

Solid-State Fermentation Of Cinnamon Bark Using *Aspergillus Awamori* To Increase Cinnamon Oil Yield Extracted Using Hydrodistillation, Maceration, And Soxhlet Extraction

Muhammad Yusuf Abduh^{1,2}, Ravelian Yulianto¹, Dennis Avima¹, Abednego Kurio Widiyanto¹, Rika Alfianny¹

¹ School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha No. 10 Bandung 40132, Indonesia

² University Center of Excellence for Nutraceuticals, Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Jalan Ganesha No. 10 Bandung 40132, Indonesia

Abstract

This research was conducted to determine the optimum fermentation time of cinnamon (*Cinnamomum burmannii*) bark using *Aspergillus awamori* to degrade lignocellulose content in the cinnamon bark. *Aspergillus awamori* inoculum, 10^8 cells/g, was added to the substrate and the fermentation was carried out at 25-30°C, ~0 W/cm² light intensity and ~99% humidity for 0, 3, 6, and 9 days. Before the fermentation, the cellulose, hemicellulose, and lignin content in the cinnamon bark substrate were 15.37%, 27.83%, and 48.04%, respectively. The biggest decrease of cellulose, hemicellulose, and lignin occurred after 9 days of fermentation: 12.37%, 16.55%, and 39.95%, respectively. Cinnamon oil extraction was carried out using hydrodistillation, maceration and Soxhlet methods. The yield of cinnamon oil after 9 days of fermentation using the hydrodistillation, maceration and Soxhlet methods were 2.09%, 2.47%, and 3.01%, respectively. Composition of the cinnamon oil was analysed using Gas Chromatography - Mass Spectrometry. The cinnamon oil primarily composed of cinnamaldehyde, and the concentration varies with fermentation time and extraction methods. A mathematical model was also developed to determine the diffusion coefficient of cinnamon oil during the extraction process that can be used predict the cinnamon oil yield. The results indicate that a higher diffusion coefficient of 2.36×10^{-11} m²/s was obtained for the hydrodistillation method, followed by Soxhlet (1.44×10^{-11} m²/s) and maceration (1.42×10^{-12} m²/s). The mathematical model can predict the cinnamon oil yield for all extraction methods reasonably well.

Keywords: *Aspergillus awamori*, Cinnamon oil, Cinnamaldehyde, Extraction, Lignocellulose

1. Introduction

Cinnamon is a type of spice obtained from the bark of plants within the genus *Cinnamomum* and belongs to the Lauraceae family [1]. There are several species of cinnamon available in the market; among them are *Cinnamomum zeylanicum*, *Cinnamomum burmannii*, and *Cinnamomum cassia* [2]. Cinnamon plants have a lot of applications in different areas. Cinnamon leaves can be used as pesticides, its wood can be used as building materials and to make particle board, meanwhile its bark, which is

most widely available, can be used as flavor, perfume base, and as an ingredient in pharmaceutical products [3].

Indonesia, China, Sri Lanka, and Vietnam are the main suppliers of cinnamon in the global market. All four countries supply up to 99% of the world's demand for cinnamon. Indonesia is the world's largest supplier of cinnamon with a total production of 87,130 tons in 2017 [4]. Cinnamon plantations in Indonesia are centered in West Sumatra Province, especially in Kerinci Regency, and are also present in Aceh, North Sumatra, Bengkulu, West Java, and East Java. Nevertheless, most of the cinnamon production in Indonesia is still at the upstream level. This is a problem because the export price of cinnamon has decreased every year, reaching USD 0.17/kg in 2008 due to excessive availability [3]. One of the efforts that can be made to overcome the excess supply of cinnamon is by processing cinnamon into its derivative products, such as cinnamon oil that causes the fragrant aroma of cinnamon [6].

Essential oils have many beneficial properties, including antiseptic, antispasmodic, and other health benefits. In addition, essential oils are also widely used in food and cosmetics, especially in the production of perfume [7]. Cinnamon oil is widely produced throughout the world with the largest producer being Sri Lanka [8]. The market price of cinnamon oil is relatively high compared to barley cinnamon, around USD 29-34/kg [9]. This high price can provide added values for the excess stock of cinnamon in Indonesia.

