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Effect Of Aspergillus Niger On Fermentation Process In Increasing The Quality Of Patchouli Oil

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Abstract

In this research, the process of fermentation of patchouli raw material was conducted to enhance the quality of patchouli oil. Patchouli fermentation was carried out using Aspergillus niger. Preparation processes were carried out prior to distillation including withering, size reducing and fermenting. Patchouli was withered for 24 hours. Patchouli was cut into pieces and weighed as much as 800 grams (3 leaves: 1 stem) after withered. Patchouli fermentation was conducted anaerobically for 20 hours. Varying the addition of A. niger used was 5 mL, 10 mL, 15 mL and 25 mL mixed in 400 mL of distilled water. The amount of A. niger colonies was 8.8 x 10^5 /mL. Extraction of patchouli oil was performed by water bubble distillation. The chemical and physical properties of the patchouli oil were analyzed and compared to the quality standard of patchouli oil according to SNI 06-2385-2006. Chemical compound of patchouli oil was identified using GC-MS. The main compound of the patchouli oil is patchoulol. The GC-MS result indicates that patchoulol content increases with the increasing addition of A. niger. The content of patchoulol was detected up to 93.75 %w/w in patchouli oil. The optimal concentration of A. niger is 1.25% that produces the optimal patchoulol content with an optimal yield of patchouli oil.

Keywords: Aspergillus niger, fermentation, Patchouli oil, patchoulol, Water bubble distillation

Abstrak

Dalam penelitian ini, dilakukan proses fermentasi bahan baku nilam dengan tujuan untuk meningkatkan kualitas minyak nilam. Fermentasi nilam dilakukan menggunakan jamur *Aspergillus niger*. Proses preparasi yang dilakukan sebelum penyulingan meliputi pelayuan, pengecilan ukuran dan fermentasi. Pelayuan nilam dilakukan selama 24 jam. Nilam dipotong-potong dan ditimbang sebanyak 800 gram (3 daun : 1 batang) setelah dilayukan. Fermentasi nilam berlangsung secara anaerob selama 20 jam. Variasi penambahan *A. niger* yang digunakan adalah 0 mL, 5 mL, 10 mL, 15 mL, dan 25 mL yang dicampurkan dalam 400 mL akuades. Jumlah koloni *A. niger adalah* 8.8 x 10^5 /mL. Ekstraksi minyak nilam dilakukan dengan cara distilasi *water bubble*. Sifat kimia dan sifat fisika minyak nilam hasil ektraksi dianalisis dan dibandingkan dengan baku mutu minyak nilam, SNI 06-2385-2006. Identifikasi senyawa kimia minyak nilam dilakukan dengan GC-MS. Hasil analisis GC MS minyak nilam menunjukkan bahwa terjadi peningkatan kadar *patchouli alcohol* dengan adanya penambahan jamur *A. niger*. Kadar patchouli alkohol dalam minyak nilam yang terdeteksi mencapai 93,75%. Penambahan *A. niger* paling optimum adalah 1,25% yang menghasilkan rendemen minyak nilam tertinggi dengan kadar patchouli alkohol optimal.

Kata kunci: Aspergillus niger, Fermentasi, Minyak Nilam, Patchouli alkohol, distilasi water bubble

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Introduction

Essential oils are also known as volatile oils, and characterized with their distinctive scent on each plant (Kusuma and Mahfud, 2016). In Indonesia, many essential oils has found from various types of plants that grow in Indonesia. Indonesia is a well-known exporter of essential oils of the world. About 90% patchouli oil in the world come from Indonesia (Gotama, 2016; Wibowo, 2017).

Patchouli (*Pogostemon cablin Bent*) is a plant grows in many tropical regions such as Indonesia (Bhau, 2016). Extraction of patchouli produces essential oils called patchouli oil. The main compound of patchouli is patchouli alcohol (patchoulol). it contains 25-40% patchoulol (Rulianah, 2012; Setiawan, 2013). The amount of patchoulol content can be used to determine the quality and price of patchouli oil (Ma'mun, 2008). Oil quality of Patchouli oil based on Indonesian National Standart is determined by color, density, ether number, acid number, patchouli alcohol and refractive index. Based on Indonesian National Standard (SNI) 06-2385-2006, minimum content of pathouli alcohol at least 30% in patchouli oil, while based on International Standard of Essential Oil Association (EOA) is at least 38%.

