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SOLID STATE FERMENTATION OF TURMERIC RHIZOMES WITH *ASPERGILLUS SP.* TO IMPROVE YIELD AND COMPOSITION OF EXTRACTED TURMERIC OIL

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ABSTRACT: This research aimed to determine the role of *Aspergillus awamori*, *Aspergillus niger*, and *Aspergillus oryzae* in degrading starch on turmeric rhizome substrate to increase the yield of turmeric oil. The substrate in the form of turmeric rhizome was given additional yeast extract of 10% weight per volume to meet the nutritional needs of fungal growth. The fungal concentration used in inoculation was $5x10^7$ cells/ml. The solid-state fermentation process was carried out in dark conditions (~0 W), temperatures of 25–28 ºC, 99% humidity, and aeration (3.5 L/min). Turmeric oil was extracted using a steam distillation method for three hours, with the substrate moisture content of 68–71% and a substrate–water ratio of 1:5. The biodegradation process was conducted for 11 days. The starch content and turmeric oil yield was determined during the fermentation particularly on days 7, 9, and 11. The results showed that the biodegradation process of starch in solid-state fermentation succeeded in increasing the yield of turmeric oil. *Aspergillus awamori* showed the most desirable starch degradation activity by 62.5% to 2.9% wet weight on the 11th day of fermentation. *Aspergillus oryzae* had the most positive effect, nearly doubling the turmeric oil yield to 3.17% dry weight after 11th day of fermentation. The main constituents of turmeric oil are β-turmerone, α-turmerone, and ar-turmerone.

ABSTRAK: Penelitian ini bertujuan bagi mengkaji peranan Aspergillus awamori, Aspergillus niger, dan Aspergillus oryzae dalam mendegradasikan kanji pada substrat rizom kunyit bagi meningkatkan hasil minyak kunyit. Substrat dalam bentuk rizom kunyit telah diberi tambahan ekstrak yis 10% mengikut berat setiap isipadu bagi memenuhi keperluan nutrisi pertumbuhan kulat. Kepekatan kulat yang digunakan dalam inokulasi adalah 5x107 sel/ml. Proses penapaian berkeadaan pepejal telah dijalankan dalam keadaan gelap (~0 W), suhu 25–28 ºC, kelembapan 99%, dan pengudaraan (3.5 L/min). Minyak kunyit diasingkan menggunakan kaedah penyulingan wap selama tiga jam, dengan kandungan lembapan substrat 68-71% dan nisbah substrat-air 1:5. Proses biodegradasi dijalankan selama 11 hari. Kandungan kanji dan hasil minyak kunyit ditentukan semasa penapaian terutamanya pada hari ke-7, 9, dan 11. Hasil kajian menunjukkan bahawa proses biodegradasi kanji dalam penapaian berkeadaan pepejal berjaya meningkatkan hasil minyak kunyit. Aspergillus awamori menunjukkan aktiviti degradasi kanji yang paling diingini iaitu sebanyak 62.5% hingga 2.9% berat basah pada hari ke-11 penapaian. Aspergillus oryzae mempunyai kesan yang paling positif, iaitu hampir dua kali ganda hasil minyak kunyit kepada 3.17% berat

kering selepas hari ke-11 penapaian. Konstituen utama minyak kunyit ialah β-turmerone, α-turmerone, dan ar-turmerone.

KEY WORDS: *Aspergillus sp., biodelignification, starch, turmeric rhizome, turmeric oil*

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) is an herbaceous plant that has been widely used as a condiment and in medicine in Asia since ancient times [1]. The main part of the turmeric plant is the rhizome which contains various types of compounds including curcuminoid compounds, turmeric oil, and oleoresin [1]. It has been reported that the rhizome contains 5-6% essential oil [2] that is commonly used in food, cosmetics, and pharmaceutical applications due to its anticancer, anti-inflammatory, antibacterial, antifungal, and antitumor effects [3-4]. Turmeric is widely cultivated in Indonesia with a total cultivation area of 7,481 ha being reported in 2018 [5]. Nevertheless, valorization of the rhizomes to produce turmeric oil is still considered to be very limited. Based on the data of the Indonesian Central Bureau of Statistics, large-scale turmeric oil processing is still in the development stage since 2018. In 2020, a production plant for producing turmeric oil was built in Pacitan, East Java, Indonesia, with a production capacity of 2.4 tons/year, relatively small as compared to a total rhizome production of approximately 193,000 tons/year [5]. This is due to the difficulty of isolating turmeric oil during the distillation process, which results in low oil recovery of approximately 0.46% [6-7].

