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Physical characterization and essential oil properties of West Sumatra mace and nutmeg seed (*Myristica fragrans* Houtt) at different ages at harvest

Mimbar Ari Saputro, Nuri Andarwulan and Didah Nur Faridah

Abstract
Myristica fragrans Houtt) is the most important species for spice and nutmeg oil in international trade. The raw materials in nutmeg oil production consist of mace, young seed, medium seed, and old seed. The nutmeg seed was classified according to the harvesting age. The physical properties of nutmeg mace and seeds were determined at different moisture content of samples. The mean length and width of seeds were (1.45, 1.56, and 2.34 cm) and (1.02, 1.77, and 2.02 cm), respectively. The mean weight of seeds were 0.77, 1.67, and 2.71 gram. The percentages of oil yield were 19.51, 8.91, 6.35, and 5.04, respectively. The mean of specific gravity, refractive index and optical rotation of oils were (0.919, 0.902, 0.923, and 0.930), (1.487, 1.481, 1,486, and 1.487), and (+6.07°, +7.51°, +6.61°, and +6.58°), respectively. Analysis of volatile components in the nutmeg oil used gas chromatography-mass spectrometry (GC-MS). In total, 40 compounds have been identified by GC-MS. The main constituents of various nutmeg oils were alpha pinene, sabinene, beta pinene, 3-carene, limonene, gamma terpinene, 4-terpineol, safrole, myristicin, eugenol, methyl eugenol, isoeugenol, and elimicine. The physicochemical properties of young nutmeg seed oil is the closest to European Pharmacopoeia (EP) and flavor and fragrance industry quality standard.

Keywords: *Myristica fragrans* Houtt, nutmeg oil, physical characteristic, GC-MS, chemical composition, EP standard

1. Introduction
Nutmeg is Indonesia's native spice, spread mainly in Maluku, Irian Jaya, Sulawesi, Java, and Sumatera. Indonesia is also the center of origin from several species of Myristica genus [1]. Thus, Indonesia needs to take a bigger role in nutmeg management, development, and use. Nutmeg species which has important economic significance is *Myristica fragrans* Houtt. The two major products are mace and seed. Nutmeg seed can be used in a many ways, for example the phenolic compound which could be beneficial antioxidant in functional food [2, 3]. According to Saxena and Patil nutmeg oil has secondary metabolites that useful in treating various infectious diseases [4]. In addition, nutmeg seed powder can be used as antimicrobial agent in candies and as anti-bacterial, anti-inflammation, antioxidant, and anti-collagenolytic agent in periodontitis treatment [5, 6]. The main raw materials in nutmeg oil production consists of mace, young, medium, and old seed.
In this date, Indonesia has exported around 26 types of essential oils, one of them is nutmeg oil. Total volume of nutmeg oil and value export in 2015 were 339 tons and valued at 14.456 million USD. Nutmeg oil as one of export commodities needs to be developed as it has provided income and jobs in the field of plantation, commerce, and industry. The main problem in nutmeg oil market is difficult to maintain the quality. According to Ma'mun, the quality of nutmeg oil is heavily determined by its chemical properties [7]. Nutmeg oil consists of several chemical compounds which are plant synthesize and accumulate large amount of specialized (or secondary) metabolites. This reaction is influenced by environmental factor and plants genetics. Chemical compound identification analysis uses gas chromatography (GC) method [8]. The quality standard is determined by international organization, among them European Pharmacopoeia (EP), British Pharmacopoeia (BP), International Organization of Standardization (ISO), and flavor and fragrance industry. In general, the standard consist of nine chemical properties parameters, which are: alpha pinene, sabinene, beta pinene, 3-carene, limonene, gamma terpinene, 4-terpineol, safrole, and myristicine. There is also additional standard that gives limit value for eugenol, methyl eugenol, isoeugenol, and elimicine in flavor and fragrance industry. Methyl eugenol and safrole are carcinogenic compound, which make them one of the most important parameter in nutmeg oil perception [9], while eugenol, limonene, and isoeugenol are allergens [10].

