents of Indian nutmeg. It contains 25-40% of fixed oil and composed of mainly trimyristin with a high proportion of essential oil. The other major sources of trimyristin are coconut oil and palm kernel oil (Purseglove *et al.*, 9; Anon., 1). Studies by various groups have given different accounts regarding the proportion of fatty acids in cultivated and wild taxa of nutmeg. Very little information is available on the

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proportion of amino acids in the leaves of cultivated and wild taxa. The present study was undertaken for an in-depth study on the fatty acid and amino acid profile of cultivated and related taxa of nutmeg.

### MATERIALS AND METHODS

Samples of nutmeg (nine accessions), mace, rind and leaves of *M. fragrans* and related taxa *M. andamanica*, *M. malabarica*, *M. magnifica*, *M. amygdalina*, *M. beddomeii*, *M. prainii* and *Knema andamanica* were collected from the Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi. The fruits were harvested at full maturity and dried. Dried nut, mace, rind and semi-dried leaves were used for the study. Total free amino acids in nutmeg, mace and leaf samples was estimated by the ninhydrin method of Yapinlee and Takahashi (12). Leaf amino acids were separated following the HPLC method. Dry leaf samples were refluxed with alcohol. The extract was evaporated to dryness and then dissolved in the amino acid diluents. The separation of amino acids was carried out in a Shimadzu LC10A HPLC adopting the post column derivatization technique employing Shimadzu FLD6A fluorescence detector. The column used was Shimpack Na. Temperature of column oven was 55°C. Amino acids were separated using three different buffers by gradient elution technique and the separated amino acids were derivatized using O- pthalaldehyde. Fat from a known quantity of the seed was extracted with petroleum ether. The solvent was then distilled off completely, dried, weighed and the percentage of fat was calculated (Sadasivam and Manikam, 10). Fatty acids present in nutmeg, mace and rind samples of *M. fragrans* and

other related taxa were extracted by incubating the fat in NaOH-methanol at 70°C for 2 hours. Free fatty acids were converted to fatty acid methyl esters for GLC analysis by incubating in methanol-HCl (Hennessey *et al.*, 3). Methyl esters of fatty acids from fat were separated in a Perkin Elmer Auto system gas chromatograph equipped with PE Nelson 1022 GC plus integrator. Flame ionisation detector temperature was 300°C and that of injection port was 200°C. The column used was Carbowax 20 M and the column oven temperature was programmed at 80-190°C @ 24°C/minute with initial holding time of one minute. Fatty acid peaks were identified using authentic Sigma grade fatty acid methyl ester standards.

### RESULTS AND DISCUSSION

Nutmeg fat is mainly considered as trimyristin. In nut, the fat content ranged from 28 to 38%. In mace, it ranged from 23 to 26% (Table 1). Generally, nutmeg is valued based on the nut yield per plant. High yielding group contains plants which yield 2000 fruits/plant, medium yielding group give 2000-1000 nuts/plant and low yielding group yield 500 fruits/plant. Fat content indicate that it does not correlate with the number of fruits/plant. Table 2 illustrate the fat content in the nut, mace and rind of *M. fragrans* and related taxa. Among the wild species, *M. prainii* possesses 33% butter (fat) in nut and *M. malabarica* has 30% of mace fat. Myristic acid dominate followed by palmitic acid in nut (*M. fragrans*) while the pattern is different in mace (Table 3). In mace, palmitic acid dominated with 52.5% followed by another fatty acids (27.5%), which may be concluded as oleic acid. Prakaschandra and Chandrasekharappa (8) reported palmitic and oleic as the major fatty acids in mace fat. Earlier reports on nutmeg fatty acid (Anon, 1) describes a profile of lauric acid, myristic acid, palmitic acid, stearic acid, hexadecanoic acid, oleic acid and linoleic acid.

**Table 1.** Estimation of fat in nut and mace of *Myristica fragrans*.

Accession No.	Fat (%)	
	Nut	Mace
A4/12 - High yielding	32.8	23.7
A9/4 - High yielding	30.9	24.8
A9/86 - High yielding	33.8	26.4
A9/18 - Medium yielding	34.9	23.4
A9/28 - Medium yielding	29.0	26.1
A9/71 - Medium yielding	28.9	24.1
A9/74 - Low yielding	28.2	23.9
A9/102 - Low yielding	32.2	24.1
A11/25 - Low yielding	38.1	25.1

**Table 2.** Fat (%) content in nut, mace and rind of *Myristica* species.

Species	Nut	Mace	Rind
<i>M. fragrans</i>	37.52	24.49	0.83
<i>M. beddomeii</i>	2.46	NA	0.45
<i>M. malabarica</i>	9.75	29.54	NA
<i>M. prainii</i>	32.79	2.17	0.57

NA - not available

Verghese (11) reported 80.6% myristic acid, 7.8% oleic acid, 7.1% palmitic acid and 1.6% lauric acid in nutmeg. He has reported 90.6% of total fatty acids as saturated with only 8.7% of unsaturated fatty acids. Saturated fatty acids with even numbered carbon atoms, like C-16, C-18 carbon atoms (palmitic and stearic acids) are predominant in plants. Most plants contain significant amounts of myristic acid and some others predominate in capric or lauric acids (Harwood, 4).

