

## BIODELIGNIFICATION OF LEMON PEELS USING *Aspergillus sp.* TO IMPROVE YIELD AND COMPOSITION OF EXTRACTED LEMON OIL

MUHAMMAD YUSUF ABDUH\*, ENJELINA NABABAN, FIRDANTA GINTING,  
JENNY JULIATI AND HUSNA NUGRAHAPRAJA

*School of Life Sciences and Technology, Institut Teknologi Bandung (ITB)  
Jalan Ganesha No. 10 Bandung 40132 Indonesia*

*\*Corresponding author: yusuf@sith.itb.ac.id*

*(Received: 24<sup>th</sup> December 2019; Accepted: 12<sup>th</sup> April 2020; Published on-line: 4<sup>th</sup> July 2020)*

**ABSTRACT:** Lemon oil obtained from lemon peels has a high market value. However, the presence of lignocellulose composed of cellulose, hemicellulose, and lignin, can inhibit the extraction process. This study aimed to determine the effect of biodelignification on lemon peels using *Aspergillus sp.* towards lignin content, yield, chemical composition, and productivity of lemon oil. A solid-state fermentation was carried out under controlled relative humidity of 99% and a light intensity of  $\sim 0$  W/cm<sup>2</sup> for 0, 3, 6, and 9 days. The number of spores used was in the range of  $0.3-5 \times 10^5$  spores/gram substrate with a ratio of spore solution and substrate of 1:1. Extraction was performed using a steam distillation method at 97-98°C for 6 hours. The results showed that the lignin content decreased with an increased fermentation time: 8.01%, 6.97-7.28%, 5.83-7.28%, and 4.35-5.44% dry weight for day 0, 3, 6, and 9, respectively. Lemon oil yield increased as the period of fermentation increased up to 0.27%, 0.29-0.31%, 0.30-0.46%, and 0.67-0.79% for day-0, 3, 6, and 9, respectively. A major component of lemon oil is d-limonene. The d-limonene content reached 72,54% for day-0, 73-99% for day-3, 75,09-84.59% for day-6, and 88,03-99% for day-9.

**ABSTRAK:** Minyak lemon yang terhasil dari kupasan lemon mempunyai nilai tinggi dalam pasaran. Walau bagaimanapun, kehadiran lignoselulosa yang terdiri daripada selulosa, hemiselulosa dan lignin, boleh merencatkan proses pengekstrakan. Kajian ini bertujuan memperolehi kesan biodelignifikasi pada kulit lemon menggunakan *Aspergillus sp.* terhadap kandungan lignin, hasil, komposisi kimia, dan penghasilan minyak lemon. Penapaian keadaan-pepejal dijalankan di bawah 99% kawalan kelembapan relatif dan keamatan cahaya  $\sim 0$  W/cm<sup>2</sup> bagi 0, 3, 6 dan 9 hari. Bilangan spora yang digunakan adalah dalam lingkungan substrat  $0.3-5 \times 10^5$  spora/gram dengan nisbah larutan spora kepada substrat adalah 1:1. Pengekstrakan dijalankan menggunakan kaedah penyulingan stim pada suhu 97-98°C selama 6 jam. Keputusan menunjukkan kandungan lignin berkurangan dengan pertambahan masa penapaian: 8.01%, 6.97-7.28%, 5.83-7.28%, dan 4.35-5.44% berat kering pada hari 0, 3, 6, dan 9, masing-masing. Hasil minyak lemon bertambah dengan pertambahan masa penapaian sehingga 0.27%, 0.29-0.31%, 0.30-0.46%, dan 0.67-0.79% pada hari 0, 3, 6, dan 9, masing-masing. Komponen major minyak lemon adalah d-limonina. Kandungan d-limonina mencapai 72,54% pada hari-0, 73-99% pada hari-3, 75,09-84.59% pada hari-6, dan 88,03-99% pada hari-9.

**KEYWORDS:** *Aspergillus sp.*; biodelignification; lemon oil; lemon peels; lignin content

## 1. INTRODUCTION

Essential oils are volatile aromatic compounds that can be obtained from natural sources, usually plants [1]. Essential oils contain the characteristic fragrance of the plant from which it is derived. The market of essential oils was valued at USD 3.4 billion in 2016 and it was projected to expand at a Compound Annual Growth Rate (CAGR) of 9.7% from 2016 to 2024 [2]. This growth was driven by increasing market demand of fragrance and flavouring.

Essential oils have high economic potential in Indonesia, one of the major sources of raw materials for essential oils including lemon [3]. Lemon (*Citrus limon* (L.) Osbeck) is an evergreen tree that belongs to the family Rutaceae with an annual production of 123 million metric ton [4]. Most lemon processing industries used lemon flesh and juice as sellable products, but other parts were thrown away as wastes. The wastes consist of peel, seed, and leftover of lemon juice [5]. The lemon peels can be further valorised as a raw material for producing essential oil, particularly lemon oil.

Lemon oil is one of the essential oils that have high demand worldwide. In 2007, lemon oil world production reached up to 9,200 metric tons [6] and the market is anticipated to expand at a 5.5% CAGR for the period of 2018-2027 [7]. Lemon oil is typically produced via steam distillation of the lemon peels and primarily composed of d-limonene which lies in the range of 72.4-94.5% [8,9]. Limonene is a relatively stable monoterpene that can find applications as a stress reliever, antibacterial, antifungal, antioxidant, and neuroprotection [10].