One of the centers of nutmeg seed commerce in Indonesia is West Sumatera. In this area nutmeg seed are sold based on its harvesting age, whether it is young seed, medium seed, or old seed. Each type has different price and in descending manner, the most valuable would be mace, young seed, medium seed, and old seed. In the process of making nutmeg oil, entire ingredients would be mixed via distillation. The chemical properties of nutmeg oil may be related to the harvesting age of nutmeg mace and seed. Several nutmeg related researches have been conducted, however research on relationship between mace, different aged nutmeg seed, and physicochemical characteristics from raw materials originated from West Sumatra had never been conducted before. This prompts for research in studying physicochemical characteristic of mace and different harvesting age of nutmeg seed via hydrodistillation technique. This research is hope to give useful information for farmers, distillers, and flavor and fragrance industry.

2. Material and Methods

2.1 Plant Material

Samples consist of mace and young, medium, and old nutmeg seed obtained directly from nutmeg sellers in Batang Kapas Districts, South Pesisir Regency, West Sumatra Province, Indonesia. Each sample that had been sorted by nutmeg seller was weighted 420 grams. This research use laboratory analysis method with two repetition technique.

2.2 Physical Characterization of Nutmeg Seed

The characteristics of seed diameter and weight were measured. Seed diameter was measured longitudinally and transversally. The number of samples were 20 seeds (n=20). Average value and deviation value were calculated.

2.3 Sample Preparation

The Nutmeg and mace samples were crushed by Xi An milling machine made in China, which followed by sieving using 30 mesh sieve. Nutmeg mace and seed moisture was measured by Sartorius Moisture Analyzer for both samples that passed and did not the sieve.

2.4 Extraction of Essential Oil

This stage covers distillation, cooling, and separation of mace and seed oil from water. (1) Distillation was done in order to separate mace and seed oil content using hydrodistillation technique, 200 grams samples were put inside round bottom flask equipped with clevenger apparatus. Afterwards water was added until the ratio of water to sample 6:1. The flask was then placed on top of heating mantle or hot plate. Distillation was done for 12 hours or until the oil content inside distilled water had run out, or considered done marked by clear distillate water. (2). Cooling was conducted to condense and cool the mixture of hot water steam and oil, so that oil could be separated from water. In this stage, the mixture of steam and oil was passed through spiral glass tube which was cooled by running water. Oil and steam would be cooled and condensed along the cooling tubes, in which distillate water would be contained in a separating funnel with oil. (3) Separation was conducted in the purpose of separating oil from water. Oil which has lighter specific gravity than water would be on top, while oil with heavier specific gravity than water would be below water. Afterwards mace and seed oil was weighted to calculate yield then analyzed, after then stored inside tightly closed glass bottle in room temperature for physical and compounds analysis.

2.5 Physical analysis of essential oil

The physical analysis that observed were specific gravity, refractive index, and optical rotation. These three parameters were included in EP standard. The amount of samples required for this analysis as 20 ml.

2.6 Essential oil composition analysis by GC-MS

Nutmeg mace and seed oil compound profile analysis was done by GC method. The equipment used was GC to determine contents in area percentage and GC-MS to determine essential oil compound composition. Essential oil quality profile was determined by matching of sample component fragmentation quality with library data on mass spectrum with 80% minimum. GC condition was set on: injector temperature at 275 ºC with split mode (split ratio 100:1) and nitrogen carrier gas with 0.5 ml/minute flow rate. Oven condition was set as: 100 ºC (10 minutes hold time) then 250-300 ºC in 5 ºC rate (15 minutes hold time). Detector condition was set as: 275 ºC, 30 ml/minute H2 flow, 400 ml/min air flow, and 25 ml/min make up flow. GC-MS condition for all essential oil sample was set as: 250 ºC ion source temperature, 200 ºC quadrupole temperature, 10-250 amu of scan mass, and around 35 µA of emission. Energy used was 70 eV with WMV < 2000 V. Carrier gas used helium with 0.5 ml/min flow. Oven and injector conditioning was the same as GC. Compound identification was done by comparing the Wiley 2008-NIST library and P. Adam data of the peaks with those reported in literature, mass spectra of the peaks with literature data.

2.6. Data analysis

Data was analyzed by using analysis of variance (ANOVA). If the result were significantly different, analysis would continue with Least Significant Difference (LSD) test on α = 0,05.

