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MORPHOLOGICAL AND ESSENTIAL OIL CHARACTERIZATION OF THE BANDA NUTMEG (*Myristica fragrans* HOUTT.) OF MOLUCCAS AND NORTH MOLUCCAS ECOTYPES

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ABSTRACT

An agronomic study is conducted to characterize morphological and essential oil properties of Banda nutmeg (Myristica fragrans Houtt.) of Moluccas and North Moluccas ecotypes. In Moluccas, three sampling sites are chosen: Banda islands, Ambon and Luhu; and in North Moluccas, the sites are Ternate, Tidore and Bacan. Twenty-one morphological characters of nutmeg trees are studied and the similarity index (SI) was calculated. For nutmeg production observations, two elevations are taken: 0 to 50 m above sea level (asl) and 250 to 300 m asl. Nutmeg oils in seed and mace are extracted by using hydro-distillation method and the resulted oils then analyzed and identified for their essential oil components by GC-MS (Gas Chromatography-Mass Spectrometry). In oil extraction, two maturity levels of seeds are used: 3 to 5 months (immature) and > 7 months (mature). Results reveal that 90% of the morphological traits observed are stabile in the six sites within the two ecotypes. The oil contents in mature seeds are 11.2 and 8.73% for Moluccas and North Moluccas, respectively. In immature seeds, however, the oil contents are much higher, i.e. 12.39% for Moluccas and 12.11% for North Moluccas. Higher oil contents are found in mace at the levels of 20.04% for Moluccas and 20.84% for North Moluccas. GC-MS analysis shows that the Banda nutmeg oil contains 28 to 31 different essential oil components. Four of them having economically important values are myristicin, elemicin, safrole, and eugenol. In conclusion, Banda nutmeg has the highest content in myristicin, i.e. 13.76%.

Key words: ***Banda nutmeg, hydro-distillation, GC-MS, essential oil***

INTRODUCTION

Nutmeg tree (*M. fragrans* Houtt.) is native to Moluccas in eastern part of Indonesia, known as the Spice Islands [1]. The tree has spread out and is cultivated for commercial purposes in other islands in the East Indian archipelago, Malaysia and in Carribean area, notably Grenada. Nutmeg growing areas in Indonesia exist in 14 provinces. In 2002, Indonesia produced approximately 20 thousand tones nutmegs in the area of 60.6 thousand ha [2]. Six nutmeg-producing countries in the world are Indonesia, Grenada, Sri Lanka, Trinidad, China and India [3]. Seventy-six percent of the world's nutmeg trades comes from Indonesia, 20% from Grenada and the remainder is shared by Sri Lanka, Trinidad and Tobago [4]

Characteristics of the nutmeg tree can be identified through their morphological and biochemical characteristics. These characters might vary depending on species and ecological factors, such as climatic and soil conditions.

There are three economically important products derived from *M. fragrans* Houtt. : shelled dried seed (nutmeg), aril or mace and nutmeg oil. The first two are commonly used as spice or condiment to flavor foods, pickles, sauces and puddings. Nutmeg oil is a component that can be extracted mainly from seed and mace by using steam or hydro-distillation method. Besides used for pharmaceutical purposes, Nutmegs oils is also utilized as a food preservation and for bioassay [5].

The first comprehensive analysis of the nutmeg essential oil is made by Power and Salway. Depending on the origin of the nutmegs, they contain between 5 to15% essential oils and 24 to 40% fixed oils [6]. The residual 45 to 60% of the nutmeg consists of solid matter including cellulose. The essential or volatile oil of nutmeg contains approximately 80% monoterpenes, 5% monoterpene alcohols, an aromatic ether fraction and small quantities of miscellaneous compounds [7].

The experiment aims to characterize morphological and essential oil properties of Banda nutmeg that originates in two ecotypes in Moluccas.

MATERIALS AND METHOD

Sampling sites and morphological characterization

For morphological observation, two different ecotypes are chosen, Moluccas and North Moluccas. In Moluccas ecotype, three sampling sites are selected, namely Banda islands, Ambon, and Luhu on

Seram island. In North Moluccas, the selected sites are Ternate, Tidore and Bacan. The study was performed during 2004 through 2005 when the nutmeg plants are in reproductive phase, which is from February to September.

Twenty-one morphological characters of the selected nutmegs are identified by using trees population having about 25 to 50 cm in circumference. Ten trees are randomly selected from the population in each sampling site for morphological identifications. The morphological characterization is accomplished following the procedure of the Tropical Fruit Descriptors [8]. For production, observations are conducted at two different levels of altitute: 0-50 m asl and 250-300 m asl. Nutmeg production is taken twice during the year. In addition, ecological observations of the sites are also conducted, including the description of the soil and climatic conditions.