Turmeric oil in the rhizome is located in secretory cells surrounded by starch granular parenchymal tissue, which can inhibit the extraction of turmeric oil. Various studies addressing the turmeric oil extraction process have been carried out. One example is a pre-treatment using enzymes to degrade polysaccharide compounds on cell walls so that oil yield can increase by 70% [8]. The enzymes are expensive and, therefore, in the large-scale production of turmeric oil, are considered to cause a significant increase in production costs [9]. Microorganisms, especially fungi, can be an alternative solution to increase the yield of turmeric oil while still saving production costs. Fungi can produce various types of extracellular enzymes that can degrade polysaccharide and consequently increase oil yield without using commercial enzymes [10]. Fungi are used because they have complete enzymatic devices, filamentous bodies that can penetrate the substrate, and accumulated ability [11].

Fungi from genus *Aspergillus*, such as *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus awamori*, are capable of being starch-degrading agents in turmeric because they are able to produce polysaccharide-breaking hydrolytic enzymes, such as amylase enzymes [12-16]. These three specific types of fungi were selected because they could survive the antimicrobial compounds of turmeric [4]. Therefore, this study was conducted to determine the species of *Aspergillus* and fermentation time that can degrade starch efficiently and produce a relatively high yield ($\geq$ 2.5%) of turmeric oil. An average yield of 2.5% is considered satisfactory for commercial productions [17].

2. MATERIALS AND METHODS

2.1. Preparations of Turmeric Rhizomes

The turmeric rhizomes used in this study were harvested at the age of 8 months from Cimasuk, Tanjung Sari, Sumedang Regency, West Java, Indonesia. They were then washed

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and sliced to a thickness of ± 0.1 cm. After that, all rhizomes were stored at room temperature (25-28 ºC) and relative humidity (50-70%).

2.2. Cultivation of *Aspergillus sp.* Inocolum

Stocks of *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus awamori* were obtained from the Microbiology Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung. Each species of fungi was cultivated in a semisolid potato dextrose agar (PDA) medium with a concentration of 39 g/L [15]. The PDA medium was sterilized using an autoclave (121 ºC, 1.5 atm). The fungi were cultivated for 4 days in a test tube at room temperature until the active fungi spores covered the entire surface of the medium.

2.3. Preparation of *Aspergillus sp.* Inocolum

The 4-day old *Aspergillus* sp. cultures were harvested with harvesting solution (0.85% NaCl + Tween80 0.1%) to obtain a 600 mL inoculum solution with a concentration of $5x10^7$ spores/ml [16, 18]. Measurement of the spore concentration was conducted using a hemocytometer under a microscope. The cell chamber of the hemocytometer was filled with a 1 mL aliquot of the spore solution. Appropriate dilutions of the aliquot were done when there were too many spores to count by eyes. The resulting number from cell counting was then multiplied by the dilution factor.

2.4. Solid-state Fermentation of Turmeric Rhizome with *Aspergillus sp.*

The fermentation process was carried out under aseptic conditions. The inoculum solution and yeast extract solution were mixed with the sterilized turmeric rhizome in the following proportions: 1 ml inoculum solution, 1 ml yeast extract solution, and 10 g turmeric rhizome [18]. The fermentation process took place in a fermenter tray (50 × 30 ×15 cm) with perforation to remove excess liquid. The fermenter tray was sealed with plastic wrap and black color plastic, so that fermentation could take place at a light intensity of ~0 W [16]. Aeration was given every 2 days for 5 minutes, at a rate of 3.5 L/min. Samples were stirred every 2 days. The fermentation process was carried out by varying the incubation time of 7, 9, and 11 days.