3. Result and Discussion

3.1 Physical characteristics of nutmeg seed

In observation, nutmeg mace is brownish red in color, has irregular shape, thin, and crumpled upon touch. Nutmeg seed is mostly dark brown, differing in diameter, weight, and shape. The result of nutmeg seed diameter measurement and weight is presented in Table 1. Young nutmeg seed is marked by the presence of wrinkles surrounding the seed and tender when pressed by the tip of our nail. Graphic of the relationship between nutmeg seed's average weight, yield, moisture, and sieved is shown in Figure 1.

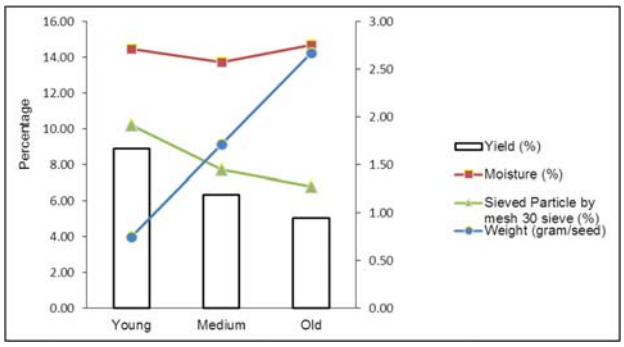

Fig 1: Graphic of the relationship between nutmeg seed's average weight, yield, moisture, and sieved

According Siregar *et al.* young nutmeg upon harvesting would then has its fruit flesh cut open to reveal greenish white

mace and seed, with is shell still tender on pressure [11]. Young nutmeg seed has high moisture, and after drying it will shrink and wrinkles. Observation on medium nutmeg seed revealed that half of the seed has hardened while half of it wrinkles. This is different with old nutmeg seed where it has strong and rough shell in oval shape. According to Soenarsih *et al.*, the shape of Banda *Myristica fragrans* nutmeg is round with oval seed [12]. The average index of the seed's diameter is 1.17. The average index was obtained from the ratio of longitudinal and transversal diameter. The measurement of old West Sumatera nutmeg seed showed 1.16 average index value. This gives hint that nutmeg seed originating from West Sumatera has genus likeness with the seed coming from Banda.

Table 1: Physical characteristic of nutmeg mace and seed oil sample

Analysis description	Mace	Nutmeg seed			EP Standard**
		Young	Medium	Old	
Mace and nutmeg seed					
Longitudinal diameter (cm)	-	1.45 ± 0.03 ^{b}	1.56 ± 0.08 ^{b}	2.34 ± 0.01 ^{a}	-
Transversal diameter (cm)	-	1.02 ± 0.07 ^{c}	1.77 ± 0.05 ^{b}	2.02 ± 0.04 ^{a}	-
Weight (gr)	-	0.77 ± 0.11 ^{c}	1.67 ± 0.01 ^{b}	2.71 ± 0.07 ^{a}	-
Moisture (%)	10.36 ± 0.23 ^{b}	14.48 ± 0.66 ^{a}	13.75 ± 0.42 ^{a}	14.71 ± 0.08 ^{a}	-
Sieved particles by mesh 30 (%)*	3.03 ± 0.03 ^{d}	10.23 ± 0.07 ^{a}	7.74 ± 0.32 ^{b}	6.83 ± 0.07 ^{c}	-
Nutmeg oil					
Yield (w/w %)	19.51 ± 0.50 ^{a}	8.91 ± 0.58 ^{b}	6.35 ± 0.17 ^{c}	5.04 ± 0.03 ^{d}	-
Specivic gravity (25/25^{0}C)	0.919 ± 0.002 ^{b}	0.902 ± 0.005 ^{c}	0.923 ± 0.001 ^{b}	0.930 ± 0.001 ^{a}	0.89 - 0.905
Refractory index (20^{0}C)	1.487 ± 0.001 ^{a}	1.481 ± 0.0017 ^{b}	1.486 ± 0.000 ^{a}	1.487 ± 0.000 ^{a}	1,475 - 1,485
Optical rotation	(+)6.07 ± (+)0.27 ^{a}	(+)7.51 ± (+)0.79 ^{a}	(+)6.61 ± (+)0.29 ^{a}	(+)6.58 ± (+)0.48 ^{a}	(+)8 - (+)18

Annotation: *finely grounded nutmeg seed **European pharmacopoeia. Superscripted letters on numbers indicated significant difference (LSD $\alpha = 0.05$).