Essential oils can be extracted through various extraction methods such as supercritical extraction and solvent extraction as well as distillation [10]. A typical issue in the extraction of essential oil is its relatively low yield. Extraction of essential oils using steam distillation method produces a yield of 1.23-2.95% [1,9] whereas hydrodistillation method produces a yield of 1.7-3.1% [11,12], while the yield for Soxhlet extraction and maceration have been reported as 3.83% [1] and 2.28% [13], respectively. A higher yield of cinnamon oil can be achieved by optimizing the operating conditions of the extraction process or through initial treatment/pre-treatment of the substrate.

Pre-treatment of the substrate can be carried out in the form of drying or degradation of components within the cell wall of the substrate [14]. Plant cell walls are composed of lignin, cellulose, and hemicellulose [15]. Degradation of these compounds can facilitate better contact between solvent and essential oil components present in the cell. Enzymes can be used to degrade compounds of the cell walls. *Aspergillus awamori* is one type of microorganisms capable of producing enzymes that can degrade lignocellulosic compounds or delignify cell walls [16]. *Aspergillus* sp. can reduce the lignocellulose content of a biomass up to 22.6% for lignin, 38.3% for cellulose, and 18.9% for hemicellulose [17].

Pre-treatment in the form of degradation of lignocellulosic compounds by *Aspergillus awamori* can degrade the lignocellulosic content of cinnamon bark to increase the yield of cinnamon oil. Hence, this study was conducted to determine the effect of biodegradation of cinnamon bark by *Aspergillus awamori* on the yield and composition of cinnamon oil. In addition, mathematical modeling of cinnamon oil extraction was also carried out to determine the diffusion coefficient and predict the cinnamon oil

yield extracted using different methods particularly hydrodistillation, maceration and Soxhlet extraction methods.

2. Materials and Methods

2.1 Materials

Cinnamomum burmannii bark samples were obtained from Gambung Tea and Tea Plantation Center (PPTK), Bandung Regency, West Java whereas *Aspergillus awamori*, potato dextrose agar, sodium chloride, n-hexane, sulphuric acid, dichloromethane, distilled water and aquades were obtained School of Life Sciences, Institut Teknologi Bandung, West Java, Indonesia.

2.2 Preparation of Cinnamon Bark

Fresh cinnamon bark was cleaned of its outer skin and vegetation. Cinnamon bark was then cut to about 3 x 3 cm and sterilized using ultraviolet light for 30 minutes [18].

2.3 Preparation of *Aspergillus awamori* Inoculum

Pure culture of *Aspergillus awamori* were subcultured to a potato dextrose agar (PDA) medium in reaction tubes using inoculation needles aseptically. The cultures were incubated at room temperature (25°C) for 7 days. After 7 days, 5 ml of NaCl solution was added into the reaction tubes to harvest *Aspergillus awamori* before being transferred to a chemical glass. Determination of the number of cells was done using a hemocytometer method [19].

2.4 Solid State Fermentation of Cinnamon Bark

Approximately 100 g of Cinnamon bark was placed in a fermentation tray. Each tray was given a solution of 10^8 *Aspergillus* spores/g of cinnamon bark substrate. After that, aquades was added with a 1:1 ratio with respect to the cinnamon bark. Small holes were provided at the bottom of the tray for drainage. The fermentation tray was covered with a plastic to maintain a low light intensity (~ 0 W/m²). The fermentation was performed at room temperature (25–27°C) for 0, 3, 6, and 9 days [17].