Extraction of essential oil

The samples of nutmegs consisting of dried seeds at two maturity levels and dried mace are placed in a flask, 200 ml distilled water added, and the hydro-distillation carried out for 2 h. The distillate is collected in 0.75-ml *n*-hexane. Octadecane is added to the samples to arrive at a final concentration of 20 g mg^{-1}. Oils obtained are dried over anhydrous sodium sulfate and stored at room temperature for later use [9].

Physico-chemical analyses

Physico-chemical analysis is performed according to the procedure described by Atti-Santos *et al.* [10] by using five parameters: specific gravity (SG), optical rotation (OR), refractive index (RI), solubility in ethanol (SE), and residue on evaporation (RE).

SG is measured with Anton Paar Modelo DMA23N densitometer, and OR measured with CETI Polaris polarimeter. Solutions of 40 g L^{-1} of the essential oil samples mixed in analytical grade chloroform (Merck). The readings are made with 10 cm dm tubes at 20°C. RI is measured with a CETI Quartz refractometer, at 20°C. SE is accomplished by complete solubilization of 1 mL of essential oil in 90% dilute ethanol, at 20°C. RE is measured by adding 3 g of essential oil sample in a petry dish, evaporating in boiling water bath, cooling in a desiccator, until two successive weights give a difference of less than 0.1%.

GC-MS analysis

GC-MS analysis follows the procedures described by Masada [11]. The analysis is carried out on a QP5000 Class-5k (Zhimatsu) by using DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness); helium as the carrier gas, flow rate of 1 mL min^{-1} and with split ratio 1:30. The injector temperature and detector temperature is 225 °C and 230°C, respectively. The column temperature is programmed from 35°C to 180°C at 3°C min^{-1} and then from 180°C to 225°C at 10°C min^{-1}. Mass spectra is recorded from 30 to 450 *m/z*. The oil components are identified by comparison of their retention indices with retention times of known compounds and also by comparison of the mass spectra published in the literature. Further identification of the separated essential oil components is also carried out by using the library of the essential oils (Lib #1 and #2) and through searching in website www.chemfinder.com.

Statistical analysis

Variance stability of the morphological characters of the plants is analyzed by using Bartlett's t test; similarity index (SI) of the characters is calculated and SI is then subjected to cluster analysis by using Minitab software [12]. Data on nutmeg productions is analyzed with ANOVA by using SAS, and a test of mean comparison is also applied by using Dunnett's t test [13].

RESULT AND DISCUSSION

Morphological traits

Cluster analysis of the morphological characters from sixty nutmeg trees (Fig. 1) shows that 90% of the trees is stabile in phenotypes (similarity index, SI= 0.9) across the six sites within the two ecotypes. The remaining 10% form two slightly different groups with respective members of four and two trees. These two groups of nutmegs are trees originating from Luhu (Moluccas ecotype) and Ternate (North Moluccas). These groups have similarity in some characteristics, but significantly different from other group.

Nutmeg production

In Moluccas ecotype, the number of fruit, fresh weight of fruit, and dry weight of mace show no significant differences at the two elevations except the fresh and dry weight of seeds. Similarly, the results reveal the same patterns in the production parameters for North Moluccas ecotype.

Fruit, seed, and mace productions of nutmeg at the two elevations originated in Ambon, Ternate, Tidore and Bacan are significantly different compared to those from Banda. Production of nutmegs (seeds and mace) of Banda is higher than that from other sites. Generally, no significant difference was found between productions in both elevations. However, seed productions show significant differences between in Ambon and Luhu and in Ternate and Tidore.

Oil contents and its essesntial components

Oil content of the seed and mace of the Moluccas nutmegs is different from that of North Moluccas. Oil content in the nutmeg seeds taken from Banda is higher than that of from other locations, except from Ambon. In mace, nutmegs from Banda contain oils in the same level as those of from Ambon, Ternate, Tidore and Bacan.

Essential oil contents in the Moluccas nutmeg oils are different from those of North Moluccas. Myristicin content, the most important component of essential oils in nutmeg, varies and dependes upon locations (ecotypes) where the nutmegs grow. Nutmegs originating in Banda contain the highest level in myristicin, i.e. 13.76%.