One of the key issues in the production of lemon is its relatively lower yield which lies in the range of 0.65-1.3% on a dry weight basis [9] due to the presence of the rigid structure of the cell wall that consists of lignin, cellulose, and hemicellulose. According to Janati et al. [11], lemon peel contains approximately 15.2% lignin which will hinder the isolation of lemon oil during the distillation process. The presence of lignin in lemon peel may be degraded by the use biological agents such as fungus to increase the lemon oil yield.

*Aspergillus sp.* can degrade complex carbohydrates into monosaccharides and disaccharides. *Aspergillus sp.* is one of the most effective fungi in terms of utilizing simple saccharides as an organic carbon source to help its growth [12]. Therefore, *Aspergillus sp.* is expected to degrade lignin and carbohydrates in order to facilitate the isolation of lemon. *Aspergillus sp.* is reported to contain lignin degradation enzymes such as laccase, lignin peroxidase, and manganese peroxidase [13-15].

A previous study on the solid-state fermentation of *Aspergillus niger* with coir waste had been carried out by Mrudula and Murugammal [16] for optimum production of cellulase. The fermentation was carried out at pH 6, 30°C, and moisture ratio of 1:2 (weight: volume) for 72 hours to optimize the biosynthesis of cellulase with an enzyme activity of 8.89 U per g of dry mycelial bran. Botella et al. [17] also has investigated the effect of particle size, initial moisture, and supplementation with carbon sources for the solid-state fermentation of grape pumice with *Aspergillus awamori* to produce high-value hydrolytic enzymes. It has been reported that xylanase activity reached a maximum value of  $40.4 \pm 15.6$  IU/gds after 24 hours of fermentation whereas cellulase reached a maximum value of  $9.6 \pm 0.76$  IU/gds within the first 24 hours of the fermentation period.

In another study, *Aspergillus oryzae*, was used in a solid-state fermentation of wheat bran and other substrates for producing glucoamylase. Optimum production of glucoamylase 1986  $\mu$ moles of glucose produced per minute per gram of dry fermented

substrate) was obtained when the fermentation was carried out at pH 6, 30°C with 1% starch and 0.25% urea on a weight basis and 100% (volume: weight) initial moisture for 120 hours. Other studies also reported that optimum conditions for the growth of *Aspergillus sp.* are at a light intensity of  $\sim 0$  W/cm<sup>2</sup> and controlled humidity of 99% [18,19].

Although previous studies on the solid-state fermentation of *Aspergillus sp.* have been reported, systematic studies that report on the biodelignification of lemon peels using different species of *Aspergillus sp.* are still very scarce. Hence, this study aims to investigate effect of solid-state fermentation of lemon peels with three different species of *Aspergillus sp.* namely *Aspergillus oryzae*, *Aspergillus niger*, and *Aspergillus awamori* on the yield and chemical composition of lemon oil.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Lemon Peels

Approximately 13.1 kg fresh lemons were squeezed to remove the lemon juice. Lemon peels were then separated from the pulps. Lemon peels were cut into square-shapes with a size of 0.5 cm x 0.5 cm. Finally, lemon peels were sterilized using an autoclave for 15 minutes at 121°C and 1.5 atm [17].

### 2.2 Preparation of Inoculum for *Aspergillus sp.*

*Aspergillus sp.* cultures in 39 g/L potato dextrose agar were mixed with 5 mL of NaCl (0.85%). Subsequently, an inoculating loop was used to retrieve spores on the medium surface. NaCl solution mixed with the spores was moved to beaker glasses and the number of spores was counted using a haemocytometer. The spores solution was finally diluted using distilled water to reach a concentration of  $0.3-5 \times 10^5$  spores/g substrate [17]. This preparation was carried out under aseptic conditions.

### 2.3 Fermentation of Lemon Peels with *Aspergillus sp.*

Sterilized lemon peels were moved into punctured trays. After that, the spore solution was added with a ratio of 0.1 mL for one g of substrate into trays and mixed [20]. Trays were then covered using black coloured plastics. Rami yarns were used to attach plastics to the trays. Subsequently, these substrate-containing trays were moved onto container trays to reduce water loss (Figure 1). Fungus inoculation on lemon peels were carried out using 2 trays for each fungi species with each tray containing approximately 2.2 kg of lemon peels. The inoculation was carried out under aseptic conditions. The fermentation was conducted for 0, 3, 6, and 9 days and the relative humidity was set constant (99%) [20,21]. Excess water collected in the container trays was returned to the substrate tray once a day throughout the fermentation process.

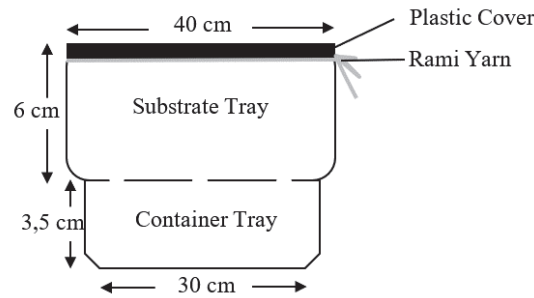


Fig. 1: Fermentation of lemon peels with *Aspergillus sp.* using two level trays (light intensity:  $\sim 0 \text{ W/cm}^2$ , relative humidity: 99%)

