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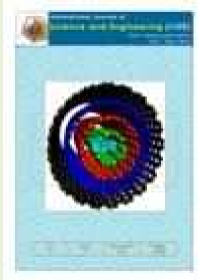

Physico-Chemical Characterization Of Maluku Nutmeg Oil

Ilyas Marzuki *,[1] Bintoro Joefrie [2] Sandra A. Aziz [2], Herdhata Agusta [2], Memen Surahman [2]

[1] Faculty of Agriculture, Pattimura University,Campus Poka, Ambon 97233
[2] Department of Agronomy, Faculty of Agriculture, Bogor Agriculture University, Darmaga, Bogor

* Corresponding author, tel/fax: 0911-322626, e-mail: marzuki64@gmail.com

Abstract - The essential oil of Banda nutmeg was extracted using hydro-distillation method to characterize their essential oil and volatile components. Banda, Ambon, and Luhu nutmegs were chosen as sample ecotypes. Nutmeg oils were subjected to physico-chemical and GC-MC analyses. Results indicated that nutmeg from the three ecotypes produced uncolored oils with the contents in mature seed were 11.69, 11.92, and 9.99%, respectively. Meanwhile those of immature seeds contained 13.32, 11.99, and 11.03% respectively. Furthermore, the physico-chemical of the oil are specific gravity 0.897 to 0.909 g/ml; refraction index, 1.489 to 1.491; and optical rotation, $+11.4^0$ to $+16,3^0$.GC-MS analysis suggested that essential oils Maluku nutmegs composed of 28 to 31 components and also showed that nutmeg from Banda comprised 52.8% monoterpene hydrocarbon (MH), 21.11% oxygenated monoterpene (OM), and 18,04% aromatic compound (AC); Ambon's 45.12% MH, 24.51% OM, and 16.97% AC; and Luhu's 56.06% MH, 27.34% OM, and 13.62% AC. Further analysis indicated that there were four important volatile oils fractions in nutmegs i.e. myristicin, elemicin, safrole, and eugenol. Maluku nutmeg contain 5.57 to 13.76% myristicin and 0.97 to 2.46% safrole. In conclusion, nutmeg oil shows a high stability in all physico-chemical properties. Nutmeg from Banda ecotype has the highest content in myristicin.

Keywords: *Banda nutmeg; ecotype; essential oil; myricticin.*

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Introduction

Nutmeg, *M. fragrans* Houtt (Myristicaceae) is an annual spice tree of Banda islands, Maluku. Nutmeg trees produce fruit containg seed and mace, which covers the seed. Most of the maces are red in colour, however, mace which yellow is also found specifically from the 'Holland" cultivar. Seed and mace of the nutmeg fruit are parts, which contain oil in a higher quantity in comparison to that of other parts of the tree.

Nutmeg oil is usually obtained through either steem destilation, water destilation or a combination of the two procedures. Generally, oil content in the nutmeg seed is in the range of 5 to 15% [1]. Nutmeg oil is clear, pale yellow or pale green in colours with specific nutmeg aroma. Nutmeg oil is sensitive to light and temperature, soluble in alcohol but not in water. Aside of oil, nutmeg also contain fat oil (or fixed oil), which is also known as oleoresin or nutmeg butter. Oleoresin mainly composed of trimyristin acid [2].

Nutmeg products (seed, mace, and essential oil) generally are grouped into two different categories; East Indian nutmegs and West Indian nutmegs. Indonesian nutmegs belong to the first group, while those of Grenada belong to the second. The two types are different in a number of characteristics including aroma. East Indian nutmegs are superior than that of West Indian type in that they produce better fragrans and have a higher ether phenil propanoid content (Masada, 1996) as well as terpene content [3]. East Indian nutmegs oil are reported to contain a high myristine content, as high as 13,5%, while those of West Indian contain less than 1% myristine content.

