

Fatty acids and leaf amino acids in *Myristica fragrans* and its wild taxa

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ABSTRACT

Myristica fragrans produces two spices, nutmeg and mace. Wild nutmeg species of importance are *M.anadmanica*, *M.malabarica*, *M.magnifica*, *M.amygdalina*, *M.beddomeii*, *M. prainii* and *Knema andamanica*. Fatty acids of nut and mace and leaf amino acids were evaluated to establish the variability among the species. Nut of *M.fragrans*, *M.beddomeii* and *M. prainii* are rich in myristic acid and mace of *M.fragrans* has high palmitic acid. *M.fragrans* and wild species except *Knema andamanica* leaf contains very high phenyl alanine. *K.andamanica* leaf is rich with aminoacids alanine and threonine.

INTRODUCTION

Myristica fragrans is a unique spice as it produces two economically important spices, nutmeg and mace. *M.fragrans*, Houtt. is native of the Eastern Moluccas. It is cultivated in the Malayan Peninsula, Penang and the Malay Islands. It belongs to the *Myristicaceae* family with about 18 genera and 300 species. Indonesia and Grenada are the major producers of nutmeg. Nutmeg was introduced in India for quite a long time. It is seen mainly in Kerala, Tamil Nadu and Karnataka. Though it is dioecious, male and female flowers are sometimes seen on the same tree (Purseglove *et al.*, 1981). Fruit is ovoid, subglobose or pyriform and 1½ - 2 inch long. The pericarp is yellow with the arillus red and lacinate (Gamble, 1967; Hooker, 1973). The other species in the family are *M.anadmanica*, *M.malabarica*, *M.magnifica*, *M.amygdalina*, *M.beddomeii*, *M.prainii* and *Knema andamanica*, Warb. *Myristica attenuata* (Gamble1967, Hooker1973, Parkinson 1972, Anonymous1962).

M.andamanica, Hook. F. is a slender handsome tree with slender horizontal branches seen in the Andaman Islands. Leaves are three to four inches in diameter and pale brown when dry on both surfaces. The leaves are pale silvery or coppery beneath.

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Fruit has the size and shape of a hen's egg. Pericarp is thick and brown with blood-red seed. (Parkinson, 1972; Hooker, 1973). *M.malabarica*, Lamk. is seen in the evergreen forests of Western Ghats, up to 1,000 ft. It is seen in the Konkan, Canara and North Malabar. It is a tree reaching 50 ft. in height with a diameter of 1.5 ft. The ripe fruits are called Bombay nutmeg and Bombay mace, which are used as adulterants of *Myristica fragrans*. They have no odour and taste. The aril is reddish yellow, irregularly lobed, lacinate and extending to the apex of the seed (Anonymous, 1962; Gamble 1967; Hooker, 1973). *M.magnifica*, Beddome Fl. Sylv. is found in the swampy ground in evergreen forests of Western Ghats, Travancore and parts of Tinnevely and Canara. It is an immense gregarious tree, 100 feet high. The fruit is oblong; up to four inch long, densely tomentose with a much deeply lacinate orange-red arillus. The red arils from seeds are used for dyeing purpose. The seeds yield oil that is used for burning and making candles (Anonymous, 1962; Gamble 1967; Hooker, 1973). *M.amygdalina*, Wall. is found in Tenasserim, Tavoy and Moulmein. It is a tall perfectly glabrous tree. Fruit is shortly peduncled and 1½ inch long. The pericarp is rather thin and glabrous. Aril is yellow and lacerate at the tip only (Hooker, 1973). *M.beddomeii* is seen in evergreen forests of Western Ghats up to 5,000 ft. It is a large tree reaching 90 ft. in height with a diameter of about 2.5 ft. Fruit is globose, 2 to 2.5 inch in diameter with a fleshy pericarp. The arillus is orange-red and lacinate with their ends separate. This tree has been used as a root stock for the vegetative propagation of *M. fragrans* (Gamble 1967; Anonymous, 1962). *M. prainii* is fairly frequent in semi-deciduous and evergreen forests with leaves of six to twelve inches long, elliptic to oblong. Flowers are small and fruit is ovoid. It is 1½ inch long with a thick pericarp with a red lacinated seed (Parkinson, 1972). *Knema andamanica*, Warb. *Myristica attenuata*, Wall. is also seen in the evergreen forests of Western Ghats up to 2,000 ft. The fruit is ovoid, 1.5 inch long and the aril has a brilliant crimson color (Gamble, 1967).

Dried nutmeg and mace are used as spices. The essential oil (also called volatile oil) and the oleoresin are the major products of interest from the spice. Nutmeg is a stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuaries; and forms a constituent of preparations for dysentery, stomachache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy. Higher doses have a

narcotic effect. Delirium and epileptic convulsions are found to occur. Mace is also used similarly. It is chewed for masking foul breath. It also prevents dental caries.