## 2.4 Measurement of Lignin Content in Lemon Peels

Lignin content in the lemon peels was determined using a Klason method [22]. Lemon peels were cut and dried in an oven until a moisture content of 4%. Dried lemon peels were then mashed up using a blender. After that, dried lemon peel powders were sieved using mesh 35 (a size of 0.5 mm). Subsequently, 1 g of sieved powders was added with 15 mL of  $\text{H}_2\text{SO}_4$  (72%) in a beaker glass at room temperature ( $25^\circ\text{C}$ ) while being stirred for 2-3 minutes. Afterwards, the beaker glass was covered using a watch crystal for 2 hours and stirred occasionally. The sample was then removed from the beaker glass and mixed with distilled water and  $\text{H}_2\text{SO}_4$  to achieve an  $\text{H}_2\text{SO}_4$  concentration of 3%. After that, the mixture was heated for 4 hours and the volume was kept constant followed by settling at room temperature and filtered with a Büchner funnel. The lignin content in the sample was calculated using equation (1).

$$\text{Lignin content (\%)} = \frac{m_{\text{studge}}(\text{g})}{m_{\text{initial sample}}(\text{g})} \times 100\% \quad (1)$$

where m is mass in gram (g).

## 2.5 Measurement of Moisture Content in Lemon Peels

The moisture content of the lemon peels was measured using an oven-drying method. Lemon peels were weighed before the oven-drying process. The drying was carried out at a temperature of  $105^\circ\text{C}$  until the weight remained constant [23]. The moisture content in the sample can be calculated using equation (2).

$$\text{Moisture content (\%)} = \frac{m_{\text{initial}}(\text{g}) - m_{\text{final}}(\text{g})}{m_{\text{initial}}(\text{g})} \times 100\% \quad (2)$$

where m is mass in gram (g).

## 2.6 Extraction of Lemon Oil using a Steam-Distillation Method

Fermented lemon peels were dried using an oven at  $50^\circ\text{C}$  until the moisture content lies in the range of 70-80%. Approximately 200 g of dried fermented lemon peels were mixed with distilled water (2:5 on a weight to volume basis). The distillation was carried out at  $96\text{-}98^\circ\text{C}$  for 6 hours [9]. The mixture of lemon oil and hydrosol were separated using a separatory funnel. Subsequently, the separated lemon oil phase was weighed, and its volume was measured. Lemon oil yield was calculated on dry weight basis using equation (3). The productivity of lemon oil can be calculated using equation (4).

$$\text{Essential oil yield (\% dry weight)} = \frac{m_{\text{lemon oil}}(\text{g})}{m_{\text{dried lemon peel}}(\text{g}) \times (1 - \text{moisture content})} \times 100\% \quad (3)$$

where m is mass in gram (g).

$$\text{Lemon oil productivity (g/day)} = \frac{m_{\text{lemon oil (g)}}}{\text{distillation time (day)} + \text{fermentation time (day)}} \times 100\% \quad (4)$$

where m is mass in gram (g) with distillation time and fermentation period in day.

## 2.7 Characterization of Lemon Oil

The density of lemon oil was calculated by measuring the weight and volume of the lemon oil. The lemon oil mass was weighed using an analytical balance whereas the volume was measured using a 5 mL graduated cylinder. The density was calculated using equation (4).

$$\text{Density (g/mL)} = \frac{m_{\text{lemon oil (g)}}}{V_{\text{lemon oil (mL)}}} \quad (5)$$

where m is mass in gram (g) and V is volume in millilitre (mL)

## 2.8 Determination of composition using a Gas Chromatography-Mass Spectrometry Method

The composition of lemon oil was analysed using a Gas Chromatography-Mass Spectrometry (GC-MS) method at the Forensic Laboratory Centre of Bareskrim Polri, Jakarta. A Shimadzu GCMS-QP2010 Ultra instrument was used for the analysis with the oven temperature of was set an initial temperature of 80°C and final temperature of 100°C in 25 minutes. The front inlet used a split model condition of 290°C, 19.23 psi, and total flow 202.2 mL/minute. The capillary column has nominal length 60 m and set at constant flow mode with a maximum temperature of 350°C, initial pressure of 19.24 psi and initial flow of 1 mL/minute. The thermal aux was operated at an initial temperature of 290°C. The MS equipment was set under a quad MS of 150°C (maximum 200°C) and a source MS of 250°C (maximum 300°C).

# 3. RESULTS AND DISCUSSION

## 3.1 Effect of Moisture Content on Lemon Oil Yield

After oven-drying at 50°C for 3-24 hours, the moisture content of the lemon peels lies in the range of 45-81%. Figure 2 shows that the lemon yield is highly influenced by the moisture content with an optimum moisture content of 77% producing a lemon oil yield of 0.71% dry weight (% dw). Drying of essential oil-bearing plants prior to distillation is important to increase the yield of isolated essential oil [24]. Low temperature and short drying time have been reported to increase essential oil yield whereas high temperature (>50°C) and long drying period (>24 hours) will decrease essential oil yield due to diffusion of the oil to the surrounding [25]. In this study, lemon peels were oven-dried at 50°C prior to the distillation to increase the lemon oil yield because the presence of water in the cells impedes the extraction of essential oil [27]. Hence, reducing the presence of water by oven-drying at 50°C increased the lemon oil yield. However, prolonged drying may result in a moisture content that too low that will disrupt the oil gland and essential oil losses along with water vapour [28,29].