2.5 Measurement of Lignocellulose Content

Measurement of lignocellulose content in the cinnamon bark was carried out using a Chesson-Datta method [20]. Approximately 1 g of cinnamon bark was weighed (a). A total of 150 ml of distilled water was added to the sample and heated using a water bath at 90-100°C for 1 hr. The mixture was filtered, and the residue was rinsed with 300 ml of distilled water. The sample was then dried with an oven until a constant weight was obtained (b). The dry residue was mixed with 150 ml of H₂SO₄ 1 N while heated using a water bath at 90-100°C for 1 hour. The mixture was given the same treatment as in the previous mixture until a constant weight was obtained (c). The dried residue was then mixed with 10 ml of 72% H₂SO₄ for 4 hr at room temperature (25–27°C). The mixture was then mixed with 150 ml H₂SO₄ (1 N) and refluxed for 1 hr. After that, the solid compound obtained was rinsed with 300 ml of distilled water then dried using an oven at 105°C until a constant weight was attained (d). The solid was then heated in a furnace until it became ash and was weighed (e). The composition of lignocellulose was then calculated using equation (1), (2), and (3).

$$\% \text{ cellulose} = \frac{(c) (g) - (d) (g)}{(a) (g)} \times 100\% \dots \dots \dots (1)$$

$$\% \text{ lignin} = \frac{(d) (g) - (e) (g)}{(a) (g)} \times 100\% \dots \dots \dots (2)$$

$$\% \text{ hemicellulose} = \frac{(b) (g) - (c) (g)}{(a) (g)} \times 100\% \dots \dots \dots (3)$$

2.6 Drying of Cinnamon Bark

Fresh cinnamon bark that had been removed from the stalk was dried using an oven until it reached a moisture content of 35-45% for extraction of cinnamon oil [9]. The water content of cinnamon bark was calculated using equation (4).

$$\text{Moisture content (\%)} = \frac{\text{cinnamon bark mass (g)}}{\text{fresh cinnamon bark mass (g)}} \times 100\% \dots \dots \dots (4)$$

2.7 Extraction of Cinnamon Oil

Extraction of cinnamon oil was carried out using four different methods particularly steam distillation, hydrodistillation, maceration and Soxhlet extraction. For steam and hydrodistillation, approximately 40 g of cinnamon bark was used, and the steam distillation was carried out at 90-100°C for 4 hr. After that, the distillate was separated using a separatory funnel and mixed with 30 ml of dichloromethane (DCM) and shaken for 1 min followed by separation of the organic phase at the bottom part of the funnel. The processes were repeated 3 times until all the essential oil in the distillate had been dissolved in the DCM followed by evaporation using a rotary vacuum evaporator to evaporate the DCM [12].

As for maceration, approximately 30 g of cinnamon bark was immersed in a glass chamber containing 180 ml of n-hexane and stirred using a shaker at 150 rpm for 24 hr. After that, the mixture was filtered, and the n-hexane was evaporated using a rotary evaporator at 60°C. The remaining semi-solid compound (concrete) was reconstituted with 20 ml of n-hexane. The solution was heated at 75°C and simultaneously stirred for 5 min and then filtered. The solution was stored at -18°C to -35°C and then filtered by a cold filtration method. The n-hexane was evaporated again with a rotary evaporator and the remaining solution was allowed to evaporate at room temperature (25–27°C) until the remaining n-hexane evaporated.

As for Soxhlet extraction, approximately 30 g of cinnamon bark was extracted using 150 ml of n-hexane for 3 hr followed by evaporation using a rotary vacuum evaporator, resulting in a semi-solid (concrete) solution. The concrete was then dissolved in 10 ml of ethanol until it was completely dissolved and then heated at 50°C using an electric stove. The concrete was stored in a freezer at -20°C until the wax solidified. After that, the concrete was filtered to separate the waxy substance and the mixture of cinnamon oil and ethanol. The filtered product was then evaporated at 40°C until all the ethanol has evaporated [1,6]. All the extracted cinnamon oil was stored in a dark bottle for further analysis. Cinnamon oil yield was determined using equation (5).

$$\text{Yield (\%)} = \frac{\text{mass of cinnamon oil (g)}}{\text{mass of cinnamon bark (g)} \times (100 - \text{moisture content})} \times 100\% \dots \dots \dots (5)$$