As shown in the Table 1, the difference in percentage between particles that passed the sieve might be related to harvesting age factor. Young nutmeg seed tissue is still tender and thus after crushing it has more small particles compared to medium and old nutmeg seed. Medium and old nutmeg seed has hard tissues, which caused their passable sized particles to be fewer than young seed. Particles that were unable to pass the sieve were mostly shell shards

3.2 Extraction of Essential oil

Figure 1 shows highest amount of nutmeg seed particles that passed sieving has highest yield, while seeds which lower particles passing the sieve has lower amount of yield. This is inversely proportional with weight, where old nutmeg seed with highest weight produced the lower percentage of yield. Particles of old nutmeg seed that did not pass the sieving were mostly shards of old nutmeg seed's shell, which influenced the percentage of nutmeg oil yield. This is in line with of a research done by Suryatmi *et al*, which stated that distillation with nutmeg shell as main ingredients does not produce nutmeg essential oil [13]. Yield percentage obtained from that research was almost the same with the one done by Pal *et al*, where old nutmeg yield percentage from Andaman Nicobar Island was 8.25 [14]. However, this research did not explain harvesting age of nutmeg seed.

3.3 Physical Analysis of Essential oil

The difference of essential oil chemical composition could cause different physical characteristic of a given essential oil [15]. Oil specific gravity is a cluster of compound molecules composing oil within a given volume and temperature. Chemical composition in nutmeg oil has intrinsic or endogenous traits depending on their molecular chemical characteristics and thus influence physical trait such as specific gravity, refractive index, and optical rotation. As shown in the Table 1, the specific gravity value of nutmeg mace, young seed, medium seed, and old seed respectively are 0.919, 0.902, 0.923, and 0.930. The refractive index values are 1.487, 1.481, 1,486, and 1.487. The value of specific gravity and refractive index showed that the older the harvesting age, the higher the value of specific gravity and refractive index of nutmeg seed. Optical rotation is molecular structure response against single light wave path. Table 1 showed that the older the seed, the smaller the value of optical rotation. If compared to EP standard, the value of nutmeg's specific gravity and refractive index meet the standard, however its optical rotation value does not. Research conducted by Ma'mun towards nutmeg oil sample from Papua obtained 0.909 specific index value, 1.487 refractive index value, and (+)15.3 optical rotation value [7].

3.4 Chemical composition of Essential Oil

A total of 40 compounds were identified for nutmeg mace and seed oil. The result of the analysis is shown in Figure 2, while the type of volatile compound and percentage of total area are shown in Table 2 and Table 3 of quantitative analysis result from young, medium, and old nutmeg seed.

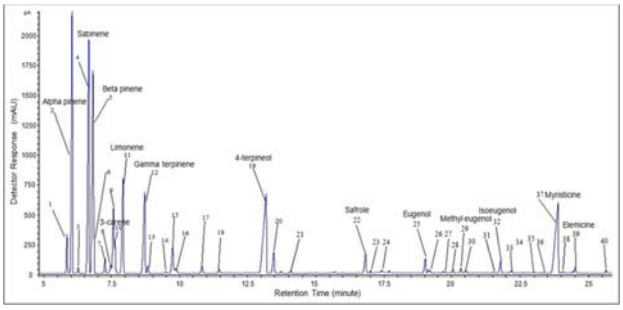

Fig 2: Identification of GC chromatogram of nutmeg oil composition

The result of GC quantitative analysis of nutmeg oil was obtained by total percentage of volatile compound in mace oil 99.41, young seed 98.63, medium seed 98.26, and old seed 98.24. From those 40 volatile compounds, there were 13 main constituents of mace and seed oil; 9 compounds which

registered in EP standard were alpha pinene, sabinene, beta pinene, 3-carene, limonene, gamma terpinene, 4-terpineol, safrole, and myristicine. The 4 other constituents were used as supporting standard in acceptance of nutmeg oil within flavor and fragrance industry. They were eugenol, methyl eugenol, isoeugenol, and elimicine.