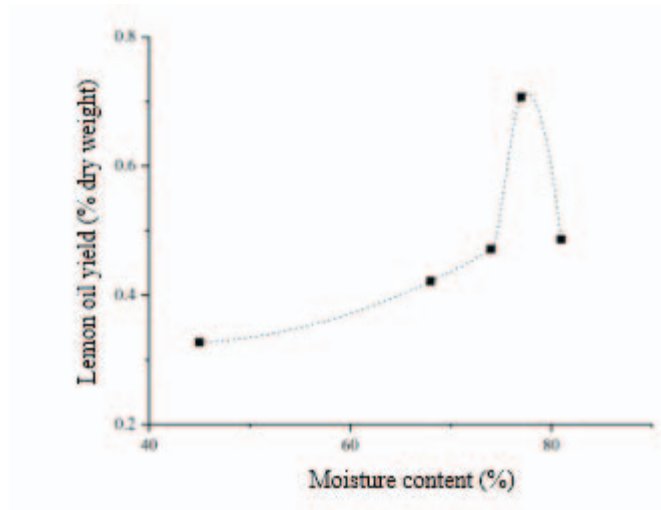


Fig. 2: Effect of moisture content on lemon oil yield

### 3.2 Effect of Fermentation Time on Lignin Content and Lemon Oil Yield

The fermentation of lemon peels using *Aspergillus niger*, *Aspergillus awamori*, and *Aspergillus oryzae* was carried out for 3, 6, and 9 days as suggested by Ferreira et al. [30] that the activity of lignin degradation enzyme (ligninase) of *Aspergillus sp.* increased along with fermentation time and reached an optimum value on the 7<sup>th</sup> day of incubation. Figure 3 shows that the lignin content in lemon peel decreased with the incubation period whereas Fig. 4 highlights that the lemon oil yield increased with the incubation period.

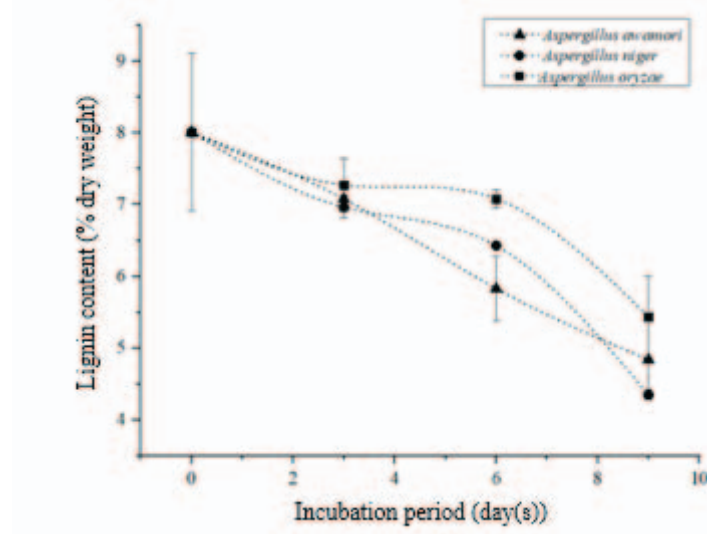


Fig. 3: Effect of fermentation with different species of *Aspergillus sp.* on lignin content in lemon peels.



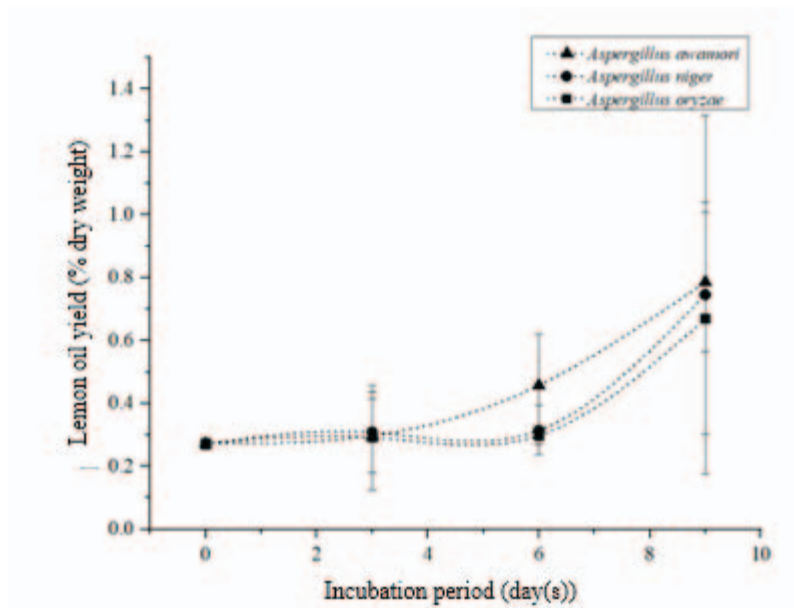


Fig. 4: Effect of fermentation with different species of *Aspergillus sp.* on lemon oil yield.

Initially, the lemon peels contained approximately  $8\% \pm 1.1\%$  dw lignin. This value resembles a lignin content of 7.6% dw reported in the literature [31]. After 9 days of fermentation with *Aspergillus sp.*, the lignin content in the lemon peels decreased to 4.4 – 5.4% dw. This may be due to the presence of various lignin degradation enzymes such as lignin peroxidase, manganese peroxidase, and laccase secreted by the *Aspergillus sp.* that were able to attack the lignin structure and caused an oxygen molecule reduction process to become water [32].