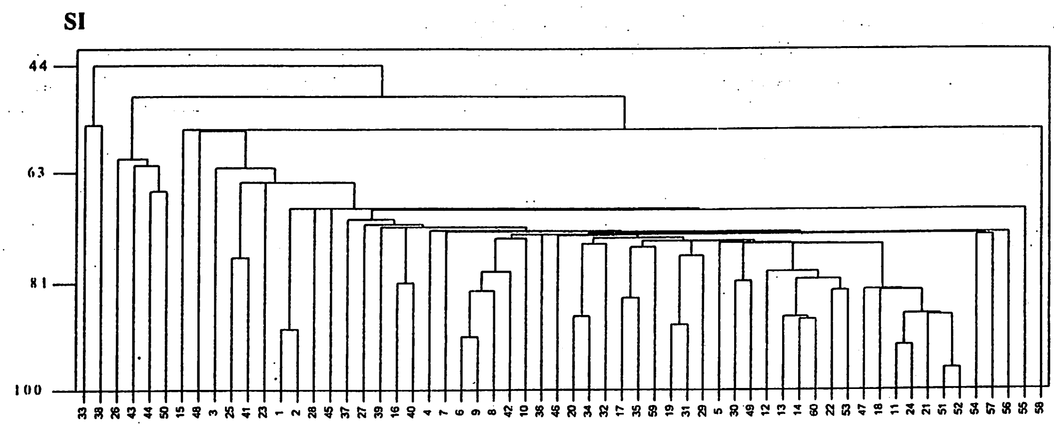

Figure 1. Dendogram for the morphological characters of the Banda nutmegs.

Table 1. Nutmeg production at the two elevations in two ecotypes

Ecotype/Site	No. fruit		Fruit FW (kg)		Seed FW (kg)		Seed DW (kg)		Mace DW (kg)	
	elev-1	elev-2	elev-1	elev-2	elev-1	elev-2	elev-1	elev-2	elev-1	elev-2
Moluccas:										
Banda	2,648 a	2,336 a	167.16 a	147.67 a	23.58 a	20.75 a	13.99 a	12.27 a	2.51 a	2.28 a
Ambon	2,145 a,*	1,820 a,*	100.65 a,*	85.24 a,*	16.19 a,*	13.80 b,*	9.60 a,*	8.18 b,*	1.61 a,*	1.39 a,*
Luhu	2,109 a,*	2,111 a,ns	120.21 a,*	118.43 a,*	15.44 a,*	15.56 a,*	9.16 a,*	9.22 b,*	2.10 a,*	2.07 a,*
North Moluccas:										
Ternate	1,720 a,*	1,631 a,*	73.35 a,*	69.50 a,*	10.68 a,*	10.12 a,*	6.33 a,*	6.00 b,*	0.72 a,*	0.69 a,*
Tidore	1,764 a,*	1,795 a,*	70.68 a,*	71.95 a,*	10.59 a,*	10.66 a,*	6.28 a,*	6.34 b,*	0.69 a,*	0.70 a,*
Bacan	1,814 a,*	1,794 a,*	89.17 a,*	88.11 a,*	12.62 a,*	12.52 a,*	7.48 a,*	7.43 a,*	1.15 a,*	1.13 b,*
Dunnett's $T_{0.05}$	*335*	*357*	*28.24*	*26.37*	*3.67*	*3.68*	*2.18*	*2.19*	*0.63*	*0,64*

Figures followed by the same letter are not significantly different at α=0,05 (comparisons are made between elev.1 and elev. 2 for the individual parameters).

* Significantly different at α=0.05 (All means in each coloum are compared to the control Banda); ns:not significant; elev: elevation; FW: fresh weight; DW: dry weight.

Table 2. Oil contents of nutmeg and their essential oil components

Ecotype/Site	Oil Content			Selected Essential Oil Component			
	Mature Seed	Immature Seed	Mace	Miristicin	Elemicin	Safrole	Eugenol
Moluccas:		(%)			%		
Banda	11.69	13.07	21.00	13.76	0.94	2.44	0.90
Ambon	11.92 ns	12.82 ns	20.42 ns	13.54	0.67	2.19	0.55
Luhu	9.99 *	11.27 *	18.69 *	5.70	2.05	0.97	0.70
North Moluccas:							
Ternate	7.95 *	13.32 *	21.98 ns	11.42	7.21	3.37	1.80
Tidore	9.61 *	11.99 *	21.01 ns	5.97	3.56	1.49	9.82
Bacan	8.64 *	11.03 *	19.53 ns	11.30	6.71	2.57	1.77
Dunnett's $T_{0.05}$	*0.46*	*1.70*	*1.87*				

* Significantly different at α=0.05 (All means are compared to the control Banda); ns: not significant;