In previous researches, various ways were performed to increase the content of patchoulol, such as by using fermentation method (Ruliah, 2012). Fermentation was carried out by an enzymatic reaction using microbes. Microbes are used to degrade the compounds of cell walls from leaf cell tissues, such as cellulose, hemicellulose and pectin (Raharjo and Retnowati, 2012; Nurwati, 2015).

Herliana (2015), conducted patchouli fermentation without the addition of fungus, however *Aspergillus sp*. and *Penicillium sp*. were detected on fermented patchouli leaves. It indicates that the fungus already existed in the patchouli sample and was active during the fermentation process. Based on the previous work, this study is aimed to ferment the patchouli using species of *Aspergillus* i.e. *Aspergillus niger*. The purpose of using *A. niger* is to increase the yield of patchouli oil. *Aspergillus niger* enables to produce cellulose enzymes, which can help the fermentation process by breaking down the patchouli cell wall (Nasarudin, 2008). Patchouli in this study was fermented using various number of *Aspergillus niger* colonies. The fermented patchouli was extracted by a

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water bubble distillation technique (Fitri, 2017). The patchouli oil produced was then characterized in accordance with SNI 06-2385-2006 as well as the analysis using gas chromatography-mass spectrometry (GC-MS).

Research Methods

Material and methods

Materials used in this research consist of leaf and stems of patchouli (*Pogostemon cablin)*, suspension of *Aspergillus niger* (strain code: A. niger fbgmu 001), 70% alcohol, KOH, $H_2C_2O_4$, anhydrous Na_2SO_4, phenolphthalein (PP), ethanol and concentrated HNO_3. All chemicals used were derived from the Merck. Patchouli plant utilized in this research was a type of *Pogostemon cablin Benth*., which is originated from Magelang district, Central Java and grown in the UII patchouli plantation at Kembangan village, Sleman regency, Yogyakarta. This type of patchouli is known to produce high patchouli oil quality (e.g. Ni'mah, 2016). Equipments used in this research include a water bubble distillation, GC-MS (shimadzu QP 2010 SE), glass tool, thermometer, scales, picnometer and refractometer.

The analytical procedures consist of (1) sample preparation and fermentation, (2) distillation process and (3) analysis of distillation result. This research was conducted at Center of Essential Oil Study, UII Integrated Laboratory.

Preparation of raw patchouli

Patchouli plants used are the leaves and stems. Before it was distilled, patchouli sample preparation was carried out. Sample preparation before the distillation can improved the quality of patchouli oil (Nasruddin, 2009). The preparation of sample was carried out by withered, reducing of size and fermentation. Fresh pathcouli withered for 24 hours and avoided from direct sunlight. After withered, patchouli samples and then cut into pieces. Patchouli was weighed by leaf ratio: stem (3: 1) as much as 800 grams. Patchouli sample was fermented using 400 mL of distilled water that was added with A.niger spore. The variation of spore addition were 5 mL; 10 mL; 15 mL; and 25 mL. The fermentation was conducted for 20 hours (Ni'mah, 2016).

Distillation process

The fermented patchouli extracted using a water bubble distillation. The distillation temperature should not exceed 100^oC and the pressure used is 0.5 Barr. The distillation process was

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carried out for 6 hours, calculated from the first droplet of distillate.

Analysis of Patchouli oil

Patchouli oil were analyzed by gas chromatography - mass spectrometer (GC-MS). The instrument operational condition of GC-MS showed in Table 1.

The physical and chemical properties of patchouli oil also tested. The results test of patchouli oil properties was compared with SNI 06-2385-2006, quality standard of patchouli oil. Figure 1 showed the preparation process, distillation using water bubble, characterization, and analysis of patchouli oil using GC-MS.

Table 1. Operational condition of GC-MS

parameter	test condition
Injection temperature	200 ^{o}C
carrier gas	Helium
size coloum	Lenght = 30 m, diameter = 0.25 m, thickness = 0.25 m
stationary phase	Rtx 5 MS

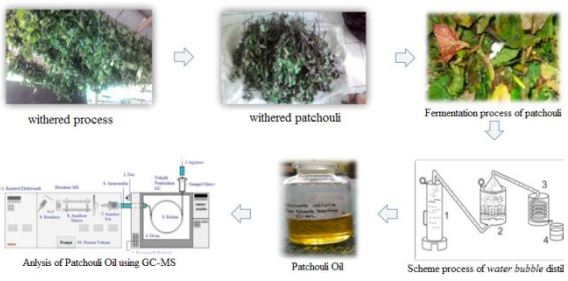

Figure 1. Preparation process of patchouli, extraction used water bubble distillation, characterization, and patchouli oil analysis using GC-MS.