As a result of the biodegradation process, the lemon oil yield increased from  $0.3\% \pm 0.02\%$  dw up to 0.67 – 0.79% dw. The increased in lemon oil yield may also be caused by the presence of other enzymes such as cellulase and pectinase that can facilitate the secretion of essential oil from lemon peels [17,33]. González et al. [33] had previously demonstrated that pre-treatment of lemon peels with cellulase increased the lemon oil yield up to 100% from 0.009 to 0.017% on a weight basis after the 3 hours of pre-treatment at 30°C [33]. Although the lemon yield reported by González et al. [33] was lower as compared to the lemon oil yield reported in other studies, a significant amount of fermentable sugars such as glucose were also produced for further valorisation.

### 3.4 Characterization of Lemon Oil

The lemon oil has a pale-yellow colour that resembles the results reported by Boughendjioua and Djeddi [34] and in line with the specification for lemon oil. The lemon oil has a density of  $0.856 \pm 0.001$  g/mL which lies in the range of 0.845-0.870 g/mL as reported in the literature [34]. The composition of lemon oil was also analysed by gas chromatography-mass spectrometry (GC-MS) and the results are shown in Table 1. From Table 1, it can be seen that the composition of lemon oil produced from the solid-state fermentation of lemon peels with different species of *Aspergillus* vary from one another. The major composition of lemon oil in the non-fermented sample (control) is d-limonene (72.5%) which resembles the composition reported in the literature. Solid-state fermentation of lemon peels with *Aspergillus awamori*; *Aspergillus niger*, *Aspergillus oryzae* increased the content of d-limonene to 73.1-99%. According to a previous study by Wilkins et al. [35], fermentation of lemon peels increased the percentage of limonene

secretion up to 38-60% due to its inhibitory effect. The secreted limonene would allow more lemon oil to be isolated during the distillation process. In another study by Lota et al. [36], *Citrus sp.* cultivated under different soil conditions and different areas proved to contain different d-limonene compositions which lie in the range of 38.1 to 95.8%.

The composition of d-limonene in the lemon oil increased from 73.1% (day-3) to 99% (day-9) when the lemon peels were incubated with *Aspergillus awamori*. In contrast, the composition of d-limonene reached its maximum value on day-3 of fermentation when the lemon peels were incubated with *Aspergillus niger* and *Aspergillus oryzae* before slightly decreasing on day-6 and slightly increasing again on day-9. The data shows that the biosynthesis of d-limonene is highly affected by the presence of *Aspergillus sp.* Nevertheless, reported studies that can explain the mechanism that results on different d-limonene compositions when lemon peels were incubated using different *Aspergillus sp.* are still very scarce. Hence, further studies are required to explain the possible mechanism of the biosynthesis of d-limonene.

### 3.5 Productivity of Lemon Oil in a Laboratory Scale

The productivity of lemon oil produced through solid-state fermentation of lemon peel followed by a steam distillation is estimated using equation (4) and the results are shown in Table 2. The estimated productivity of lemon in a laboratory scale for the non-fermented sample (control) is of 0.5 g/day which resembles a lemon oil productivity of 0.48 g/day reported by Ferhat et al. [37]. From table 1, it can be observed that the productivity of lemon oil decreased with fermentation since a longer time was required to isolate the lemon oil. The highest productivity of lemon oil for the fermented samples is 0.044 g/day, which is around 91% lower when the lemon peel was incubated with *Aspergillus niger* for 3 days with a d-limonene content of 93.7%. The productivity of lemon oil decreased as a result of long fermentation time that was incomparable to the increase of lemon oil as suggested by Díaz [38]. Although the productivity of lemon oil decreased for the fermented samples, but the composition of d-limonene in the oil increased up to 37.7% as a result of biodegradation of lignocellulosic component by the *Aspergillus sp.* [39]. After the 9<sup>th</sup> day of fermentation with *Aspergillus awamori*, the productivity of lemon oil is 0.039 g/day with a d-limonene content of approximately 99%. Hence, a compromise is required between productivity and quality of the desired lemon oil.

Table 1: Composition of lemon oil for non-fermented and fermented lemon peel with *Aspergillus sp.*

No.	Compounds	Composition (%)									Ref. [8]	
		Day-0	Day-3			Day-6			Day-9			
			<i>Aa</i> <sup>1</sup>	<i>An</i> <sup>2</sup>	<i>Ao</i> <sup>3</sup>	<i>Aa</i> <sup>1</sup>	<i>An</i> <sup>2</sup>	<i>Ao</i> <sup>3</sup>	<i>Aa</i> <sup>1</sup>	<i>An</i> <sup>2</sup>		<i>Ao</i> <sup>3</sup>
1	$\alpha$ -Thujene	-	-	-	-	-	-	-	-	0.12	-	0.27
2	$\alpha$ -Pinene	-	-	-	-	-	-	0.23	-	-	0.54	0.98
3	$\beta$ -Pinene	-	-	2.32	-	0.30	-	-	-	0.58	-	6.60
4	Mycrene	1.33	-	1.04	-	1.65	1.16	1.33	-	3.15	2.69	1.73
5	Limonene	72.54	73.10	93.66	99.00	81.97	75.09	84.56	99.00	88.03	89.43	72.48
6	(Z)- $\beta$ -Ocimene	1.59	6.30	-	-	-	4.42	1.46	-	-	-	0.07
7	Linalool	-	-	-	-	-	-	-	-	-	-	0.13