Table 3. Physico-chemical properties of the distillated nutmeg oils

Ecotype/Site	SG (g ml^{-1})	RI	OR (°)	ER (%)
Moluccas:				
Banda	0.906	1.490	+6.3	0.7
Ambon	0.897 ^{ns}	1.491 ^{ns}	+13.0 *	0.8 ^{ns}
Luhu	0.909 ^{ns}	1.489 ^{ns}	+11.4 *	0.9 ^{ns}
North Moluccas:				
Ternate	0.910 ^{ns}	1.486 ^{ns}	+21.5 *	1.0 ^{ns}
Tidore	0.884 ^{ns}	1.491 ^{ns}	+40.0 *	0.9 ^{ns}
Bacan	0.909 ^{ns}	1.488 ^{ns}	+18.2 *	0.9 ^{ns}
SNI standard	0.876-0.919	1.488-1.495	(+8) – (+26)	≤ 3.0
Dunnett's $T_{0.05}$	*0.03*	*0.01*	*1.38*	*0.60*

Notes: SG, specific gravity; RI, refraction index; OR, optical rotation index; ER, evaporation residual.
All means in each coloum are compared to the control Banda.

Physico-chemical properties of the nutmeg oil

The specific gravity (SG), refraction index (RI) and evaporation residual (ER) of the nutmeg oils from Banda are not significantly different from those of other locations. The optical rotation index (OR) of nutmeg oil from Banda, however, is significantly different compared to that of from the remaining sites. Nevertheless, the four physico-chemical characteristics of the nutmeg oil are still in an acceptance range of the SNI standard. These accepted values of physico-chemical properties of the nutmeg oils are important for agro-industry and trade purposes [14].

The stability in morphological characteristics or phenotypes showed by Banda nutmeg in the six locations in the two ecotypes is anadvantage in the context of agronomy and quality of nutmeg production. In addition, this phenomenon verifies that genetic stability in Banda nutmeg is highly conserved although the plant is open-pollinated. The conserved features in morphology of the trees are probably determined by the homogeneity in the nutmeg population where almost 100% of the nutmeg plants in Moluccas and North Moluccas belongs to *M. fragrans* species. Nevertheless, intercross between Fragrans species and other species of nutmeg is possible. Few changes in the morphological characters have occured within the nutmeg trees in North Moluccas ecotype. These changes had been observed by Hadad and Hamid [15] in the same ecotype. Although the characteristics of the morphology of the nutmeg are not fully stabile in the environment, plant morphology is still widely used as classical characterization method [16].

In average, nutmeg production in Banda islands is much higher than that in the remaining sites. Type and properties of the ecotypes studied are considered affecting the plant stability and the nutmeg production. The similar effects of the ecotype on nutmeg were also reported by Flamini *et al.* [17] on *Rosmarinus officinalis* L. in two ecotypes.

Hydrodistilled nutmeg oil of Tidore-origin nutmegs shows pale yellow color, while others are colorless with a characteristic odor of nutmeg. Sometimes, the color of nutmeg oil is yellow to pale green. The colors of distilled nutmeg oil strongly correlate to the thermo-stability of the nutmeg oil during distillation process. Changed color of nutmeg oils could be potentially caused by higher content of safrole [18]. Oil content in the seed between Banda origin and others is the same level, except for nutmeg from Ambon. Oil content in mace, however, is similar to that from both ecotypes, except from Luhu (see Table 2). In the context of the essential oil components, distillated nutmeg oil from Banda's nutmegs has the higherst content in miristicin (13.76%), but relatively low in saprole (2.44%) and eugenol 0.90%). In addition, nutmeg grown in Ternate has a high level in safrole (3.37%). Contents of the essential oil of nutmeg are dependent on the ecotype and the species. In terms of physico-chemical properties, nutmeg oils from Moluccas and North Moluccas generally have SG, RI, ER, and OR indices within an acceptance range of the SNI standard. However, nutmeg oil originated in Tidore shows a higher value of OR (+40.0). Higher index in OR is also reported by Purseglove *et al.* [1].

Apparently, the pale yellow color of the nutmeg oil originated from Tidore ecotype related to the higher OR value. This phenomenon is also found by other researchers [19,20].

CONCLUSION

1. Banda nutmeg in both Moluccas and North Moluccas ecotypes shows high stability in its morphological phenotypes;
2. Oil content in the seed and mace of the nutmeg is ecotype-dependent;
3. Physico-chemical properties of the nutmeg oils from Moluccas are still suitable for international trade purpose; and
4. Nutmeg originated in Banda islands contains myristicin component at the highest level.

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