Results and Discussion

Preparation of raw patchouli

The first step in this research is patchouli sample preparation. The process of withered was objective to minimize water content in patchouli. The wilted patchouli facilitates and accelerates the distillation process. The process of withered should not be too long, so that the patchouli leaves become dry, because the fermentation process requires moisture (Ni'mah, 2016). The process of withered produce the color change on the patchouli leaves, to gray or brown and bring up a distinctive sharp aroma of patchouli (Ma'mun, 2008).

The next step, was fermentation using *Aspergillus niger*. The fermentation process was intended to facilitate the degradation cell wall of patchouli leaf tissue, so that patchouli oil more easily out of the oil glands of the plant (Nurwati, 2015). In this experiment, there were 5 variations of *A. niger* addition volumes were 5 mL, 10 mL, 15 mL, and 25 mL (were given J0 for control without *A.niger*, J5, J10, J15, and J25 notation). The greater the volume of the fungus, the more fungal spores are added. According to the

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calculations performed, it was known that in 1 mL the suspension of A. niger fungal spores contains $8.8x10^5$ /mL colonies. The fermentation process was carried out for 20 hours in a sealed dark container (Ni'mah, 2016). In this study, the temperature increased from 28°C to 30°C. The increasing temperature was indicated the fermentation process was going well.

The next step, extraction of fermented patchouli raw materials using distillation water bubble technique. The advantage of the technique of water-bubble distillation is that the patchouli alcohol content was increased significantly, since the semi-polarized essential oil compounds will dissolve in the distillate. The process of distillation water bubbles was conducted for 6 hours, according the previous research results (Fitri, 2017).

Characterization of Patchouli Oil

The colour of patchouli oil produced was golden yellow (J0-J15) and greenish yellow for J25 (Figure 2). High quality patchouli oil has coloured golden yellow. This indicates, that the addition of A. niger in the fermentation process can affect the color of patchouli oil produced. Based on the color of patchouli oil produced, the addition of A. niger in the recommended fermentation process is 5-15 mL.

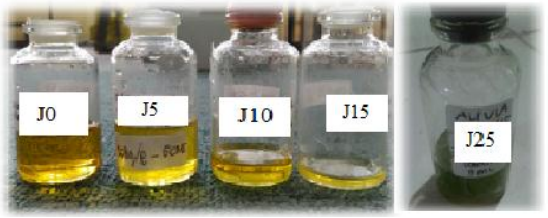

Figure 2. Patchouli Oil J0, J5, J10, J15, and J25.

The results of patchouli oil was characterized according to SNI-06-2385-2006 and analyzed using GC-MS. Characterization of Physics Chemistry of Patchouli Oil based on SNI 06-2385-2006 showed in Table 2.

Table 2. showed the color of the oil produced was accordance with the quality of the SNI (golden yellow) except on patchouli J25 (25 mL additional fungus), which results in a greenish yellow color. More brown color of patchouli oil produced, indicate the patchouli oil content is low (Supriono, 2014). The yellow light patchouli oil shows a high content of patchouli alcohol.

The acid number is a number of milligrams of 0.1N KOH used to neutralize the free fatty acids present in 1 gram of essential oil or fat (Sastrohamidjojo, 2004). The acid number indicates the free acid content in the sample. Free acid in patchouli oil can affect the quality of oil. The free

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acid can ignite with oxygen from the air and can affect the smell of patchouli oil. Based on SNI 06-2385-2006, the acid number in patchouli oil is maximal 8. Based on the 5 sample of patchouli oil analysed, the acid number of all samples were less than 0.015. The result indicates that the patchouli oil produced is high quality without free acid content.

Based on Table 2. it was known that the relative content of patchoulol were greater than 43%. Results obtained showed the high quality of patchouli oil. Relative content of patchouli alcohol has appropriate with SNI, at least 30% and the quality standards of patchouli oil according to the Essential Oil Association (EOA), which requires 38% concentration of patchouli alcohol.