8	Nonanal	0.84	-	-	-	-	-	-	-	-	-	<0.05
9	Citronellal	0.86	-	-	-	-	-	-	-	-	-	0.07
10	Decanal	-	-	-	-	0.50	-	-	-	-	-	0.05
11	Nerol	6,13	-	-	-	0.28	-	-	-	-	-	0.07
12	Undecanal	0.29	-	-	-	0.27	-	-	-	-	-	<0.05
13	Neryl acetate	-	-	-	-	-	0.56	-	-	0.15	-	1.21
14	Geranyl acetate	-	-	-	-	2.41	-	-	-	-	-	0.59
15	trans- $\alpha$ -Bergamotene	1.42	-	1.66	-	2.74	4.89	2.09	-	2.39	2.30	0.41
16	(E)- $\beta$ -Farnesene	-	-	-	-	0.55	0.38	-	-	0.35	0.16	<0.05
17	$\beta$ -Bisabolene	2.12	-	1.32	-	4.78	7.29	2.96	-	3.07	2.82	1.22
18	Spathulenol	-	-	-	-	-	-	-	-	-	-	0.05
19	Caryophyllene oxide	-	-	-	-	-	-	0.22	-	-	0.22	<0.05
20	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	2.18	-	-	-	-	-	-	-	-	-	-
21	1-nonanol	0.50	-	-	-	-	-	-	-	-	-	-
22	$\beta$ -Fencyl-alkohol	2.52	-	-	-	-	0.71	-	-	-	-	-
23	2,6-Octadienal, 3,7-dimethyl-, (Z)	7.28	-	-	-	2.26	1.09	-	-	-	-	-
24	Siklooktana	0.39	9.49	-	-	-	-	-	-	-	-	-
25	2-(5-methyl-5-vinyltetrahydro-2-furanyl)-2-propanol	-	11.11	-	-	-	-	-	-	-	-	-
26	1,6-Octadien-3-ol, 3,7-dimethyl-, propanoate	-	-	-	-	-	-	-	-	-	-	-
27	Dodecanal	-	-	-	-	0.44	0.49	0.28	-	-	0.28	-
28	$\alpha$ -Bisabolene	-	-	-	-	0.45	0.77	0.26	-	0.11	0.26	-
29	$\alpha$ -Bisabolol	-	-	-	-	0.68	0.97	0.44	-	0.38	0.44	-
30	1,5,9-Decatriene, 2,3,5,8-tetramethyl	-	-	-	-	0.43	-	-	-	0.33	-	-
31	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	-	-	-	-	0.28	-	-	-	-	-	-
32	Decanol	-	-	-	-	-	1.27	-	-	-	-	-
33	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	-	-	-	-	-	0.69	-	-	0.14	-	-
34	Santolina epoxide	-	-	-	-	-	0.52	-	-	-	-	-
35	4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal	-	-	-	-	-	0.68	-	-	-	-	-
36	2,3-Dehydro-1,8-cineole	-	-	-	-	-	-	-	-	0.14	-	-
37	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	-	-	-	-	-	-	-	-	0.12	-	-
38	Bicyclo[2.2.1]heptane, 2-methyl-3-methylene-2-(4-methyl-3-pentenyl)	-	-	-	-	-	-	-	-	0.35	-	-
39	Patchouli alcohol	-	-	-	-	-	-	2.95	-	0.12	-	-

40	Cyclohexanebutanal, 2,2-dimethyl-6-methylene-	-	-	-	-	-	-	-	-	0.27	2.95	-
41	1-vinyl-1-(4-methyl)pentan-3-enyl-cyclopropane	-	-	-	-	-	-	-	-	0.13	-	-
42	1,2,4-Methenoazulene, decahydro-1, 5,5,8a-tetramethyl-	-	-	-	-	-	-	-	-	0.09	-	-
43	Nonadienol	-	-	-	-	-	-	0.42	-	-	-	-

Note: <sup>1</sup>*Aspergillus awamori*; <sup>2</sup>*Aspergillus niger*; <sup>3</sup>*Aspergillus oryzae*

Table 2: Estimated productivity of lemon oil for non-fermented and fermented lemon peel with *Aspergillus sp.*

Parameter	Day-0	Day-3			Day-6			Day-9		
		Aa <sup>1</sup>	An <sup>2</sup>	Ao <sup>3</sup>	Aa <sup>1</sup>	An <sup>2</sup>	Ao <sup>3</sup>	Aa <sup>1</sup>	An <sup>2</sup>	Ao <sup>3</sup>
Oil mass (g)	0,125	0,137	0,142	0,134	0,211	0,145	0,137	0,362	0,343	0,308
Oil yield (% dry weight)	0,27	0,30	0,31	0,29	0,46	0,32	0,30	0,79	0,75	0,67
Oil productivity (g /day)	0,5	0,042	0,044	0,041	0,034	0,023	0,022	0,039	0,037	0,033

Note: <sup>1</sup>*Aspergillus awamori*; <sup>2</sup>*Aspergillus niger*; <sup>3</sup>*Aspergillus oryzae*

#### 4. CONCLUSION

A solid-state fermentation of lemon peels using *Aspergillus sp.* has been carried out to investigate the effect of biodelignification towards yield and chemical composition of lemon oil isolated from the lemon peels. The results show that the lignin content in lemon peel decreased from 8% to 4.4–5.4% whereas the yield increased from 0.27% to 0.67-0.79% as the fermentation time increased. The results obtained in this study highlights that the fermentation of lemon peels with *Aspergillus oryzae*, *Aspergillus niger*, and *Aspergillus awamori* increased the yield of lemon oil isolated from lemon peels. The major content of lemon oil, particularly d-limonene, also increased from approximately 73% up to 99%, depending on the species of *Aspergillus* used in the fermentation. The largest reduction of lignin content (49%) and increment of yield (193%) were obtained when the lemon peels were subjected to fermentation with *Aspergillus awamori* for 9 days which results to an increase of d-limonene up to 99%.