2.5. Extraction of Turmeric Oil using a Steam-Distillation Method

Fermented turmeric rhizome was dried using an oven at 50 ºC for 24–26 hours to reach a moisture content of 68–71% [19]. Moisture content was measured using a Mettler Toledo Infrared Moisture Content Analyzer. Turmeric oil was extracted using a steam distillation method with a ratio of 100 g substrate to 500 mL of distilled water for 3 hours [20-23]. Distillates were collected in a 500 mL separating funnel, and the oil and hydrosol were separated. The obtained turmeric oil yield was calculated using Eq. (1):

$$Yield\ (\%w_d) = \frac{m_{m\,(g)}}{w_d(g)} \times 100 \quad (1)$$

where m_m is the mass of oil in grams, and w_d is the dry weight of the substrate in grams. The value of w_d can be calculated using Eq. (2):

$$w_d(g) = (1 - moisture\ content) \times m(g) \quad (2)$$

where moisture content is in percent, and m is the substrate mass in grams at the initial moisture content (~90%).

2.6. Determination of Turmeric Rhizome Starch Content

The starch content was determined using a Luff Schoorl method (SNI 01-2891-1992). The preparation of the Luff Schoorl reagent was carried out by mixing the following: 143.8 g

of anhydrous Na_2CO_3 in 300 mL of distilled water, 50 g of citric acid in 50 mL of distilled water, and 25 g of $CuSO_4.5H_2O$ in 100 mL of distilled water. The solution was stirred until homogeneous.

Next, 3% HCl (200 mL) was added to 5 g of fermented turmeric rhizome. The mixture was boiled using a reflux extractor for 3 hours and then cooled down, and the pH was adjusted to 7.0 using a 30% NaOH solution. The solution was diluted to a volume of 500 mL and filtered. 10 mL of this filtrate was mixed with 25 mL of the Luff Schoorl reagent and 15 mL of distilled water and then boiled for 10 minutes using an upright cooler. 15 mL of KI 20% and 25 mL of 25% H_2SO_4 were slowly added. The final solution was titrated with $Na_2S_2O_3$ (0.1 N), and its volume increase was measured. Starch solution 0.5% weight–volume (w/v) was used as an indicator.

The calculation of starch content was carried out using Eq. (3), where w_1 is the sample mass of turmeric rhizome (mg), w is glucose (mg) contained for every milliliter of $Na_2S_2O_3$ used, f_p is a dilution factor, and the value 0.9 is a glucose conversion factor for starch. The value of w was determined using the sugar conversion table according to SNI.

$$\text{Starch content (\%)} = \frac{0.9w_1\ (mg) f_p}{w\ (mg)} \times 100\% \quad (3)$$

2.7. Characterization of Turmeric Oil

The oil characteristics were determined particularly color, refractive index, and density. The color of turmeric oil was observed subjectively by eye. The refractive index value of turmeric oil was tested using a refractometer. The density was determined by measuring the volume v of oil (mL) using a measuring cup and oil mass m (g) on the analytic balance and calculated using Eq. (4).

$$\text{Density} = \frac{m_{turmeric\ oil}\ (g)}{V_{turmeric\ oil}\ (mL)} \quad (4)$$

2.8. Determination of composition using a Gas Chromatography-Mass Spectrometry

The composition of turmeric oil was analyzed using a gas chromatography–mass spectrometry (GC–MS) device. The initial temperature was set at 60 ºC, then raised to 280 ºC at a rate of 8 ºC/min. The samples were injected in split mode with a split ratio of 200. The rate of the column was set to 1.31 mL/min with a linear speed of 41.7 m/s. The analysis was carried out at the Instrumental Chemistry Laboratory, Universitas Pendidikan Indonesia, Bandung, Indonesia.