Table 2: Chemical compound profile in nutmeg mace and young, medium, and old seed oil

Peak number	Chemical compound	m/z	Nutmeg mace oil (%)	Young nutmeg seed oil (%)	Medium nutmeg seed oil (%)	Old nutmeg seed oil (%)
1	Alpha thunjene	136	1.78 ± 0.03 ^{c}	2.27 ± 0.03 ^{a}	2.22 ± 0.04 ^{ab}	2.11 ± 0.00 ^{b}
2	Alpha pinene*	136	19.77 ± 0.06 ^{a}	19.28 ± 0.12 ^{a}	17.75 ± 0.26 ^{b}	16.16 ± 0.25 ^{c}
3	Camphene	136	0.36 ± 0.01 ^{a}	0.37 ± 0.01 ^{a}	0.33 ± 0.01 ^{b}	0.31 ± 0.01 ^{b}
4	Sabinene*	136	12.45 ± 0.14 ^{a}	12.23 ± 0.65 ^{a}	11.09 ± 0.07 ^{a}	11.07 ± 0.35 ^{a}
5	Beta pinene *	136	14.77 ± 0.13 ^{ab}	14.78 ± 0.37 ^{a}	13.37 ± 0.02 ^{b}	12.26 ± 0.08 ^{c}
6	Beta myrcene	136	2.30 ± 0.02 ^{a}	2.30 ± 0.06 ^{a}	2.06 ± 0.01 ^{b}	1.94 ± 0.02 ^{b}
7	Alpha phelandrene	136	0.93 ± 0.03 ^{a}	0.54 ± 0.01 ^{b}	0.49 ± 0.01 ^{c}	0.49 ± 0.02 ^{c}
8	3-carene*	136	1.53 ± 0.03 ^{a}	0.42 ± 0.01 ^{b}	0.40 ± 0.01 ^{b}	0.40 ± 0.02 ^{b}
9	Alpha terpinene	136	3.21 ± 0.05 ^{b}	3.56 ± 0.09 ^{a}	3.25 ± 0.05 ^{b}	3.18 ± 0.01 ^{b}
10	Beta ocimene	136	0.41 ± 0.01 ^{c}	1.73 ± 0.05 ^{a}	1.35 ± 0.06 ^{b}	1.24 ± 0.04 ^{b}
11	Limonene*	136	5.63 ± 0.10 ^{a}	5.84 ± 0.18 ^{a}	5.12 ± 0.04 ^{b}	4.93 ± 0.03 ^{b}
12	Gamma terpinene*	136	4.98 ± 0.08 ^{b}	5.54 ± 0.16 ^{a}	5.19 ± 0.06 ^{ab}	5.12 ± 0.00 ^{b}
13	Cis sabinene hydrat	136	0.16 ± 0.00 ^{c}	1.08 ± 0.04 ^{a}	1.05 ± 0.02 ^{a}	0.91 ± 0.03 ^{b}
14	Cymenene	132	0.05 ± 0.00 ^{b}	0.07 ± 0.00 ^{a}	0.07 ± 0.01 ^{a}	0.07 ± 0.00 ^{a}
15	Alpha terpinolen	136	2.24 ± 0.04 ^{a}	1.49 ± 0.05 ^{b}	1.41 ± 0.01 ^{b}	1.40 ± 0.01 ^{b}
16	Trans sabinene hydrat	154	0.13 ± 0.00 ^{c}	0.99 ± 0.05 ^{a}	1.00 ± 0.02 ^{a}	0.85 ± 0.03 ^{b}
17	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	154	0.35 ± 0.00 ^{c}	0.65 ± 0.02 ^{a}	0.63 ± 0.00 ^{ab}	0.60 ± 0.01 ^{b}
18	1-Methyl-4-isopropyl-3-cyclohexen-1-ol	154	0.25 ± 0.00 ^{c}	0.44 ± 0.01 ^{a}	0.42 ± 0.00 ^{ab}	0.40 ± 0.01 ^{b}
19	4-terpineol*	154	7.87 ± 0.20 ^{c}	11.47 ± 0.24 ^{a}	10.35 ± 0.08 ^{b}	10.39 ± 0.04 ^{b}
20	Beta fhencol	154	0.94 ± 0.02 ^{c}	1.23 ± 0.04 ^{a}	1.12 ± 0.01 ^{b}	1.14 ± 0.01 ^{b}
21	Trans piperitol	154	0.11 ± 0.00 ^{b}	0.20 ± 0.01 ^{a}	0.21 ± 0.00 ^{a}	0.21 ± 0.00 ^{a}