Physico-chemical characteristics of nutmegs oil generally are different between East indian type and West Indian type as reported by Guenther [2]. East Indian and West Indian nutmeg types have a similar specific grafity and refractive index, but noticebly different in the optical rotation, ester's number, solubility in 90% alcohol solution. The range of optical rotation of East Indian nutmegs are wider than that of West Indian, similar to that of ester's number.

Nutmeg's oil can also be obtained through extraction from mace and leaves. Mace can produce 4 to 17% oil while leaves can produce oil in a very low content of 1,7% [4]. The oil content in the leaves are less than 1%, and therefore, destilation of nutmeg oil through leaves is not economical [1]. Nutmeg's oil produced from leaves, eventhough, chemically similar to that produced from seeds and maces, have lower taste and fragrans qualities [1]. Quality standard of nutmeg's oil is regulated by National Standardization Body (NSB), which determine Indonesian National Standard (SNI), with SNI number of 06-2388-19998 [4]. SNI is the guide for the quality standard used to ensure commercial nutmeg's oil as shown in Table 1.

Table 1. The quality requirements of the Indonesian nutmeg's oil according to Indonesian standars (SNI)

Quality component	Designation	Requirement
Specific grafity, 20 °C	g/ml	0.876 - 0.919
Refractive Index. 20 °C/20 °C	-	1.488 - 1.495
Optical rotation, 20 °C	degree	(+8) - (+26)
Solubility in 90% ethanol	%	1:3 clear, further clear
Evaporating residue (Example 4.8 - 5.2 g)	%	Maximum 3%
Fat	-	negative
Additional alcohol	-	negative
Pelican oil	-	negative
Terpentin oil	-	negative

Nutmeg's oil contain several essential components, most of them are quite high grade industrial compounds. Components of nutmeg essential oil have pharmacological characteristics, and therefore, nutmeg's oil is used further as antibacterial compounds [5,6,7,8], as well as in the examination of insulin activity and metabolism.

Materials and Methods

Nutmeg's oil was extracted from the seeds obtained at two different harvestable stages, which were 3 to 5 months and more than 7 months. Samples were taken from three ecotypes: Ambon, Banda and Luhu.

Destilation procedure

Nutmeg's oil destilation was accomplished by the use of hydro-destilation method utilizing heating erlemeyer with the capacity of 500 ml. Procedure implemented in the nutmeg's oil destilation was similar to that described by [9].

Physico-Chemical analysis

Destillated nutmeg's oil was used as the materials in physico-chemical analysis. The analysis was accomplished in the laboratory of BALITTRO, Bogor. The physico-chemical characteristics of nutmeg's oils were determined based on the procedure produced by Indonesian National Standardization Body [4].

Identification and characterization of components in the nutmeg's oil were accomplished through the use of GC-MS OP500 17A Shimadzu. A sample of 1 µl oil obtained from water destilated nutmegs was injected into the GC column and caried by helium vapor. The collumn is made of methyl xilaksan with a diameter of 0.25 µ and 30 meters in length. The sample was moved within the column carried by helium vapor with the current speed of 1 meter per second.

GC-MS Analysis

The GC-MS data were analized using 5k class program, in which each chromatographic peak produced by the analysis was the representation of one essential compound, identitified by comparing it with the library of the essential compound available. The chemical identity of every compound was than compared with two different checks of standard chemical compound library, library #1: NISST62.LIB-20 and #2: NIST12.LIB-20. After fitting to the chemical compound library, the identity of the tested essential oil components were than presented, which include similarity index, molecular weight and molecular name.

Results and Discussions

Content of mature nutmeg seed is varied from 7.95 up to 11.92%. The nutmeg oil obtained through destilation of nutmeg seeds which are harvested at two different stages of maturity are shown in Table 2 below.

Table 2. Essential oil content (%) of the nutmeg seeds.

Ecotype		Mature seed	Immature seed
1.	Banda	11.69 ns	13.07 ns
2.	Ambon	11.92ns	12.82 ns
3.	Luhu	9.99*	11.27 *
	Dunnett =	0.46	1.70

Note: * Significantly different based on Dunnett test at α=0.05; ns not significantly different.