3. RESULTS AND DISCUSSIONS

3.1. Effects of Moisture Content on Turmeric Oil Yield

The effects of moisture content on the yield of turmeric oil are shown in Fig. 1. A dry weight base was used for this study so that the values of different moisture content could be compared. The results indicate that the yield of turmeric oil increases with decreasing moisture content of the material before decreasing after reaching an optimum moisture content. A lower moisture content requires longer drying time and consequently more turmeric oil is susceptible to evaporation during the drying process. The turmeric oil yield with 87.9% moisture content was 1.27% dry weight (dw). Lower moisture content in the range of 74–78% resulted in more turmeric oil. Specifically, the yield of turmeric oil at 77.9% moisture content was 1.70% dw, and at 74.3% moisture content, it was 1.69% dw. The optimum moisture content was at 69.2% (obtained after drying for 24 hours) which resulted in a yield of 1.89% dw.

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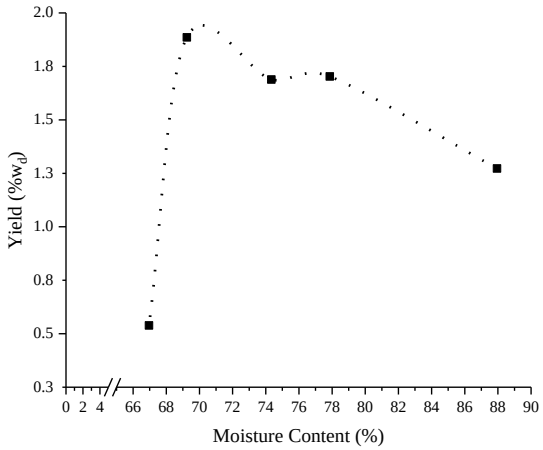

Fig. 1. Effects of moisture content on turmeric oil yield.

The drying process was carried out using an oven at 50 ºC. The selection of the drying method considered two important parameters that affected the extraction of essential oils, namely drying time, and temperature [24]. The higher the temperature in the drying process, the lower the yield of essential oil due to evaporation. According to Hamrouni-Sellami et al. [25], the drying process at a temperature of 50 ºC can produce a high amount of volatile oils, while temperature above 50 ºC will reduce their yield. For medicinal plants, high temperatures (>50 ºC) may cause a drastic decrease in their essential oil yield. An increase in temperature by 20 ºC can cause the loss of essential oils up to 92.3% [25]. This is because high temperatures can damage the biological structure of oil glands and epithelium cells in medicinal plants and reduce the permeability of plasma membranes [25].

The effect of drying time on the yield of essential oils is related to the rate of evaporation of essential oils, which is greater with the length of the drying time. For example, the process of drying *Tymus* subsp. *Doenensis* leaves uses the shade drying method, which causes the reduction of essential oils to increase. The yield of essential oils by drying treatment using an oven at 50 ºC amounted to 1.46%, while the yield by shade drying was only 0.91%. This is due to the long drying time that caused the loss of essential oils as they diffused into the air [24].

The moisture content measured in this study ranged from 66–87% (Fig. 1). A moisture content of 69.24% resulted in the optimal yield of turmeric oil of 1.89%. After reaching the optimum yield at 69% moisture content, the oil yield decreased as the moisture content was reduced. The dried rhizome to 66.96% moisture content resulted in a yield of only 0.54% dw due to the length of drying time, which caused longer evaporation of turmeric oil. The high yield of turmeric oil obtained at 69% moisture content or 24 hours drying (1.89% dw) is in accordance with the literature, which states that drying for 24 hours will result in the extraction yield of essential oils from rhizome plants in the range of 1.6–2.2% [26]. Hence, a moisture content range of 68–71% was used in this study.

