The role of pre-destilation fermentation in increasing patchouli oil productivity (*Pogostemon cablin benth*)

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Abstract: Mechanical cell destruction through the patchouli leaves and stems chopping has been done, but the damage is still macro and has not been able to damage it to the celular level. Cells destruction through a pre-distillation fermentation process makes it possible to damage the oil cell network to the celular level. The pre-distillation fermentation process used 2 grams of tempe mushrooms (rhizopus origosporus) per kg of raw material for 2, 4, 6, 8, and 10 days and continued with the distillation process at a temperature of 95 ^{0}C. Dry patchouli was 2.25 kg per process with a composition of 1.6 kg (71%) of chopped patchouli stem 5 - 10 cm mixed with 0.65 kg (29%) patchouli leaves. The result is that the dry patchouli distillation process at 95 °C after fermentation results in a higher volume of patchouli oil than without fermentation. The volume of patchouli oil increases with increasing fermentation time from 2 to 8 days, then the volume drops back to 10 days fermentation. The time needed for the distillation process after fermentation 2 days, 4 days and 6 days at 95 °C is 3 hours. Whereas fermented 8 days and 10 days require distillation up to 4 hours. Pre-distillation fermentation process does not have an effect on decreasing patchouli alcohol (PA) levels below 30% which means that it still meets SNI standards. The index value of refined patchouli oil from distillation at 95 °C after fermentation of 2 days to 10 days was between 1.507 - 1.515 (nD20) which means that it meets SNI standards. While the value of the refractive index of patchouli oil distilled 95 °C without fermentation was below 1.507 (nD20). The density of patchouli oil (25 °C / 25 °C) distilled at 95 °C after fermentation of 6 days and 8 days was between 0.95 - 0.975 which means that it meets SNI standards. While the density of patchouli oil fermented 2 days, 4 days and 10 days distilled 95 ° C was above 0.975.

Keywords: pre-distillation fermentation, patchouli oil content, distillation time, patchouli oil quality.

1. Introduction

Patchouli (Pogostemon cablin Benth) is a plant from the Lamiaceae family, which is famous for its medicinal and aromatic properties. Patchouli is a perennial, branching, scented plant with fragrant leaves [1]. This plant in Indonesia is known as nilam. The patchouli oil production process has not optimal yet because of the fact that there is still oil in the cell network that cannot be removed. Patchouli oil is synthesized in glandular cells in plant network and some are formed in resin vessels

[2]. To remove the oil content from oil gland cells, efforts are needed to force the oil to diffuse out of the cell before being distilled.

The cell destruction process before being distilled can be performed mechanically, thermally, electrically, chemically and others. The damage of network destruction through the chopping process is still macro and has not been able to damage it to the cellular level. The freezing process can cause cell damage in both plant and animal network [3, 4]. Fermentation is one way to destroy cell network in plants. The fermentation process allows damaging the network to the cellular level. The fermentation principle is the patchouli oil isolation by breaking the glandular hair cells walls from patchouli leaves using enzymes found in microorganisms. The destruction of cell walls and glandular hair results in patchouli oil being separated from the leaves and can be isolated more easily.