2.8 Cinnamon Oil Composition Analysis

The composition of cinnamon oil was analyzed using a Gas Chromatography - Mass Spectrometry (GC-MS) method at the Central Forensic Laboratory, Police of the Republic of Indonesia. The GC-MS equipment consists of an oven, back inlet, and capillary column. The oven was operated at 80 ° C for 30 min. The back inlet was operated using a split method with a split ratio of 30:1. The capillary column contains 5% phenyl methyl silox. The capillary column has a length of 30 m, a diameter of 0.25 mm, and a thickness of 0.25 μm. The analysis of the composition of cinnamon oil was carried out based on the database of National Institute of Standards and Technology.

2.9 Determination of Diffusion Coefficient for Extraction of Cinnamon Oil

A mathematical model was developed out to determine the diffusion coefficient for extraction of cinnamon oil using a second Fick's law of diffusion[22] as shown in equation (6).

$$\frac{d\phi}{dt} \left(\frac{g}{cm^3 s} \right) = D \left(\frac{cm^2}{s} \right) \frac{d^2\phi}{d^2x} \left(\frac{g}{cm^3} \right) \dots \dots \dots (6)$$

where D is the diffusion coefficient (cm²/s), φ is the concentration (g/cm³), and x is the position (cm). Relevant assumptions used in constructing the model to determine the diffusion coefficient for extraction of cinnamon oil are as follows:

1. The value of D is constant
2. There is no diffusion resistance in the solution around the particles
3. There is no oil concentration on the particle surface due to washing by solvent
4. The concentration of oil in the particles is uniform
5. The geometry of the plate-shaped particles is uniform with a thickness of L
6. The diffusion process occurs in 1 dimension

Equation (6) can be derived using the number of exponential decays to yield equation (7) to compare the experimental values with those based on the model. This model is valid for the acquisition value when t is greater than 0.

$$\frac{M_t(\%)}{M_\infty(\%)} = 1 - Ae^{(-B(s^{-1}) \times t(s))} \dots \dots \dots (7)$$

where M_t is the yield at time t, M_∞ is the yield at steady state, A is the model constant (A = 8/π² for plate geometry), t is time, and B is the diffusion rate constant. Equation (7) can be simplified to equation (8) by changing the yield fraction of cinnamon oil to a constant E [23].

$$E = 1 - \frac{M_t(\%)}{M_\infty(\%)} = Ae^{(-B(s^{-1}) \times t(s))}$$

$$\ln E = \ln A - B(s^{-1}) \times t (s) \dots \dots \dots (8)$$

Parameter B was determined using a curve fitting feature on MATLAB® based on equation (8). The value of parameter B was used to determine the value of D, the diffusion rate coefficient that can be determined using equation (9), where L is the particle width used in this study (1.25 x 10⁻³ m)[24].

$$D \left(\frac{\text{cm}^2}{\text{s}} \right) = \frac{B(\text{s}^{-1}) \times L(\text{cm})^2}{\pi^2} \dots\dots\dots(9)$$

3. Results and Discussion

3.1 Effect of Different Extraction Methods on Cinnamon Oil Yield

Essential oil is typically produced using a steam distillation method. In this study, different extraction methods were used to extract essential oil from unfermented cinnamon bark and the results are shown in Table 1. Extraction of cinnamon oil using the steam distillation method produces a cinnamon oil yield of 1.17%, which is lower than previously reported cinnamon oil yield of 3.2% (dry weight) [9]. This difference can be due to the smaller particle size of the cinnamon bark used in the previous study. Smaller particle sizes will have a larger surface area so that the diffusion rate is higher and consequently lead to a higher yield [25]. From Table 1, it can be observed that cinnamon oil yield of the hydrodistillation method (1.37%, dry weight) is higher than the steam distillation method. This is because in the hydrodistillation method, the substrate was immersed in water and consequently has a better contact with the solvent as compared to the steam distillation method. It has been reported that increasing the contact time between the substrate and the solvent will increase the yield [26].