3.2. Effects of Fermentation Time on Starch Content of Biodegraded Rhizomes and Turmeric Oil

The test results on the content of turmeric rhizome starch fermented by the fungus *Aspergillus* sp. are shown in Fig. 2. The content of the control rhizome starch (day 0 of fermentation) was measured to be 7.8% wet weight (ww). The starch content in fermented rhizomes by all three species showed the same tendency. The content of rhizome starch

continued to decrease along with the length of fermentation time. In the fermentation process using the *Aspergillus niger*, starch content decreased in the range of 14.7–30.6% ww to 6.6% ww on the 7th day, 5.8% ww on the 9th day, and 5.4% ww on the 11th day. Meanwhile, the decrease of starch content in the fermentation by *Aspergillus oryzae* was 11.8–59.3% ww to 6.9% ww on the 7th day, 4.2% ww on the 9th day, and 3.2% ww on the 11th day. Fermentation using *Aspergillus awamori* showed a better starch degradation activity. *Aspergillus awamori* successfully reduced starch in the range of 31.8–70.6% with the starch content to decrease to 5.4% ww on the 7th day, 3.1% ww on the 9th day, and 2.9% ww on the 11th day.

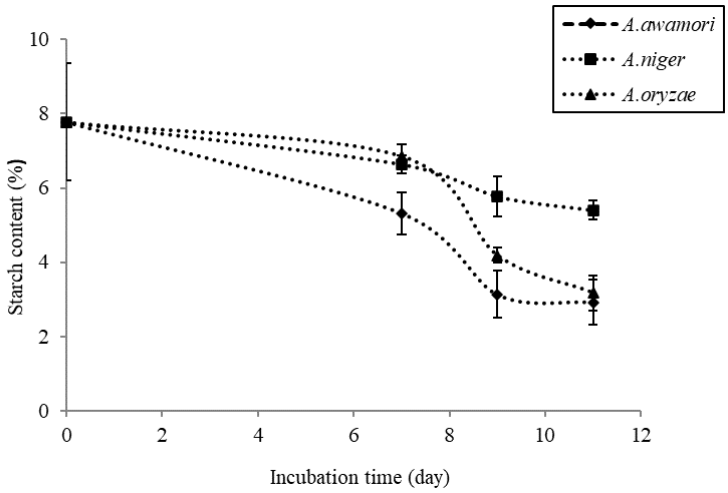

Fig. 2. Effects of fermentation on rhizome starch content.

The profile of turmeric oil obtained in this study is shown in Fig. 3. Turmeric oil yield for the control sample (day 0 of fermentation) was 1.06% dw. The fermentation process of each fungal species yielded an increase in turmeric oil with the increasing fermentation time. The *Aspergillus oryzae* showed a significant increase in the yield of turmeric oil compared to the other two fungal species. The 7th day fermentation resulted in the acquisition of turmeric oil by 2.17% dw and continued to increase to 2.60% dw on the 9th day and 3.17% dw on the 11th day. The fermentation process using *Aspergillus awamori* produced turmeric oil of 2.38% dw on the 7th day, 2.50% dw on the 9th day, and 2.62% dw on the 11th day. Meanwhile, fermentation using *Aspergillus niger* had the lowest yield of 1.44% dw on the 7th day, 1.82% dw on the 9th day, and 1.92% dw on the 11th day.

According to the study by de Castro [27], the amylase hydrolytic enzyme group stabilizes after 4 days of fermentation and reaches a maximum point after 6 days. The test results of the control rhizome starch content (Fig. 2) of 7.8% ww were similar to the research of Kusbiantoro and Purwaningrum [28], which obtained the value of turmeric rhizome starch content of 8% ww. A decrease in the starch content occurs with the ongoing fermentation process, owing to the growth and metabolic activity of the fungus *Aspergillus* sp.

Hydrolytic enzymes, especially amylase, are enzymes that correlate closely with growth [26]. *Aspergillus* spp. secrete enzymes as an effort to extract nutrients that support colony growth and regulation of metabolism [29]. The secretion of hydrolytic enzymes in the process of metabolic fungi results in the cutting of the glycosidic bonds of starch compounds on the cell wall, causing the breakdown of complex compounds into simpler ones, namely glucose, and a decrease in starch content [29-30]. It is known from the research of Reyes et al. [31] that the fermentation time of 3 days is sufficient to degrade starch granules in the material.