Essential oil is one of the results of the rest of the metabolic processes in plants that are formed due to the reaction between various chemical compounds in the presence of water. Essential oils contained in leaves, flowers, stems and plant roots are synthesized in gland cells in plant network or formed in resin vessels [2]. To remove the oil content from network cells, effort is needed to force the oil to diffuse out of the cell. Patchouli drying temperature affects the capacity and quality of distilled patchouli oil. Conventional drying can result in shrinkage of the outer network which results in decreased permeability, so that the fluid in the cell is difficult to evaporate [5]. The drying process of patchouli before distillation by air flowing at 45 °C results in more oil production and better quality than patchouli which is dried in a shaded environment [6].
In general, patchouli oil is obtained from patchouli leaves hydrodistillation. Most industries still use patchouli oil destillators made from metal. Essential oil from Pogostemon cablin leaves extracted through the hydrodistillation process with the help of microwaves and analyzed by gas chromatography-mass spectrometry (GC-MS) is known to contain nineteen compounds in various concentrations. The main compound of patchouli leaves is patchoulol (26.32%) [7]. Natural Patchouli alcohol (PA) can be isolated from Patchouli oil (PO) under solvent-free conditions using fractional distillation and crystallization techniques. The results of PA, in terms of their purity and separation time, indicate that this procedure is more effective and environmentally friendly compared to other synthesis and separation methods. PO which is fractionally distilled by cooling and centrifuges produces more than 80% PA. Furthermore, the smell of PO with this procedure will not be damaged and can be used as an fragrance ingredient [8]. During the distillation process it is recommended to keep the temperature of the destilator room stable. The fluctuating distillation temperature yields a low patchouli oil yield of around 1.12% [9]. Unstable distillation temperatures can also reduce the essential oils quality from vetiver, especially in terms of it smells [10]. A stable distillation temperature can increase the volume of each kg of dry patchouli weight. The yield of patchouli oil from post-freezing distillation can be increased by increasing the distillation temperature. The lower freezing temperature can increase the patchouli oil yield and can reduce the distillation time to a maximum of 3 hours [11]. Destilator pressure also affects patchouli oil production. From variations in distillation pressure 0.1; 0.2; 0.3; 0.4; 0.5 kg/cm2 with a distillation time of 6 hours, it is known that the pressure of 0.4 kg/cm2 produces the highest rendemen of 2.00%, the patchouli alcohol level is 40.06% and density of 0.961 [12]. Microwave-Assisted Hydrodistillation (MAHD) is a sophisticated technique for patchouli oil extraction processes, where microwaves are used for heating processes during the extraction process. The MAHD method has been proven to reduce extraction time and increase oil production. MAHD requires less energy than HD, which is HD energy consumption 30% higher than MAHD. MAHD power has a significant effect on the final yield and length of extraction time. Higher MAHD power produces more oil productivity [13].

Fermentation is a method to destroy cell network in plants. The fermentation principle in the patchouli oil isolation is by breaking the walls of the glandular hair cells of the leaves and patchouli stem using enzymes contained in microorganisms. The destruction of the cell wall and glandular hair results in patchouli oil being separated from the leaves and stems so that it can be isolated more easily.

International patchouli oil quality standards have not uniformed yet, because every producing and importing country determines patchouli oil quality standards themselves. Patchouli oil quality standards from Indonesia refer to SNI-06-2385-2006 with quality as in table 1.

Table 1. Indonesian patchouli oil quality requirements are based on **SNI- 06-2385-2006**

No	Test types	Unit	Requirements
1	Color	-	Light yellow - reddish brown
2	Specific Gravity (25°C/ 25°C)	-	0.950 – 0.975
3.	Refractive index (nD20)	-	1.507 – 1.515
4.	Solubility in 90% ethanol at a temperature of 20 ^{o}C ± 3 ^{o}C	-	A clear solution or mild expansion in a volume ratio of 1:10
5	Acid number	-	Max. 8
6	Esters number	-	Max. 20
7.	Optical rotation	-	(-)48^{o} – (-)65^{o}
8.	Patchouli alcohol (C15H26O)	%	Min. 30
9	Alpha copaene (C15H24)	%	Maximum 0.5
10	Iron content (Fe)	mg/kg	Maxsimum 25

Patchouli alcohol is an alcoholic sesquiterpen which can be isolated from patchouli oil. Not soluble in water, but can dissolve in alcohol, ether or other organic solvents. The boiling point is 140 ^{o}C at a pressure of 8 mHg. The formed crystal has a melting point of 56 ^{o}C. Patchouli alcohol also called patchouli camphor or octahidro-4, 8a, 9.9-tetrametil-1.6-metanonaphalene, has a molecular weight of 222.36 with the molecular formula $C_{12}H_{26}O$.