Nutmeg Oil Characteristics

Physico-chemical characteristics of nutmeg's oil which generally described are specific grafity, refractive index, optical rotation, and evaporating residue. Specific grafity of the nutmegs show no significant change in three locations examined. The specific grafity of the nutmeg's oil are between 0.884 and 0.909 g/ml. These values agree with the SNI for nutmegs oil. It is shown that variation of the value of specific grafity is very small between and within the ecotypes.

Table 3. Physico-chemical properties of the nutmeg oil.

	Ecotype	Specific grafity	Refractive Index	Optical Rotation
1.	Banda	0.906	1.490	+16.3
2.	Ambon	0.897 ns	1.491ns	+ 13.0*
3.	Luhu	0.909 ns	1.489 ns	+ 11.4*
	Dunnett =	0.03	0.01	1.38

Note: * Significantly different based on Dunnett test at α=0.05; ns not significantly different.

Refractive indices of the Maluku nutmeg's oil show small variation in their values. Statisticly, refractive index of nutmeg's oil of Banda ecotype is similar to that of other ecotypes. Within Maluku ecotypes, the refractive indices of nutmeg's oil are ranged from 1.489 to 1.491 with the average of 1.490. Different than that of refractive index, optical rotation of nutmeg's oil of Banda ecotype (16.3) statistically shows significant difference in comparison to that of other ecotypes. Maluku nutmeg's oil show optical rotation with the values ranged from 13.00 and 16.30. The average values of optical rotation is 13.30. Positive signs indicating the right turing of optical rotations.

Analysis of GC-MS of Maluku nutmegs indicate that Banda nutmeg's oil is composed of 28 volatile components. These components consist of 52.8% hydrocarbon monoterpene (HM), 21.11% oxigenated monoterpene (OM), 18.04 arometic fractions (AF) and 7.27% others. The most encountered essential's component is myristicin with the value of 13.76%.

Chromatogram analysis of Banda nutmeg's oil show 28 peaks in which, every peak represent one volatile component. Several peaks are not identified (not numbered). The unidentified peaks are the dirts, which are other compounds than the essential components. Analysis indicates that pinen (15.30%) is the main compound composing HM fraction followed by osime (10.22%) and mirse (3.42%). OM components, whose identity detected are terpinolene (1.54%) and eukaliptol (1.76%). Myristicin is a component, which compose aromatic compound with the highest content (13.76%) followed by safrole (2.44%). In nutmeg of Banda ecotype found myristate acid in a consentration of 6.55%.

The results of GC-MS chromatogram analysis of nutmeg's oil of Banda ecotype show 28 peaks, in which every peak represent one essential component. Wide to hight ratio of the peaks are proportional to the content of each essential component in nutmeg's oil. A number of peaks are not identified (not numbered). The unidentified peaks are the dirts, which are other compounds than the essential components.

Similar to that of Banda ecotype, Ambon ecotype nutmegs contain volatile components which are almost similar in kind except that the amount are 31 compounds. GS-MS analysis show that Ambon ecotype nutmeg's oil is composed of 45.12% MH, 24.52% OM, 16.97% AF and 11.75 other compounds. The greatest component found in the essential oil of Ambon ecotype nutmegs, which are identified are pinene (14.70%), followed by myristicin (13.54%). Monoterpene hydrocabon component in nutmegs of Ambon ecotype mainly composed of pinen (17.08), osimen (7.8%), and terpenine (4.6%). Meanwhile, aromatic fraction mainly is myristicin.

Nutmegs of Ambon ecotype are also rich in miristic acid (that is a component composing oleoresin fatty acid, which is important in the formation of nutmeg's butter (fixed oil).

Essential oil of Luhu ecotype nutmegs contain 56.06% of MH, 27.34% of OM, 13.62% of AF and the remain 11.70% of acid compounds (Table 4). Among the HM, pinene is the dominan one with 17.43%. Meanwhile, in the group of OM, $C_{10}H_{18}O$ is the highest in concentration. In the group of aromatic compounds, myristin (5.57) has the hignest concentration observed in the essential components of nutmeg's oil of Luhu ecotype.