#### REFERENCES

- [1] Pratiwi A, Utami L. (2018) Isolasi dan Analisis Kandungan Minyak Atsiri pada Kembang Leson. Bioeksperimen 4 (1): 42-47. <https://doi.org/10.23917/bioeksperimen.v4i1.5930>
- [2] Grand View Research. (2018) Essential Oils Market Size, Share & Trends Analysis Report by Product (Orange, Corn, Mint, Eucalyptus, Citronella, Pepper Mint, Lemon, Clove Leaf, Lime, Spearmint), by Application, and Segment Forecasts, 2018-2025. <https://www.grandviewresearch.com>
- [3] Rosiantiningsih D. (2017) Minyak Atsiri, Emas Terpendam Indonesia. <http://marketplus.co.id/2017/03/minyak-atsiri-emas-terpendam-indonesia/>.
- [4] González-Molina E, Domínguez-Perles R, Moreno DA, García-Viguera C. (2010) Natural Bioactive Compounds of Citrus limon for Food and Health. J. Pharmaceutical and Biomedical Analysis, 51 (2): 327-345. <https://doi.org/10.1016/j.jpba.2009.09.049>
- [5] Nurlaila A. (2001) Upaya Pemanfaatan Limbah Jus Jeruk Lemon (*Citrus limon* (Linn) Burm. F.) sebagai Bahan Baku Pengolahan Jeruk Lemon. Bogor, Institut Pertanian Bogor. <http://repository.ipb.ac.id/handle/123456789/13152>
- [6] Sonneman T. (2012) Lemon A Global History. London, Reaktion Books Ltd.

- Transparency Market Research. (2019) Lemon Essential Oil Market to Expand at 5.5% CAGR During 2018-2027. <https://www.prnewswire.com>
- [7] Gök A, Kirbaşlar IFGK. (2014) Comparison of Lemon Oil Composition After Using Different Extraction Method. *Journal of Essential Oil Research*, 27(1): 17-22. <https://doi.org/10.1080/10412905.2014.982872>
- [9] Sikdar D, Nikila R. (2017) Extraction of Citrus Oil from Lemon (*Citrus limon*) Peels by Steam Distillation and Its Characterizations. *International Journal of Technical Research and Applications*, 5(2): 29-33. <https://pdfs.semanticscholar.org>
- [10] Dosoky NS, Setzer WN. (2018) Biological Activities and Safety of *Citrus spp.* Essential Oils. *International Journal of Molecular Sciences*, 19 (7): 1966-1991. <https://doi: 10.3390/ijms19071966>.
- [11] Janati SSSF, Beheshti H, Feizy J, Fahim N. (2012) Chemical Composition of Lemon (*Citrus limon*) and Peels Its Consideration as Animals Food. *GIDA-Journal of Food*, 37(5): 267-271. <https://dergipark.org.tr>
- [12] Chen H. (2014) *Biotechnology of Lignocellulose: Theory and Practice*. Dordrecht, Springer.
- [13] Ahammed S, Prema P. (2002) Influence of media nutrients on synthesis of lignin peroxidase from *Aspergillus sp.* *Applied Biochemistry and Biotechnology*, 327-336. <https://doi.org/10.1385/ABAB:102-103:1-6:327>
- [14] dos Santos TC, dos Santos RN, Silva TP, Machado FD, Bonomo RC, Franco M. (2016) Prickly palm cactus husk as a raw material for production of ligninolytic enzymes by *Aspergillus niger*. *Food science and biotechnology*, 25(1): 205-211. <https://doi.org/10.1007/s10068-016-0031-9>
- [15] Shah MP, Reddy GV, Banerjee R, Babu PR., Kothari IL. (2005) Microbial degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi (*Phylosticta spp.* MPS-001 and *Aspergillus spp.* MPS-002). *Process Biochemistry*, 40(1): 445-451. <https://doi.org/10.1016/j.procbio.2004.01.020>
- [16] Mrudula S, Murugammal R. (2011) Production of cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. *Brazilian Journal of Microbiology*, 7:1119-1127. <https://doi.org/10.1590/S1517-83822011000300033>
- [17] Botella C, Diaz A, de Ory I, Webb C, Blandino A. (2007) Xylanase and Pectinase Production by *Aspergillus awamori* on Grape Pomace in Solid State Fermentation. *Process Biochemistry* 42 (1): 98-101. <https://doi.org/10.1016/j.procbio.2006.06.025>
- [18] Steviani S. (2011) Pengaruh penambahan molase dalam berbagai media pada jamur tiram putih (*Pleurotus ostreatus*). Surakarta, Universitas Sebelas Maret. <https://digilib.uns.ac.id>
- [19] Silva R. (2016) *Mycology: Current and Future Developments: Fungal Biotechnology for Biofuel Production Volume 1*. Sharjah, Benthan Science Publishers.
- [20] Zambare V. (2010) Solid state fermentation of *Aspergillus oryzae* for glucoamylase production on agro residues. *International Journal of Life Sciences*, 16-25. <https://doi.org/10.3126/ijls.v4i0.2892>
- [21] Abduh MY, Zuliansyah W, Aprina L, Arazella N. (2019) Effect of pectin biodegradation with *Aspergillus niger* on total flavonoid content of *Citrus limon* L. *J Biodjati*, 4(2):194-203. <https://doi.org/10.15575/biodjati.v4i2.4537>
- [22] Gwalingo E, Rahman NSA, Al-Marzouqi AH, Omar MMA, Khaleel AA. (2018) Klason method: an effective method for isolation of lignin fractions from date palm biomass waste. *Chemical and Process Engineering Research*, 57:46-58. <https://www.iiste.org/Journals/index.php/CPER/article/view/41830>
- [23] Carneiro JD, Nogueira RM, Martins MA, Valladão DM, Pires EM. (2018) The Oven-Drying method for Determination of Water Content in Brazil Nut. *Bioscience Journal* 34 (3), 595-602. <https://doi.org/10.14393/BJ-v34n3a2018-37726>
- [24] Kandi M, Sefidkon F. (2011) The influence of drying methods on essential oil content and composition of *Laurus nobilis* L. *Journal of Essential Oil Bearing Plants*, 14 (3), 302-308. <https://doi.org/10.1080/0972060X.2011.10643938>