Patchouli oil distillation is the process of separating components in the form of liquids or solids from 2 or more kinds of mixtures contained in the leaves and stems of patchouli plants due to differences in the vapor points. The amount of patchouli oil that evaporates together with water vapor is determined by 3 factors, namely the amount of temperature or vapor pressure used, the molecular weight of each component in oil, and the ease of oil coming out of the cell. At the beginning of distillation, the distillation results consist mostly of low boiling point oil components, and then followed by higher boiling point components, and at the end of distillation the amount of refined oil will be smaller. The process of refining oil can be accelerated by increasing the temperature and pressure or using the "superheated steam" technique. In the patchouli oil processing development, it has been known 3 distillation systems types [14]:

a) *Water distillation*
b) *Water and Steam Distillation*
c) *Steam Distillation*

Some processes are carried out first on patchouli raw material before distillation to obtain a higher patchouli oil yield, including through drying, chopping, withering, cutting and so on. Patchouli leaf drying aims to improve the raw materials quality and the patchouli oil quality produced. Refining fresh leaves will produce a low oil yield because the oil inside the leaves cannot come out, since it is blocked by the water content in the leaves. The isolating patchouli oil process by direct drying is not perfect because patchouli oil is still bound to leaf network. Patchouli oil pre- distillation fermentation is one method to destroy oil cells network to obtain high patchouli oil rendemen. One of the microorganisms that can be used in the fermentation process is tempe mushrooms (Rhizopus oligosporus). Molds are microbes in a group of fungi

shaped like filaments. Molds are very easy to breed and often cause damage to food so that it is often used in the fermentation process.
The content in patchouli oil includes, patchouli alcohol, eugenol, benzaldehyde, cinamic aldehyde, and cadinene. But the most determining component of patchouli oil is patchouli alkhohol because it is the main characteristic [15]. Patchouli alcohol is sesquiterpene alcohol which can be isolated from patchouli oil. Not soluble in water, soluble in alcohol, ether or other organic solvents. Has a boiling point of 280.37 ° C and the crystals formed have a melting point of 56 ° C.

2. Method

Pre-distillation fermentation process using tempe mushrooms (Rhizopus oligosporus) for 2, 4, 6, 8, and 10 days followed by a distillation process at a temperature of 95 ^{0}C. The fermentation and distillation process was carried out on 2.25 kg dry patchouli per process with a composition of 1.6 kg (71%) chopped patchouli stem 5 cm - 10 cm and 0.65 kg (29%) patchouli leaves. The fermentation process used rhizopus oligosporus in anaerobic conditions with a percentage of 2 grams rhizopus oligosporus every 1 kg of patchouli raw material. The distillation process was carried out until the oil content in saturated steam has run out. The results are viewed in terms of volume/ content, distillation time and patchouli oil quality based on national patchouli oil quality standards **(SNI-06-2385-2006).**

Figure 1.. Destilator tool for research

3. Results and Discussions

Patchouli Oil Content from Post Fermentation Distillation

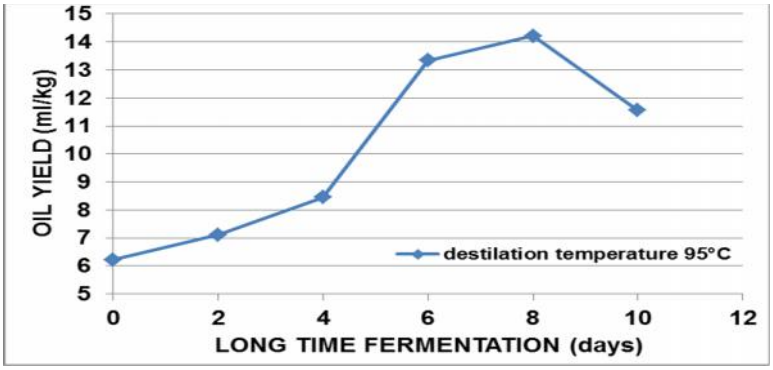


Figure 2. The oil yield of fermented patchouli oil distilled at 95 ° C

The rendemen of patchouli oil from post fermented dried patchouli distillation is shown in Figure 2.