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The previous study used pure starch whereas this study used turmeric rhizome as the substrate, which is a complex medium. In complex media, fungi consume simple sugars at the initial time to support their growth before finally degrading starch complexes. Therefore, they take longer to degrade than pure starch substrates [32].

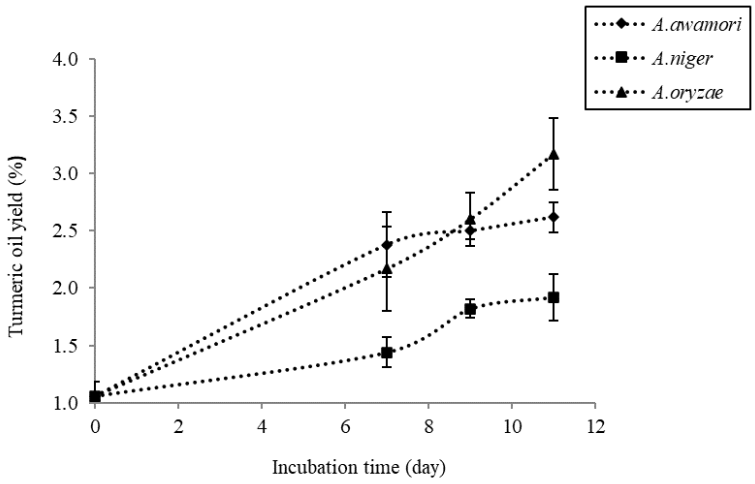

Fig. 3. Effect of fermentation on turmeric oil yield.

In this study, the growth of fungal hyphae was not seen until it reached the 2nd day. Aeration to the system was given after the emergence of hyphae, which was evenly distributed on the 2nd day of fermentation to allow the fungal hyphae to grow around the entire surface of the turmeric rhizome in the fermenter tray. More evenly distributed fungal growth was observed qualitatively on the 3rd to 9th day. After the 9th day, there was no mycelium growth observed owing to the absence of any remaining organic matter in the turmeric rhizome that *Aspergillus* could take as nutrition. In addition, the metabolic activity of the fungus allowed a decrease in pH on the substrate, so that the growing environment was no longer suitable for fungi [33]. In other words, fungal growth had entered a decreasing phase in which metabolic activity did not degrade starch [33].

The results of the starch content revealed that the *Aspergillus awamori* fungus showed the greatest reduction in starch content relative to the other two species, as shown in Fig. 2. This was predicted to occur because of the differences in the enzyme activity of the three species. The amylase enzyme activities measured in solid-state fermentation systems by *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus awamori* were 467.8, 500, and 589.7 IU/g, respectively [34-35]. Greater enzyme activity in *Aspergillus awamori* causes the fungus to degrade more starch [36]. *Aspergillus awamori* can degrade starch in the range of 31.8–70.6%, with a maximum decrease occurring on the ninth day of 62.5%. This data is in accordance with the results of research by Umsza-Guez et al. [37], which states that *Aspergillus awamori* fungi can degrade up to 64% in a span of six days in solid-state fermentation systems.

The increase in turmeric oil yield was caused by the decreased presence of starch in the rhizome cell wall that had been degraded. The *Aspergillus* fungal metabolism results in secretory cells in the parenchyma tissue, that had been initially protected, to become more open. Changes in the structure around the secretory cells allow the turmeric oil to be more easily extracted [29, 38]. The results of fermented turmeric oil measurements in Fig. 3 show that the fermentation with *Aspergillus oryzae* produced the largest yield of turmeric oil. Even though the degradation of starch produced by *Aspergillus oryzae* was lower than *Aspergillus awamori*, the yield of oil produced was higher. During fungal metabolism, other hydrolytic

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enzymes are involved that can degrade complex compounds other than starch on cell walls, such as endo-1, 4-β-glucosidase, or β-glucosidase, which act as catalysts in degrading cellulose [39].