- [25] Rahimmalek M, Goli SA. (2013) Evaluation of Six Drying Treatments with Respect to Essential Oil Yield, Composition and Color Characteristics of *Thymys daenensis* subsp. *daenensis*. Celak Leaves. Industrial Crops and Products, 42(1): 613-619. <https://doi.org/10.1016/j.indcrop.2012.06.012>
- [26] Ambrose DC, Annamalai S, Naik R. (2013) Effect of Drying on Volatile Oil Yield of Patchoulli. Indian Journal of Science and Technology, 6(12): 5559-5562. <http://52.172.159.94/index.php/indjst/article/view/43613>
- [27] Hobir Y, Nuryani E, Anggraeni. (2003) Peningkatan Produktivitas dan Mutu Minyak Nilam Melalui Perbaikan Varietas dan Teknik Pengolahan. Balitro, Bogor.
- [28] Hamrouni-Sellami I, Wannas WA, Bettaieb I, Berrima S, Chahed T, Marzouk B, Limam F. (2011) Drying Sage (*Salvia officinalis* L.) Plants and Its Effects on Content, Chemical Composition, and Radical Scavenging Activity of the Essential Oil. Food and Bioprocess Technology, 5(8): 2978-2989. <https://pubag.nal.usda.gov/catalog/448611>
- [29] Hasmita I, Adisalamun A, Alam PN, Satriana S, Mahlinda M, Supardan, M. D. (2015) Effect of Drying and Hydrodistillation Time on the Amount of Ginger Essential Oil. International Journal on Advanced Science, Engineering, and Information Technology, 5(5): 300-303. <http://dx.doi.org/10.18517/ijaseit.5.5.567>
- [30] Ferreira FL, Dall'Antonia CB, Shiga EA, Alvim LJ, Pessoni RA. (2018) Sugarcane Bagasse as a Source of Carbon for Enzyme Production by Filamentous Fungi. Hoehnea, 45(1): 134-142. <https://doi.org/10.1590/2236-8906-44/2017>
- [31] Mamma D, Christakopoulos P. (2013) Biotransformation of *Citrus* By-Products into Value Added Products. Waste Biomass Valorization, 5(4): 529-549. <https://doi.org/10.1007/s12649-013-9250-y>
- [32] Brijwani K, Rigdon A, Vadlani PV. (2010) Fungal Laccases: Production, Function, and Applications in Food Processing. Enzyme Research, 1: 1-10. <https://doi.org/10.4061/2010/149748>
- [33] Chávez-González ML, López LI, Rodríguez-Herrera R, Contreras-Esquivel JC, Aguilar CN. (2015) Enzyme-Assisted Extraction of *Citrus* Essential Oil. Chemical Papers, 70(4): 1-10. <https://doi.org/10.1515/chempap-2015-0234>
- [34] Boughendjioua H, Djeddi S. (2017) Organoleptic and Physicochemical Properties of Algerian Lemon Essential Oil. World Journal of Applied Chemistry, 2(3): 96-100. <https://doi.org/10.11648/j.wjac.20170203.14>
- [35] Wilkins MR, Grohmann K, Widmer WW. (2006) Effect of d-limonene on the Fermentation of *Citrus* Peel Waste. American Society of Agricultural and Biological Engineers, 1(1): 1-7. <https://doi.org/10.1080/15435075.2017.1307753>
- [36] Lota ML, de Rocca SD, Tomi F, Jacquemond C, Casanova J. (2002) Volatile Components of Peel and Leaf Oils of Lemon and Lime Species. Journal of Agricultural and Food Chemistry, 50(4): 796-805. <https://doi.org/10.1021/jf010924l>
- [37] Ferhat MA, Meklati BY, Smadja J, Chemat F. (2006) An Improved Microwave Clevenger Apparatus for Distillation of Essential Oils from Orange Peel. Journal of Chromatography A, 1112 (1): 121-126. <https://doi.org/10.1016/j.chroma.2005.12.030>
- [38] Díaz AB, Ory ID, Caro I, Blandino A. (2012) Enhance Hydrolytic Enzymes Production by *Aspergillus awamori* on Supplemented Grape Pomace. Food and Bioprocess Technology, 90(1): 72-78. <https://doi.org/10.1016/j.fbp.2010.12.003>
- [39] Tang PL, Hassan O, Maskat MY, Badri K. (2015) Production of Monomeric Aromatic Compounds from Oil Palm Empty Fruit Bunch Fiber Lignin by Chemical and Enzymatic Methods. Biomed Research International, 5: 1-14. <https://doi.org/10.1155/2015/891539>