GC-MS analysis revealed that nutmeg's oil of Maluku contain 4 to 6 aromatic compounds. Within the aromatic component of Banda nutmegs found the most important are myristin, elemicine, safrole, and eugenol.

A significantly different content of thearomatic compound of the three ecotypes showed by the (iso) eugenol content, which ranged from 0.55% to 0.90%. Aromatic fractions present in essential oil arethe second highest component after hydrocarbon monoterpene and oxigenized monoterpene. Aromatic fractions, specifically aromatic ether is the component responsible for the fragrans aroma of the nutmegs oil.

Nutmeg oil content is generally determined by species (genetic factors), plant age, ecological conditions (climate and soil fertility), the extraction method used, and the cultivation practices. A study by [10] on *Rosmarinus officinalis* in two ecotypes found that the characteristics of essential oils produced in different geographical locations or ecotypes, in which the plants grow,were varied. Similar conclusionswerealso obtained by [11] and [12]. Moreover, [13] reported that redberry oil content was higher in the Summer than Winter, Spring, and Fall. In addition to ecological factors or geographic location, nutmeg's oil production is also determined by the cultural practices applied. Cultivation of nutmegs in Maluku has been done without fertilization and therefore, increasing the content of nutmeg's oil through the application of chemical fertilizers may be possible. In addition, other ecological factors such as soil fertility can also cause the difference in the content and quality of essential oil. It is known that Banda, Ambon, and Luhu are located in a region characterized by soil with a quite high fertility levels (high in organic carbon, total nitrogen, and phosphorous). In addition, the difference in seed weight may be another factor that can lead to differences in oil content.

The study concluded that the chemical composition of essential oils related toclimatic conditions, growing season, geographicc condition and land fertility, harvesting prosedure, and distillation techniques used. Essential oils are generally composed of various mixtures of chemical compounds formed by the elements of carbon, hydrogen, oxygen, and other elements such as nitrogen and sulfur [14]. In general, there are three constituent groups in the volatile components of nutmeg's oil: hydrocarbons, oxygenated hydrocarbons, and aromatic compounds. Hydrocarbons mostly are of terpene compound; oxygenated hydrocarbons composed mainly of compounds that contain alcohol, ketone, or ester, whereas the aromatic compounds, which responsible for the distinctive aroma of nutmeg, consist of aromatic ether compounds.

Maluku nutmeg contains 28 to 31 components in the oil. Thee components are mainly composed of monoterpene hydrocarbons (45.12 to 56.06%). Monoterpenes found in the nutmeg are the dominant volatile components. [15] reported that nutmeg's oil can contain up to 80% monoterpene components. Geographical location is reported related to the variation of oil composition of the volatile components [16] and organoleptic characteristics [12] of nutmegs. [13] also found that level of volatile components in red berry mirsen increased in the spring and the summer.

Myristicin, safrole and elemicine possess hallucinogenic properties [17]. This characteristic is very useful in the pharmaceutical industry. Aromatic compounds are also very important because they are the determining factors of the aroma and quality of essential oils. Another component of essential oils is myristic acid. In general, myristic acid increased with increasing duration of storage. Therefore, myristic acid can be used as an indicator of nutmeg's oil quality related to storage [18].

Table 4 Main essential oil components of Maluku nutmeg.

Maluku		Main essential oil component (%)			
		Myristicin	Elemicin	Safrole	(Iso)eugenol
1.	Banda	13.76	0.94	2.44	0.90
2.	Ambon	13.54	0.67	2.46	0.55
3.	Luhu	5.57	2.05	0.97	0.70

Conclusions

Maluku nutmegs produce essential oils with the content ranged from 9.99 to 11.92% andhave physico-chemical properties that are relatively stable except for the optical rotation. Monoterpen hydrocarbon component of the oils is pinen, while the aromatic components are myristicin, elemicine, safrole , and (iso) eugenol. Myristicin is the highest aromatic component in the nutmegs of Banda ecotype (13.76%).

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