3.3. Characteristics and Composition of Turmeric Oil

The physical-chemical characteristics of turmeric oil obtained in this study are shown in Table 1. Turmeric oil from fermented rhizome was yellow in color with a density of 0.89 g/mL. The measured refractive index of turmeric oil was 1.45. The results are in accordance with the results of a previous study by Naibaho [40] and comply to the national standard in Indonesia. The composition of turmeric oil is shown in Table 2. This study found 30 components of constituent compounds of turmeric oil that comprise monoterpenoids and sesquiterpenoids. The main compounds found were ar-turmerone, α-turmerone, and β-turmerone. In general, the solid-state fermentation process caused some components of minor compounds not to be detected in turmeric oil. Fermentation by different *Aspergillus* sp. resulted in varied amounts of components of the main compounds in turmeric oil.

Table 1: Physical-chemical characteristics of turmeric oil

Characteristics	This study	SNI	Reference [40]
Color	Yellow	Yellow Orange	-
Density (g/mL)	0.89 ± 0,02	0.82-0.92	0.92
Refractive index	1.45 ± 0.07	1.46-1.47	1.47

The main compounds of fermented turmeric oil are ar-turmerone, 10.4%, α-turmerone, 16.2%, and β-turmerone, 33.8%. In the fermentation using *Aspergillus awamori*, there was a decrease in the ar-turmerone compound but an increase in α-turmerone and β-turmerone compounds. The ar-turmerone compound was measured in the range of 1.6–9.4%. The α-turmerone compound was measured in the range of 14.5–18.1%. The β-turmerone compound was measured in a larger range, 41.9–48.5%. The fermentation process using *Aspergillus niger* showed the same tendency. The ar-turmerone compound decreased to 0.98–9.88%. The α-turmerone and β-turmerone compounds were measured in the range of 11.59–15.29% and 20.97–45.36%, respectively. *Aspergillus oryzae* had a different influence on the content of the main compounds of turmeric oil. The fermented turmeric rhizome using *Aspergillus oryzae* did not have any detrimental effect on the content of the main compounds. Fermentation using *Aspergillus oryzae* increased ar-turmerone, α-turmerone, and β-turmerone compounds to the ranges of 10.09–13.50%, 14.47–16.38%, and 26.07–37.52%, respectively.

Turmeric oil from the biodegraded rhizomes of the three species generally had a relatively similar compound-content profile. β-turmerone was the highest concentration, followed by α-turmerone, then ar-turmerone. *Aspergillus niger* and *Aspergillus awamori* had relatively the same effect on changes in turmeric oil compound concentrations: decreasing the concentration of ar-turmerone on the seventh day, before an increase due to the transformation of α-turmerone and β-turmerone. However, the treatment using *Aspergillus oryzae* produced turmeric oil with decreases in ar-turmerone.

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Table 2: Composition of turmeric oil for fermented rhizome with *Aspergillus* sp.