22	Safrole*	162	2.30 ± 0.00 ^{a}	1.23 ± 0.06 ^{c}	1.24 ± 0.01 ^{c}	1.40 ± 0.00 ^{b}
23	Alpha bornyl acetate	196	0.13 ± 0.01 ^{b}	0.20 ± 0.01 ^{a}	0.19 ± 0.02 ^{ab}	0.15 ± 0.00 ^{ab}
24	p-Pentylanisole	178	0.13 ± 0.01 ^{b}	0.14 ± 0.01 ^{b}	0.27 ± 0.02 ^{a}	0.30 ± 0.01 ^{a}
25	Eugenol**	164	0.39 ± 0.01 ^{a}	0.26 ± 0.01 ^{b}	0.29 ± 0.02 ^{b}	0.35 ± 0.00 ^{a}
26	Citronelyl acetate	198	0.19 ± 0.01 ^{b}	0.19 ± 0.01 ^{b}	0.22 ± 0.01 ^{a}	0.23 ± 0.00 ^{a}
27	Alpha terpenyl acetate	196	0.02 ± 0.00 ^{c}	0.07 ± 0.00 ^{b}	0.09 ± 0.01 ^{a}	0.07 ± 0.00 ^{b}
28	Alpha cubebene	204	0.06 ± 0.01 ^{c}	0.12 ± 0.00 ^{b}	0.16 ± 0.01 ^{a}	0.13 ± 0.00 ^{ab}
29	Methyl Eugenol**	178	0.35 ± 0.00 ^{a}	0.24 ± 0.03 ^{b}	0.34 ± 0.01 ^{a}	0.38 ± 0.01 ^{a}
30	Neryl acetate	196	0.02 ± 0.01 ^{b}	0.07 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.07 ± 0.02 ^{a}
31	Beta caryophyllene	204	0.01 ± 0.00 ^{c}	0.03 ± 0.00 ^{b}	0.04 ± 0.00 ^{ab}	0.05 ± 0.01 ^{a}
32	Isoeugenol**	164	1.38 ± 0.12 ^{a}	0.44 ± 0.18 ^{b}	0.98 ± 0.05 ^{a}	1.21 ± 0.00 ^{a}
33	Alpha bergamotene	204	0.02 ± 0.00 ^{c}	0.11 ± 0.01 ^{b}	0.14 ± 0.01 ^{a}	0.13 ± 0.01 ^{ab}
34	Beta farnesene	204	0.00 ± 0.00 ^{ni}	0.03 ± 0.00 ^{ni}	0.05 ± 0.00 ^{ni}	0.04 ± 0.00 ^{ni}
35	Methyl isoeugenol	178	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{b}	0.07 ± 0.00 ^{a}	0.07 ± 0.00 ^{a}
36	Germacrene	204	0.01 ± 0.01 ^{c}	0.05 ± 0.00 ^{b}	0.07 ± 0.01 ^{a}	0.06 ± 0.00 ^{ab}
37	Myristicine*	192	13.83 ± 0.71 ^{a}	8.67 ± 1.90 ^{b}	14.02 ± 0.17 ^{a}	15.61 ± 0.33 ^{a}
38	Beta bisabolene	204	0.02 ± 0.00 ^{d}	0.09 ± 0.00 ^{c}	0.13 ± 0.01 ^{a}	0.11 ± 0.00 ^{b}
39	Elemicine**	208	0.25 ± 0.03 ^{b}	0.24 ± 0.09 ^{b}	0.57 ± 0.02 ^{a}	0.66 ± 0.01 ^{a}
40	Methoxy eugenol	194	0.14 ± 0.06 ^{c}	0.02 ± 0.01 ^{c}	0.55 ± 0.15 ^{b}	2.12 ± 0.06 ^{a}
	Total		99.41 ± 0.05	98.63 ± 0.11	98.26 ± 0.03	98.24 ± 0.04

Annotation: *European Pharmacopoeia standard, *flavor and fragrance industry standard. m/z = molecular mass compared to protonated molecular charge. Superscripted letters on numbers indicated significant difference (LSD $\alpha = 0.05$).