From Figure 2, it appears that pre-distillation fermentation can increase the patchouli oil yield compared to patchouli distillation without the fermentation process. Patchouli oil yield increased with pre-distillation fermentation from 2 days to 8 days, then the rendemen droped back to 10 days fermentation. The optimal fermentation time was 8 days and then distillation at 95 °C. This is because the fermentation process for 8 days causes the glandular hair cells of the leaves and patchouli stem to be damaged by the enzyme phytase produced by tempe mushrooms. 8-day fermentation, which is the optimum fermentation time for phytase enzymes, can hydrolyze cellulose and lignin, while fermentation time of 2 days, 4 days and 6 days more occurs in phases of phytase enzyme adaptation to fermented patchouli leaves and stems. Cellulose and lignin are one of the inhibitors of the oil release from cells when distilled because the cellulose natural crystal structure is very strong and insoluble, making it difficult to hydrolyze quickly. The destruction of the cell wall and glandular hairs of the leaves and patchouli stem causes patchouli oil to separate from the leaves and patchouli stems, so that when carried out distillation at 95 °C the volume of oil that evaporates is increasing. Phytase enzymes produced by tempe mushrooms aim to degrade lignin (delignification) and hemicellulose and open the crystalline cellulose structure, so that when carried out distillation causes oil bound in vacuola, oil glands, vessels, oil sacs or glandular hair to get out more easily. So that the rendemen produced in the 8-day fermentation treatment at 95°C distillation temperature is more than that produced by fermentation 2 to 6 days. The decrease in oil yield in the 10-day fermentation treatment was due to the evaporation of some oil molecules that had low boiling points at low temperatures during fermentation and began to enter the phase of death of phytase-producing fungi, so that when the distillation process was carried out the rendemen of patchouli oil were less than those fermented 8 days. Volatile chemical compounds are acyclic hydrocarbons and isocyclic hydrocarbons, as well as hydrocarbon derivatives that have bind oxygen.

Duration of Patchouli Distillation Process After Fermentation

In general, the time needed for the distillation process after fermentation is not more than 4 hours as shown in Figure 3.

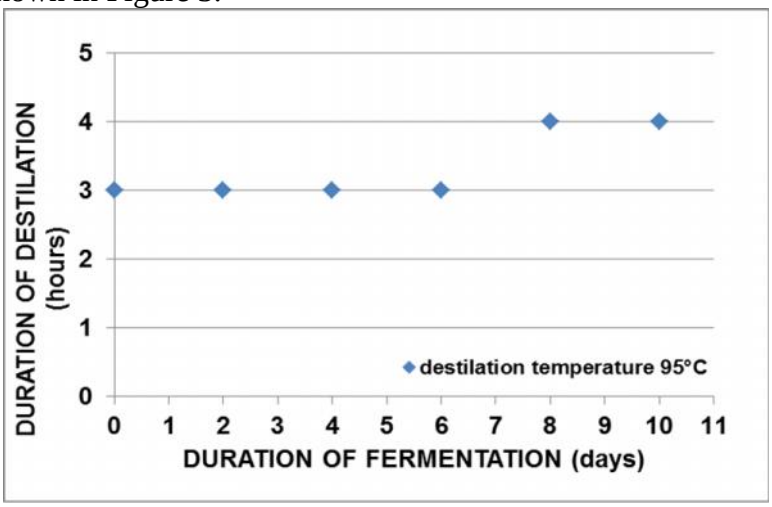


Figure 3. Effect of fermentation duration on distillation period at 95 °C

The maximum time needed for the patchouli distillation process is only 4 hours with a distillation temperature of 95 °C. The duration of fermentation 2 days, 4 days, 6 days and without fermentation requires a 3 hour distillation time. Distillation time is less than 4 hours with the rendemen of patchouli oil which is more than that which is not fermented due to the enzyme phytase produced by tempe mushrooms is capable of degrading lignin and hemicellulose and opening the crystalline cellulose structure during fermentation time. So that the time needed to release patchouli oil that is bound in vacuola, oil glands, vessels, oil bags or glandular hair of post fermentation through the distillation process is shorter.

Levels of Patchouli Alkohol (PA) in Patchouli Oil from Post Fermentation Distillation

Patchouli alcohol (PA) content is the most determining component of patchouli oil quality because it is the main characteristic [15]. PA levels in patchouli oil are considered to meet the SNI-06-2385-2006 standard if more than 30%. The results of the PA test results from pre-distillation fermented patchouli oil are shown in the following graph.