Nutmeg has the following composition. 14.3% moisture, 7.5% protein, 36.4% ether extracts, 28.5% carbohydrates and 11.6% fibre. Mace, on the other hand, has following composition: 15.9% moisture, 6.5% protein, 24.4% ether extract, 47.8% carbohydrate and 3.8% fibre. It has 1.6% of mineral content with 0.18% calcium, 0.10% phosphorous and 12.6 mg% of iron. It has 4 to 15% of essential oil, 25% amylopectin, reducing sugars, pectin and resins. The mace oil resembles nutmeg oil in odour, flavour and composition. Thus no distinction is made between them in trade (Anonymous, 1962).

Nutmegs contain 25-40 % of fixed oil, otherwise called *oleum myristiceae expressum*. It is a highly aromatic, orange-yellow coloured fat with the consistency of fat at room temperature. It has the odour and taste of nutmeg. It is composed of mainly trimyristin with a high proportion of essential oil. It is an ester of glycerol and tetradecanoic acid (myristic acid).

The other major sources of trimyristin are coconut oil and palm kernel oil. (Purseglave *et al.*, 1981, Anonymous, 1962). Studies by various groups have given different accounts regarding the proportion of fatty acids in cultivated and wild taxa of nutmeg. Similarly very little information is available on the proportion of amino acids in the leaves of cultivated and wild taxa. Maya *et al.*, (2004) has reported variability in the concentration of essential oil constituents of East Indian and West Indian nutmeg. This has prompted for an in- depth study on the fatty acid and amino acid profile of cultivated and wild taxa of nutmeg.

MATERIALS AND METHODS

Samples of nutmeg, mace, rind and leaves of *M. fragrans* and the wild species were collected from the IISR experimental farm Peruvannamuzhi. The fruits were harvested at full maturity and dried. Dried nutmeg, mace, rind and semi dried leaves were used for the study. Total free amino acid in nutmeg, mace and leaf samples was estimated by the ninhydrin method of Yapinlee and Takahashi (1966).

HPLC of leaf aminoacids: Dry leaf samples were refluxed with alcohol. The extract was evaporated to dryness and then dissolved in the amino acid diluent. 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 amino acids were separated using three different buffers by gradient elution technique and the separated amino acids were derivatized using O- phthalaldehyde.

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 & Manikam, 1992).

Fatty acids present in nutmeg, mace and rind samples were extracted by incubating in NaOH-methanol at 70⁰C for 2 hours. Free fatty acids were converted to fatty acid methyl esters (FAMES) for GLC analysis by incubating in methanol-HCl (Hennessey *et al.*, 1983).

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 (FID) temperature was 300⁰C and that of Injection port was 200⁰C. Column used was Carbowax 20 M. Column oven temperature was programmed at 80-190⁰C @ 24⁰C/ minute with initial holding time of 1 minute. Fatty acid peaks were identified using authentic Sigma fatty acid methyl ester standards.

RESULTS AND DISCUSSION

Nutmeg fat is mainly considered as trimyristin. Table 1 account the level of fat in nutmeg and mace of *M.fragrans*. In nutmeg the fat content range from 28 to 34%. In mace it range from 23 to 26%. Table 2 illustrates the details of fatty acid profile in the nut, mace and rind of *M.fragrans*. Myristic acid concentration dominate followed by palmitic acid in nutmeg while the pattern is different in mace. In mace palmitic acid dominate with 52.5% followed by another fatty acid of 27.5% which couldn't be identified. From the reports it may be concluded as oleic acid. Prakaschandra and Chandrasekharappa (1984) reported palmitic and oleic as the major fatty acids in mace fat.

Table 1. Estimation of fat in nutmeg and mace

No.	Accessions		Fat (%)	
			Nutmeg	Mace
1	A4 / 12	High yielding	32.8	23.7
2	A9 / 4	High yielding	30.9	24.8
3	A9 / 86	High yielding	33.8	26.4
4	A9 / 18	Medium yielding	34.9	23.4
5	A9 / 28	Medium yielding	29.0	26.1
6	A9 / 71	Medium yielding	28.9	24.1
7	A9 / 74	Low yielding	28.2	23.9
8	A9 / 102	Low yielding	32.2	24.1
9	A11 / 25	Low yielding	38.1	25.1

Table 2. Fatty acid compositions of nutmeg, mace and rind

	Laurate %	Myristate %	Palmitate %	Unidentified %	Stearate %	Unidentified %
<i>M. fragrans</i> – nutmeg	8.00	55.10	14.87	2.52	7.3	2.36
<i>M. fragrans</i> – mace	1.31	8.11	52.56	-	7.98	27.52
<i>M. fragrans</i> –rind	2.86	11.09	4.25	50.00	-	-

Earlier reports on nutmeg fatty acid (Anon, 1962) describes a profile of lauric acid, myristic acid, palmitic acid, stearic acid, hexadecanoic acid, oleic acid and linoleic acid. Verghese (2001 b) 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.

Verghese (2001b) has reported oleic acid (42.3%) as the predominant fatty acid in mace. It is followed by 30.7% of palmitic acid and 17.2% of linoleic acid. Thus it has 38% of saturated fatty acids and 62.1% of unsaturated fatty acids. However, previous

report (Anonymous, 1962) 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 2001 b).