Table 3: Main constituents of nutmeg oil compared to literature

Method / Parameter	Nutmeg mace oil (%)	Young nutmeg seed oil (%)	Medium nutmeg seed oil (%)	Old nutmeg seed oil (%)	Nutmeg oil from Sulawesi* (%)	Nutmeg oil from Java* (%)	Nutmeg oil from Andaman Nicobar** (%)	EP Standar*** (%)	Flavor and fagrance industry standard (%)
Extraction method	Hydrodistillation	Hydrodistillation	Hydrodistillation	Hydrodistillation	Steam distillation	Steam distillation	Hydrodistillation	-	-
EP standard parameter***									
Alpha pinene	19.77 ± 0.09 ^{a}	19.28 ± 0.18 ^{a}	17.75 ± 0.36 ^{b}	16.16 ± 0.36 ^{c}	19.07	19.33	9.40	15 - 28	15 - 28
Sabinene	12.45 ± 0.20 ^{a}	12.23 ± 0.93 ^{a}	11.09 ± 0.10 ^{a}	11.07 ± 0.49 ^{a}	19.07	23.44	41.70	14 - 29	14 - 29
Beta pinene	14.77 ± 0.18 ^{ab}	14.78 ± 0.51 ^{a}	13.37 ± 0.03 ^{b}	12.26 ± 0.11 ^{c}	15.71	15.86	7.30	13 - 18	13 - 18
3-carene	1.53 ± 0.04 ^{a}	0.42 ± 0.01 ^{b}	0.40 ± 0.01 ^{b}	0.40 ± 0.02 ^{b}	0.61	1.05	0.60	0.5 - 2	0.5 - 2
Limonene	5.63 ± 0.13 ^{a}	5.84 ± 0.25 ^{a}	5.12 ± 0.06 ^{b}	4.93 ± 0.04 ^{b}	6.25	5.87	3.70	2 - 7	2 - 7
Gamma terpinene	4.98 ± 0.10 ^{b}	5.54 ± 0.23 ^{a}	5.19 ± 0.09 ^{ab}	5.12 ± 0.01 ^{b}	4.73	3.7	2.90	2 - 6	2 - 6
4-terpineol	7.87 ± 0.28 ^{c}	11.47 ± 0.34 ^{a}	10.35 ± 0.10 ^{b}	10.39 ± 0.05 ^{b}	5.73	4.01	5.80	2 - 6	2 - 6
Safrole	2.30 ± 0.00 ^{a}	1.23 ± 0.09 ^{c}	1.24 ± 0.01 ^{c}	1.40 ± 0.01 ^{b}	1.6	1.64	1.40	0 - 2	0 - 2
Myristicine	13.83 ± 1.00 ^{a}	8.67 ± 2.68 ^{b}	14.02 ± 0.25 ^{a}	15.61 ± 0.47 ^{a}	10.12	10.74	2.70	8 - 12	8 - 12
Total	83.11 ± 0.23	79.44 ± 0.58	78.51 ± 0.11	77.33 ± 0.17	82.89	85.64	75.50	83.25	83.25
Additional standard parameter									
Eugenol	0.39 ± 0.01 ^{a}	0.26 ± 0.01 ^{b}	0.29 ± 0.03 ^{b}	0.35 ± 0.01 ^{a}	0.17	0.32	0.50	-	0 - 0.5
Methyl eugenol	0.35 ± 0.01 ^{a}	0.24 ± 0.03 ^{b}	0.34 ± 0.02 ^{a}	0.38 ± 0.01 ^{a}	0.65	0.4	1.50	-	0 - 0.5
Isoeugenol	1.38 ± 0.16 ^{a}	0.44 ± 0.25 ^{b}	0.98 ± 0.08 ^{a}	1.21 ± 0.00 ^{a}	0.59	0.82	0.00	-	0 - 1
Elemicine	0.25 ± 0.03 ^{b}	0.24 ± 0.11 ^{b}	0.57 ± 0.03 ^{a}	0.66 ± 0.00 ^{a}	0.59	0.49	0.80	-	0 - 0.5
Total	2.35 ± 0.05	1.17 ± 0.10	2.17 ± 0.04	2.59 ± 0.01	2.00	2.03	2.80	-	1.25
Total sum	85.46 ± 0.14	80.61 ± 0.34	80.68 ± 0.08	79.91 ± 0.09	84.89	87.67	78.30	-	84.50