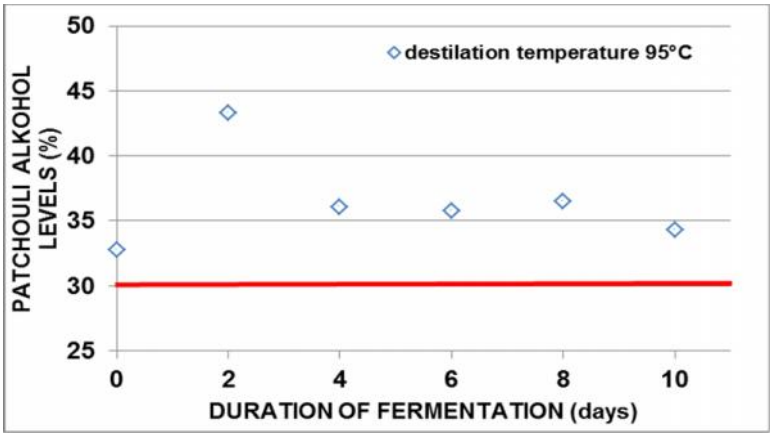


Figure 4. Level of PA distilled fermented patchouli oil at 95 ° C

Figure 4 shows that patchouli alcohol levels from all post fermented distillated oil specimens distilled at 95°C meet SNI standards. This shows that the pre-distillation fermentation process does not affect the patchouli content of patchouli oil below 30%.

Patchouli Oil Refractive Index Results of Post Fermentation Distillation

The refractive index of patchouli oil is considered to meet the Indonesian patchouli oil quality standards (SNI-06-2385-2006) if the refractive index ranges from 1.507 - 1.515 (nD20). Data on the refractive index of patchouli oil from post fermented distillation results are shown in the following graph.

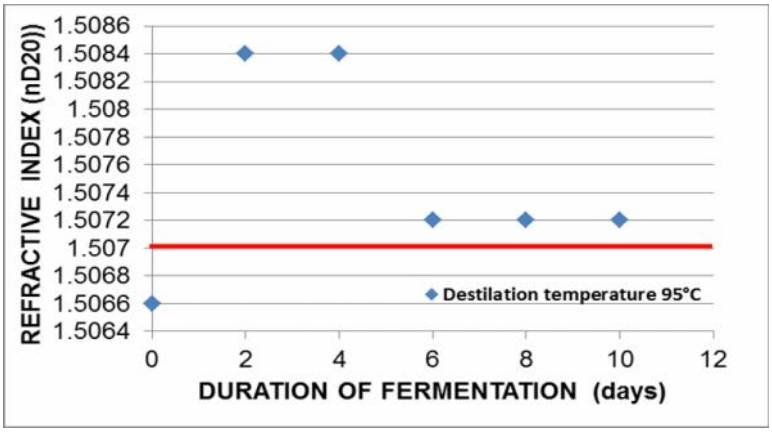
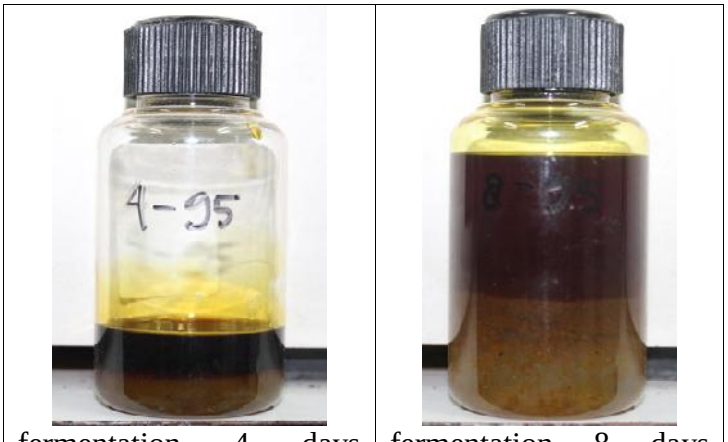


Figure 5. The refractive index of distilled patchouli oil at 95 °C after fermentation