Table 3. Fat content of different fruit parts in wild *Myristica*

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

Table 3 present the percentage of fat in nut, mace and rind of *M. fragrans*, and the wild species. *M. prainii* possess almost the same content of butter as in *M. fragrans* in nutmeg while *M. malabarica* had the same mace fat as that of *M. fragrans*.

Table 4. Fatty acid composition of fruits

	Laurate %	Myristate %	Palmitate %	Unknown %	Stearate %	Unknown %
<i>M. fragrans</i> – nutmeg	8.00	55.10	14.87	2.52	7.3	2.36
<i>M. fragrans</i> – mace	1.31	8.11	52.56	-	7.98	27.52
<i>M. fragrans</i> – rind	2.86	11.09	4.25	50.00	-	-
<i>M. beddomeii</i> - nutmeg	4.76	78.20	6.28	-	0.55	2.46
<i>M. beddomeii</i> - rind	9.07	38.34	22.17	2.91	5.40	-
<i>M. malabarica</i> – nutmeg	0.49	19.06	1.34	12.33	57.64	0.31
<i>M. malabarica</i> – mace	0.08	3.22	2.23	20.71	59.39	9.22
<i>M. prainii</i> – nutmeg	1.9	61.39	1.43	6.33	9.38	-
<i>M. prainii</i> – mace	12.08	31.97	16.95	5.38	9.14	1.42

Table 4 illustrates the fatty acid composition in the wild species. Myristic acid concentration was rich in nut and rind of *M. beddomeii* and nut and mace of *M. prainii*. *M. malabarica* nutmeg and mace contain stearic acid as the predominant fatty acid. *M. fragrans* rind contain about 50% fatty acid which couldn't be identified. It may be palmitioleic acid. This is in contrast to the previous study which state stearic acid as dominant in *M. beddomeii* and myristic acid in *M. malabarica*.

Table 5. Level of total free amino acids in leaves of wild taxa

No	Acc No	(µg/100mg)%
1	<i>M. fragrans</i>	139.64
2	<i>Knema andamanica</i>	198.43
3	<i>M. amygdalina</i>	135.28
4	<i>M. andamanica</i>	179.01
5	<i>M. beddomeii</i>	174.66
6	<i>M. fatua</i> var. <i>magnifica</i>	127.65
7	<i>M. malabarica</i>	148.39
8	<i>M. prainii</i>	215.21

Mean 164.78 ±31.90; 132.88 -196.68

Table 6. Amino acid profiles of leaves of wild taxa

Amino acid (µg/mg)	Asp	Thr	Ser	Glu	Ala	Val	Ile	Leu	Tyr	Phe	His
<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>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
<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. fatua</i> var <i>magnifica</i>	2.3	11.3	3.7		49.1	26.6			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

Using the HPLC technique 11 amino acids were separated (Table 6). Total free amino acids in *M.fragrans* and wild taxa (Table 5) indicate *M.fatua* variety *magnifica* as the lowest and *M.prainni* having the highest amino acid content. In the amino acid profile *M.fragrans* contain very high concentration of phenyl alanine (Table 6). While *M.magnifica* the lowest phenyl alanine. Among the wild, *M.prainni* had the highest phenyl alanine. *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.

REFERENCES

- Anonymous 1962 The wealth of India – a dictionary of Indian Raw materials and Industrial Products. Volume VI. L-M, CSIR, New Delhi. pp. 473-480.
- Gamble J S 1967 Flora of the Presidency of Madras. Volume II. Botanical Survey of India. pp. 848-851.
- Hennessey T M, Andrew D and Nelson D L 1983 Biochemical studies of the excitable membrane of *Paramecium tetraurelia* VII. Sterols and other neutral lipids of cilia. *Journal of Lipid Research*. Vol. 24 (5) : pp. 575-587.
- Hooker J D 1973 Flora of British India. Periodicals experts, Vivek Vihar, New Delhi-32.p.102.
- Maya K M, Zachariah T J and Kishnamoorthy B 2004 Chemical composition of essential oil of nutmeg accessions. *J. of Spices and Aromatic Crops* 13 (2):135-139
- Parkinson C E 1972 A forest flora of The Andaman Islands – an account of trees, shrubs and principal climbers of the Islands. Iayyed Press, Ballimaran, New Delhi. Pp. 223-224.
- Prakaschandra and Chandrasekharappa (1984)
- Purseglove J W, Brown E G, Green C L and Robbins S R J 1981 Spices. Vol. I. Longman, London.
- Sadasivam S & Manikam A 1992 Biochemical methods for agricultural sciences. Wiley Eastern Limited, Madras.pp. 1-246.
- Verghese J 2001b Nutmeg and Mace – III. Nutmeg and mace butter. *Spice India*, June, 14-16.

Yapinlee and Tunekazu Takahashi (1966). Estimation of free amino acids, *Analytical Biochemistry*, 14, 71-77.