Table 1 Cinnamon oil yield for different extraction methods

Method	Yield (% dry weight)
Steam distillation	1.17 ± 0.22
Hydrodistillation	1.37 ± 0.31
Maceration	0.94 ± 0.35
Soxhlet	1.96 ± 0.13

A lower cinnamon oil yield 0.94% (dry weight) was obtained from the maceration method. Two– stage maceration with ethanol as a solvent may further increased the cinnamon oil yield as reported in another study [13]. However, the use of ethanol as a solvent may extract other non-volatile compounds together with the volatile compounds, thereby reducing the quality of the extracted oil [21]. Cinnamon oil extracted by the Soxhlet extraction method using n-hexane as the solvent produced a higher yield than the distillation method. The results resemble the findings in previous studies that extraction of cinnamon oil using Soxhlet extraction produces a higher yield as compared to the steam distillation method [1,6]. As such is due to the much greater solubility of cinnamon oil in n-hexane as compared to water [24].

3.2 Effect of Fermentation on Lignocellulose Content of Cinnamon Bark and Cinnamon Oil Yield

The effect of solid-state fermentation of cinnamon bark with *Aspergillus awamori* on lignocellulose content of cinnamon bark and cinnamon oil yield are shown in Figure 1-2. From Figure 1, it can be observed that the lignocellulosic content of the cinnamon bark shows a decreasing profile with increasing fermentation time. As such demonstrates that the *Aspergillus awamori* is able to degrade lignocellulose in the cinnamon bark into simpler compounds. Initially, the lignin content in the unfermented cinnamon bark was 51.14% gradually decreased to 39.95% after 9 days of fermentation. The highest decrease of lignin was observed between day 3 and 6, most probably due to the presence of laccase enzyme secreted by *Aspergillus awamori*, that typically reaches its highest activity after fermentation for 6 days [27,28].

Prior to fermentation, hemicellulose content in the cinnamon bark was 27.83%. The value gradually decreased to 16.55% after fermentation for 9 days with the highest decrease occurring between day 3 and day 6. As such is most probably due to the presence of xylanase enzyme secreted by *Aspergillus awamori* that typically reaches its highest activity after fermentation for 5 days [29,30]. The reduction in hemicellulose content was greater than that of other lignocellulosic compounds. This happened because *Aspergillus awamori* is a soft rot fungus that can attack carbohydrates in wood more effectively by forming holes in the secondary cell walls and causing erosion of the entire secondary cell wall [31]. In contrast to lignin and hemicellulose, cellulose content in the cinnamon bark only slightly decreased from 15.37% to 13.20% after fermentation for 3 days and became stagnant at 12.37-12.67% after fermentation for 9 days which may be due to the limited amount of cellulose degrading enzyme secreted by *Aspergillus awamori* during the fermentation process [32,33].

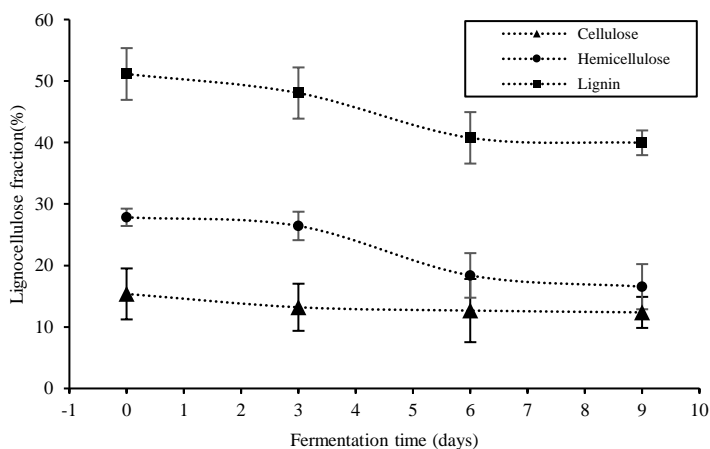


Figure 1 Effect of fermentation time by *Aspergillus awamori* on lignocellulose content in cinnamon bark