Table 2. Chemical Physical Characterization of Patchouli Oil based on SNI 06-2385-2006

Parameter	SNI	J0	J5	J10	J15	J25
Colour of Patchouli Oil	Yellow-brown reddish yellow	Yellow-brown reddish yellow	Yellow-brown reddish yellow	Yellow-brown reddish yellow	Yellow-brown reddish yellow	Yellow-brown reddish yellow
Refractive Index (nD^{20})	1.507-1.515	1.505	1.507	1.508	1.508	1.507
Acid Number	Maks. 8	0.0077	0.0074	0.0143	0.0104	0.0074
PA ($C_{15}H_{26}O$) (%)	Min. 30	79.17%	85.96%	87.97%	43.37%	93.75%
Peak Area	-	15,843,608	25,875,925	21,758,876	46,688,661	22,350,900
Fe content (mg/kg)	Maks. 25	0.3	0.26	0.28	0.37	0.55
Yield	-	1.43%	1.57%	0.73%	0.8%	0.98%

Information :
J0 = control (without A.niger)
J5 = 1.25% A.niger
J10 = 2.50% A.niger
J15 = 3.75% A.niger
J25 = 6.25% A.niger

Relative content of patchoulol showed comparison of components in patchouli oil, while the concentration of patchoulol was indicated by the large peak area. The peak area produced by the chromatogram relates to the amount of component concentration in a mixture. Peak area was directly proportional to concentration. The larger the peak area, the greater component concentration in the oil. Table 3. showed the largest peak area belongs to the sample J15. It means that the optimal addition of *A.niger* in the fermentation process is 1.88% v/b. *Aspergillus niger* helps the biodelignification process during the fermentation process. Adding *A. niger* with the right concentration can help opened of oil glands.

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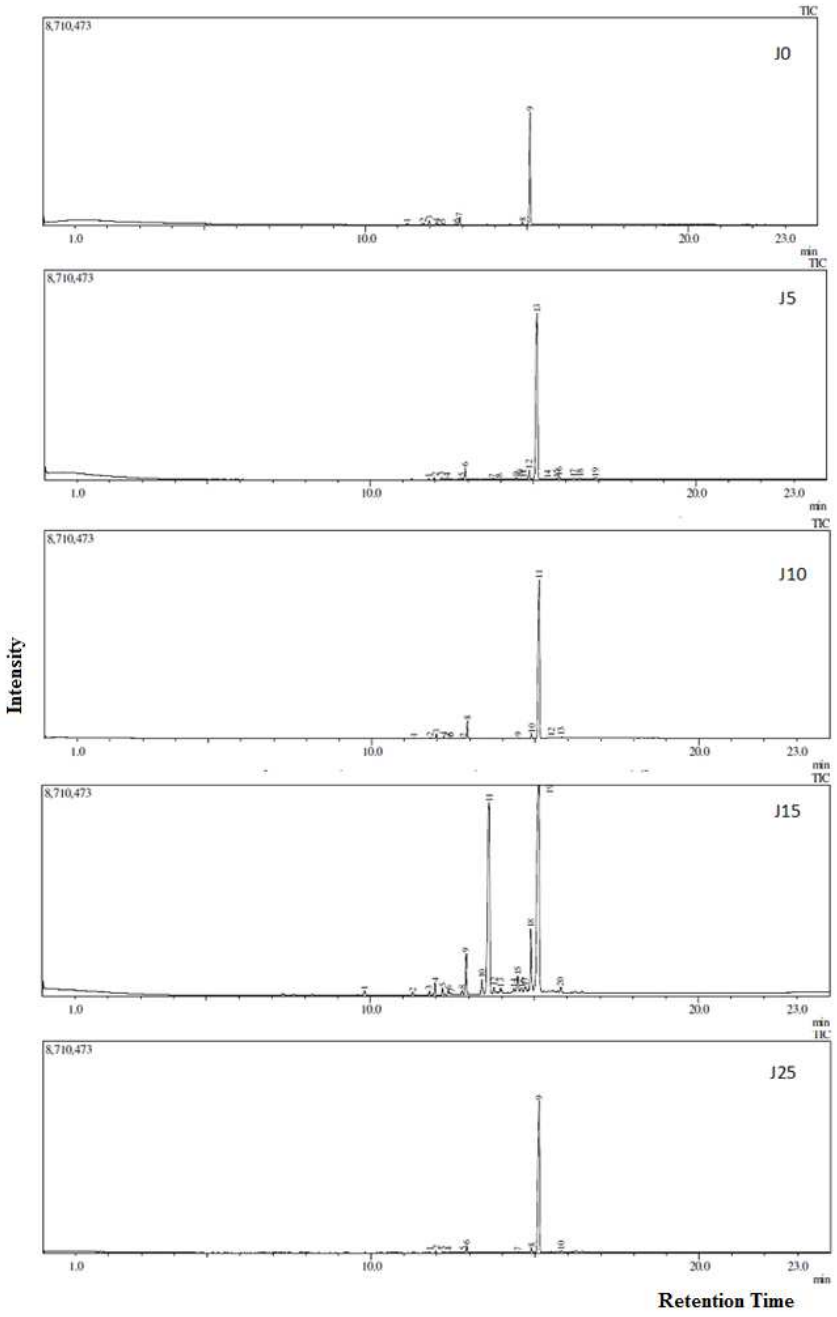