Annotation: *Riyadi *et al.* (2014), **Pal *et al.* (2011), ***European Pharmacopoeia. Superscripted letters on numbers indicated significant difference (LSD $\alpha = 0.05$).

Table 3 showed the amount of alpha pinene, sabinene, beta pinene, 3-carene, limonene, and gamma terpinene gradually decreases as the harvesting age gets older while the value of safrole, myristicine, eugenol, methyl eugenol, isoeugenol, and elimicine increase. This shows that the older nutmeg seed turned into, the lower the development of secondary metabolites and what afterwards increase is primary metabolites, which resulted to production of compound essential for plant growth [16]. This analysis shows the value of safrole, isoeugenol, myristicine, and elimicine, to be higher than standard value of EP and flavor and fragrance industry. According to Schenk and Lamparsky, safrole, isoeugenol, myristicine, and elimicine compound are among aromatic compound group consisting oxygenated hydrocarbon content, thus having high value of specific gravity and refractive index [17]. The amount of monoterpenoid hydrocarbon compounds such as alpha pinene, beta pinene, sabinene, and 3-carene were lower than the amount shown in EP standard. This influenced optical rotation value to be small.

According to Riyadi *et al.*, the chemical compounds of nutmeg oil originated from Sulawesi and Java meets the quality standard by 85% and 100%, respectively [9]. Research conducted by Pal *et al.* using main ingredient originated from Andaman Nicobar meets the quality standard by 69% via hydrodistillation process [14]. Nutmeg oil from Java and Sulawesi have a better quality. This opens the possibility of nutmeg oil extraction by using steam distillation while influenced by environmental factors and plant genetics.

Chemical compounds of mace oil that meets EP standard and flavor and fragrance industry standard were 62%, or 8 out of 13 compounds; which are alpha pinene, beta pinene, 3-carene, limonene, gamma terpinene, eugenol, methyl eugenol, and elimicine. For young seed oil the value was 77%, or 10 out of 13 compounds; which are alpha pinene, beta pinene, limonene, gamma terpinene, safrole, myristicine, eugenol, methyl eugenol, isoeugenol, and elimicine. For medium seed oil the value was 62% or 8 out of 13 compounds, which are alpha pinene, beta pinene, limonene, gamma terpinene, safrole, eugenol, methyl eugenol, and isoeugenol. Finally, old seed oil the value was 46% or 6 out of 13 compounds which are alpha pinene, limonene, gamma terpinene, safrole, eugenol, and methyl eugenol. From here we observe that young nutmeg seed oil has a quality near EP and flavor and fragrance industry standard, nearer compared to mace oil, medium seed oil, and old seed oil.

4. Conclusion

Weight and size of nutmeg seed is directly proportional to harvesting age; whereas nutmeg oil yield is inversely proportional. Nutmeg oil physical characteristics showed that the older the seed, the higher the value of specific gravity and refractive index, while optical rotation will lower. As many as 40 chemical compounds have been identified as constituents of nutmeg mace, young seed, medium seed, and old seed oil. The main chemical compounds of nutmeg oil were alpha pinene, sabinene, beta pinene, 3-carene, limonene, gamma terpinene, 4-terpineol, safrole, myiristicine, eugenol, methyl eugenol, isoeugenol, and elimicine with different compound composition for every nutmeg type. Terpenes group compound decrease as nutmeg seed gets older however aromatic component will increase. Various nutmeg oil does not qualify EP and flavor and fragrance industry physicochemical quality standard. The quality of young nutmeg seed oil is the closest to EP and flavor and fragrance industry standard, compared to mace oil, medium seed oil, and old seed oil. Further study is needed in order to obtain quality that meets the standard, whether of processing or of raw materials composition.

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6. References

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