From the graph it is known that the refractory index value of patchouli oil from distillation at 95°C post fermentation meets SNI standards. While the refractive index value of patchouli oil distilled 95°C without fermentation is below 1.507 (nD20). Patchouli oil from fermented distillation has a low refractory index. This shows that only light oil molecules are capable of being evaporated during the distillation process. While heavy molecules still cannot be released from vacuola, oil glands, vessels, oil sacs or glandular hair. From the graph also shows that the fermentation times of 6, 8 and 10 days tend to produce a lower refractive index than those fermented 2 days and 4 days. In 2 days and 4 days fermentation produces a high refractive index, which shows that the density of oil molecules is also high so that the rays are more difficult to be transmitted in the patchouli oil medium which causes the index to be high. The oil molecules density is caused by damage to oil cells due to the delignification of phytase enzymes during fermentation 2 days and 4 days so that when distilled the patchouli oil is more easily evaporated from oil cells. The refractive index of patchouli oil from distillation after 6 days, 8 days and 10 days is lower than those fermented 2 days and 4 days. This is because there has been evaporation of some oil molecules that have a low boiling point at low temperatures during fermentation, so that the resulting oil concentration is lower and the refractive index is also lower. Figure 6 shows that patchouli oil distilled from 95°C after 4 days of fermentation is much darker in color than patchouli oil which is fermented 8 days.



fermentation 4 days before distillation 95°C | fermentation 8 days before distillation 95°C

Figure 6. Patchouli oil distilled 95°C post fermentation 4 days and 8 days

Specific gravity of patchouli oil from distillation post-fermentation

Spesific gravity of patchouli oil is considered to meet the **SNI-06-2385-2006** standard if it has a specific gravity value (25°C/25°C) between 0.950 - 0.975. Data from the test results of patchouli fermented pre-distillation oil samples are shown in the graph in Figure 4.7 below.

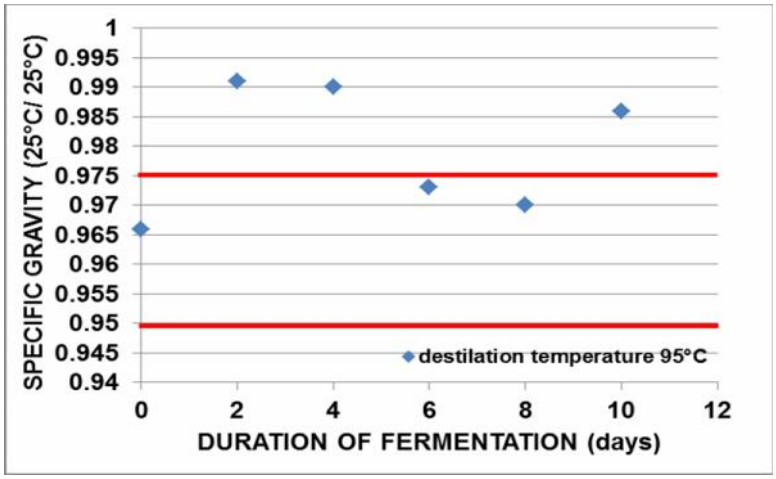


Figure 7. Specific gravity of patchouli oil distilled post fermentation which is distilled at 95°C

From the graph, it is known that not all samples of patchouli oil from the type of distillation post fermentation meet the SNI standard. The spesific gravity of patchouli oil (25°C/25°C) distilled at 95°C post fermentation of 6 days and 8 days and patchouli oil without fermentation is between 0.95 - 0.975 which means that it meets SNI standards. While the spesific gravity of patchouli oil fermented 2 days, 4 days and 10 days distilled 95°C is above 0.975.

4. Conclusions

1. Pre-distillation fermentation process can increase the patchouli oil yield compared to non-fermented patchouli distillation for distillation temperatures of 95°C. Patchouli oil yield rises with increasing fermentation time from 2 days to 8 days, then the oil yield decreases again in 10 days fermentation.
2. The 2-day, 4-day, 6-day fermentation duration and without distilled fermentation at 95°C requires a 3-hour distillation time. While the fermentation time of 8 days and 10 days requires distillation to 4 hours
3. Pre-distillation fermentation process does not have an effect on decreasing patchouli content of patchouli oil below 30%, which means it still meets SNI standards.
4. The refractive index value patchouli oil from distillation at 95°C post fermentation of 2 days to 10 days is between 1.507 - 1.515 (nD20) which means that it meets SNI standards. While the refractive index value of patchouli oil distilled 95°C without fermentation is below 1.507 (nD20).
5. Specific gravity of patchouli oil distilled at 95°C post fermentation of 6 days and 8 days, and patchouli oil without fermentation is between 0.95 - 0.975 which means that it meets SNI standards. While the spesific gravity of patchouli oil fermented 2 days, 4 days and 10 days distilled 95 ° C is above 0.975.

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