Figure 3. Chromatogram of Patchouli Oil Analysis. Patchouli without A.niger J0, and with A.niger J5, J10, J15 and J25

Chromatogram of the patchouli oil is shown in Figure 3. As results, 20 peaks were detected in J0 with content of patchoulol 79.14%, appearing at the 17th peak. 19 peaks were identified in J5, with content 85.96% patchouol; 13 peaks were detected in J10 with 87.97% patchoulol content. Also 20 peaks and 10 peaks were identified in J15 and J25, respectively (Figure 3). The high

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peak was identified as patchoulol. This is due to the nature of the non-polar patchoulol, so it will elute longer in the Rtx 5MS column which is semipolar.

Table 3. Composition of Patchouli Oil and its peak area

Number	Retention Time (minute)	Peak Area					Compund's name
		J0	J5	J10	J15	J25	
1	11.7	113.024	64.248	79.331	336.788	36.420	trans-geraniol
2	11.9	31.765	218.435	358.324	930.722	171.124	alpha-Humulene
3	12.1	347.332	199.290	183.254	622.367	76.721	alpha-Guainene
4	12.3	88.906	164.047	117.215	490.173	35.963	trans-Carryophyllene
5	12.8	166.717	145.768	93.273	473.461	37.714	Patchollane
6	12.9	842.562	1.037.016	1.327.247	3.141.628	532.546	delta-Guainene
7	14.4	236.554	238.140	81.872	2.032.795	67.422	alpha-selinen
8	14.8	739.797	995.367	495.959	7.087.798	446.484	beta-selinen
9	**15.1**	**15.843.608**	**25.875.925**	**21.758.876**	**46.688.661**	**22.350.900**	**Patchouli alcohol**
10	15.7	95.158	238.126	95.039	522.832	86.723	Aromadenderen
Increasing of Patchouli Alcohol content (%)*			**63,3%**	**37,3%**	**194,6%**	**41,1%**	

*percentages of patchoulol content compare to J0.

Iron content in patchouli was analyzed using AAS. Prior to analysis, patchouli oil samples were dried. The iron content of patchouli sample J0; J5; J10; J15 and J25 were appropriated with the standard quality of SNI, that is below 25 mg / kg (Table 2). Table 2 shows that the iron content are 0.30; 0.26; 0.28; 0.37; and 0.55 ppm respectively for the patchouli oil sample J0; J5; J10; J15 and J25. Very low iron content, show patchouli oil is not contaminated with iron. It is supported by a distilled instrument made of stainless steel. Patchouli oil with yellow colour was an indication of the absence of oxidation process in patchouli oil.

The main factor in the production of patchouli oil is the high yield. A high yield can be economically advantageous. Many research have been conducted to increase the yield. Based on Table 1, the highest yield (1.57%) was obtained on the J5 sample.

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5 patchouli oil samples have the same components commonly found in patchouli oil, namely trans-geraniol, alpha-Humulene, alpha-Guainene, trans-Carryophyllene, Patchollane, delta-Guainene, alpha-selinen and Aromadenderen (Table 2). It can be also seen in Table 3, the patchoulol content proportional to the magnitude of the peak area. Comparing to J0, the patchoulol content in J5, J10, J15 and J25 increased to 63.3%, 37.3%; 194.6% and 41.1%, respectively. Based on the peak area, the highest patchoulol content was in J15. This indicated that the optimal addition of *A. niger* in fermentation process was 3.75%.. Patchouli alcohol is the main component of patchouli oil, which is often used as parameter to determine the patchouli oil quality. The higher of patchouli alcohol content, indicates the better the quality of patchouli oil. Patchoulol gives a distinctive aroma to patchouli oil (Purwaningrat, 2008).

Conclusion

Based on research, it can be concluded, *Aspergillus niger* activated the fermentation process of pathcouli. Fermentation was carried out to opened oil glands on patchouli leaves. The opening of oil glands spur the patchouli oil faster out and the extraction process become efficient. The optimal concentration *A. Niger* in fermentation process was 1.25%.

The effective method of water bubble distillation was used to increase the level of patchouli alcohol and improved the quality of patchouli oil. The polar compounds dissolve into the water using water bubble distillation technique.

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