Verghese (11) has reported oleic acid as the predominant fatty acid in mace of *M. fragrans*. It is followed by palmitic acid and linoleic acid. Thus, it has 38% of saturated fatty acids and 62.1% of unsaturated fatty acids. However, previous report (Anon, 1) describes a fatty acid composition similar to that of nutmeg. The differential composition of fatty acids in nutmeg and mace is reflected in the consistency and organoleptic properties of the fat. Mace fat by virtue of its high unsaturated fatty acid content has a lower melting point (Verghese, 11). Among the wild species, myristic acid concentration was rich in nut and rind of *M. beddomeii* and nut and mace of *M. prainii*. *M. malabarica* nut and mace contain stearic acid as the predominant fatty acid. *M. fragrans* rind contain about 50% fatty acid which may be palmitoleic acid. This is in unison with the previous study which state stearic acid as dominant fatty acid in *M. beddomeii* and myristic acid in *M. malabarica* (Anon, 1).

Total free amino acids in *M. fragrans* and related taxa indicate that *M. prainii* has the highest amino acid content and *M. magnifica* the lowest (Table 4). Using the HPLC technique 11 amino acids were separated. High concentration of phenylalanine was observed in *M. fragrans*. Among the related taxa; *M. magnifica* contains the lowest phenylalanine and *M. prainii* had the highest phenylalanine content. *Knema andamanica* contained highest alanine followed by threonine. The relatively low yield of essential oil by the wild nutmeg species may have some relation to the amino acid levels.

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**Table 3.** Fatty acid composition of nut, mace and rind of *Myristica* species.

Species	Lauric acid (%)	Myristic acid (%)	Palmitic acid (%)	Stearic acid (%)	Unidentified-1 (%)	Unidentified -2 (%)
<i>M. fragrans</i> - nut	8.00	55.10	14.87	7.30	2.52	2.36
<i>M. fragrans</i> - mace	1.31	8.11	52.56	7.98	NA	27.52
<i>M. fragrans</i> - rind	2.86	11.09	4.25	NA	50.00	NA
<i>M. beddomeii</i> - nut	4.76	78.20	6.28	0.55	NA	2.46
<i>M. beddomeii</i> - rind	9.07	38.34	22.17	5.40	2.91	NA
<i>M. malabarica</i> - nut	0.49	19.06	1.34	57.64	12.33	0.31
<i>M. malabarica</i> - mace	0.08	3.22	2.23	59.39	20.71	9.22
<i>M. prainii</i> - nut	1.90	61.39	1.43	9.38	6.33	-
<i>M. prainii</i> - mace	12.08	31.97	16.95	9.14	5.38	1.42

NA = Not available.

**Table 4.** Amino acid profiles in leaves of *Myristica* species and related taxa ( $\mu\text{g}/\text{mg}$ ).

Genotype	Aspartic acid	Threonine	Serine	Glutamic acid	Alanine	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine
<i>M. fragrans</i>	3.8	11.6	5.8	3.5	51.1	39.6	15.5	14.2	8.1	134.7	5.1
<i>M. amygdalina</i>	15.8	21.0	4.8	5.5	52.7	39.8	12.5	11.9	5.4	118.4	3.3
<i>M. andamanica</i>	4.2	21.4	7.5	2.8	55.8	61.4	23.1	15.3	36.9	64.3	3.4
<i>M. magnifica</i>	2.3	11.3	3.7	N.A	49.1	26.6	N.A	N.A	33.6	6.4	2.3
<i>M. malabarica</i>	11.6	18.2	3.8	8.6	60.7	34.8	9.6	9.1	19.9	119.6	1.1
<i>M. prainii</i>	2.2	7.0	4.8	1.6	39.3	30.6	12.5	13.1	4.8	207.7	9.5
<i>Knema andamanica</i>	40.6	139.2	16.1	38.5	269.1	58.5	20.8	18.5	17.4	88.6	5.5

NA = Not available.

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