Compounds	Percentage of Compounds (%)									Ref.
	Fermentation Day-7			Fermentation Day-9			Fermentation Day-11			
	AN	*AO*	*AA*	*AN*	*AO*	*AA*	*AN*	*AO*	*AA*	[3,4
α-turmerone	15.3	16.4	14.6	15.1	15.1	16.8	11.6	14.5	18.1	13
β-turmerone	45.4	37.5	48.5	34.1	36.2	41.8	21.0	26.1	46.2	7.1
ar-turmerone	1.0	13.5	1.6	9.9	10.2	9.4	6.3	10.9	2.8	17-
1-phellandrene	0.8	0.5	0.6	1.7	0.8	1.5	0.6	0.4	1.0	0.
α-terpinolene	0.2	-	0.2	0.4	0.2	0.3	0.2	-	0.3	0
trans-caryophyllene	0.8	0.8	0.8	0.8	1.1	0.6	0.6	0.4	0.6	2.
zingiberene	2.5	1.3	2.1	2.8	1.9	3.5	1.7	1.7	2.1	0.2-
α-bisabolene	0.6	-	-	0.6	0.3	-	0.4	-	-	0
β-bisabolene	0.8	0.3	0.7	0.7	0.6	0.5	0.5	0.4	0.5	0.
β-sesquiphellandrene	2.5	1.4	2.2	2.4	1.9	1.9	1.7	1.6	1.8	5.6
(-)-caryophyllene oxide	-	-	1.0	-	-	0.5	-	-	-	
β-myrcene	-	-	-	-	-	-	-	-	-	0
δ-3-carene	-	-	-	-	-	-	-	-	-	0
α-terpinene	-	-	-	-	-	-	-	-	-	1
1,8-cineole	-	-	-	0.2	-	0.1	-	-	-	0.9
γ-terpinene	-	-	-	0.1	-	-	-	-	-	
ar-curcumene	2.5	1.8	-	1,8	2.2	-	1.4	1.3	-	1.4
γ-curcumene	-	-	-	-	-	-	-	-	0.1	
α-santalol	0.3	-	-	0.3	0.5	3.7	0.2	1.2	-	0
β-santalol	-	-	-	-	-	-	-	-	-	
(-)-α-pinene	-	-	-	-	-	-	-	-	-	0
α-patchoulene	-	-	-	-	-	-	-	-	0.4	
(+)-α-atlantone	-	-	1.3	-	-	2.3	-	-	4.7	
β-himachalene	-	-	-	-	-	0.1	-	-	-	
farnesol	-	-	-	-	-	0.1	-	-	0.1	

AN: *A. niger*, AO: *A. oryzae*, AA: *A. awamori*

The concentration of each of the three main compounds in turmeric oil, as a result of the biodegradation of the three *Aspergillus* spp., increased and decreased on different fermentation days. The increase in the content of the antioxidant compounds can be due to starch degradation and other structural polymers that made antioxidant compounds more accessible during distillation [45]. However, in all samples with 11 days of fermentation time, there was no increase in the content of α-turmerone and β-turmerone compounds, owing to lower starch content, compared to the other timeframes.

With a higher concentration of ar-turmerone, a decrease in the content of α-turmerone and β-turmerone was found. α-turmerone and β-turmerone are unstable compounds that will turn into a more stable aromatic form (ar-turmerone) with continuous exposure to air. The content of ar-turmerone compounds decreased in turmeric oil by biodegradation treatment of the three *Aspergillus* spp., a genus that can carry out biotransformation of ar-turmerone to a more oxidized form [46]. This decrease was discovered on days 7 and 11 for *Aspergillus niger* and *Aspergillus oryzae* and day 9 for *Aspergillus awamori*. Previous studies reported that the largest compound in turmeric oil was ar-turmerone, followed by α-turmerone and β-turmerone in relatively equal amounts [3, 41]. Such results are slightly different with the findings obtained in this study and may be caused by several factors, such as geographical origins, microclimate conditions, soil content, and the differences of turmeric root age [41-47].

4. CONCLUSION

The treatment of biodegradation in turmeric rhizomes using fungi from the genus *Aspergillus* can reduce starch content in turmeric rhizomes, thereby increasing the yield of turmeric oil. *Aspergillus awamori* reduced starch content more than the other two species,

reaching 2.9% ww. *Aspergillus oryzae* resulted in the highest turmeric oil yield compared to the other two species of 3.2% dw after 11 days of fermentation. Of the three *Aspergillus* spp., the greatest decrease in starch content and increase in the oil yield occurred on the 9th day. Incubation of turmeric rhizomes by *Aspergillus* reduced the content of ar-turmerone but increased the content of α-turmerone and β-turmerone compounds, which could be transformed into ar-turmerone. The use of *Aspergillus oryzae* as a biological agent for the biodegradation process showed the most positive influence on the extraction of turmeric oil compared to the other two species, with the maximum increase in oil yield and decrease in starch content on the 11th day.

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