Figure 2 shows that the yield of cinnamon oil continues to increase along with an increasing fermentation time for all extraction methods, reaching a maximum yield of 3.01%, 2.09%, and 2.47% dry weight after 9 days of fermentation for extraction of cinnamon oil using Soxhlet, hydrodistillation, and maceration, respectively. As such indicates that solid-state fermentation of the cinnamon bark with *Aspergillus awamori* is able to degrade the lignocellulosic components of the cell wall and facilitate better

mass transfer for the extraction of cinnamon oil [34]. A higher cinnamon oil yield obtained by the Soxhlet extraction may be due to the reflux of solvent causing the solvent not to be saturated with the dissolved material. Unsaturated solvents can extract more dissolved material because there is a larger concentration gradient that can increase the mass transfer rate between the solvent and the dissolved material [35]. Figure 2 also shows that the maceration method produces a higher yield than the hydrodistillation method. As such may be due to the use of non-polar organic solvent particularly n-hexane. Cinnamon oil is non-polar so its solubility in n-hexane is higher than in water [36].

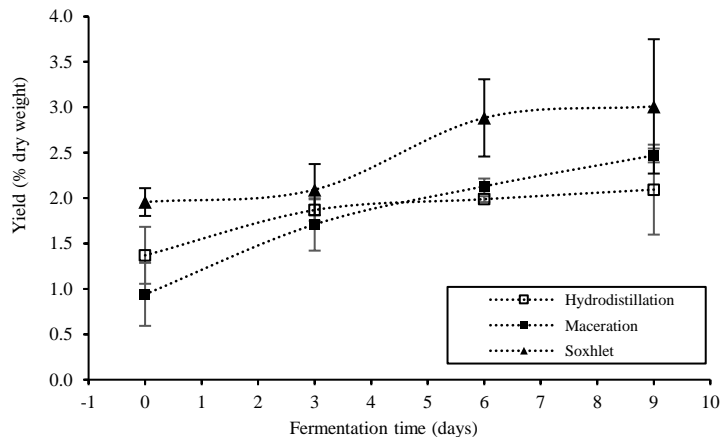


Figure 2 Effect of fermentation with *Aspergillus awamori* on cinnamon oil yield for different extraction methods

3.3 Characterization of Cinnamon Oil

Relevant properties of cinnamon oil were characterized and compared with the Indonesian National Standard (SNI) and the results are shown in Table 2. The color and aroma of cinnamon oil obtained in this study are already in accordance with the SNI 06-3734-2006 however the density is slightly larger. The cinnamaldehyde content also satisfies a minimum requirement of 50%. Table 3 presents the effect of fermentation of cinnamon bark with *Aspergillus awamori* on the cinnamaldehyde content in cinnamon oil extracted using different methods. In general, the cinnamaldehyde content varies for different extraction methods and tend to decrease upon fermentation. Nevertheless, the cinnamaldehyde content still lies in the range 42.23-96.01% as reported in previous studies [1,37,38]. The lower cinnamaldehyde content for fermented samples could have been caused by the presence of other compounds that are also extracted due to the degradation of the lignocellulosic compounds in the sample causing more compounds to be extracted. The number of identified compounds also varies with an increasing fermentation time as shown in Table 3. As such because the composition of essential oil is highly influenced by various factors including internal and external factors that can cause the composition of cinnamon oil to vary even though the substrate of cinnamon comes from the same source [39].

Table 2 Physical dan chemical properties of cinnamon oil

Property	Experiment Result	SNI 06-3734-2006
Color	Dark yellow	Pale yellow
Aroma	Cinnamon	Cinnamon
Density (g/mL)	1.032-1.063	1.008-1.030
Cinnamaldehyde content (%)	53.63-93.19%	Minimum 50%

From Table 3, it can also be observed that cinnamon oil extracted via the hydrodistillation method contains more ketones and carboxylic acids whereas cinnamon oil extracted via the Soxhlet method contains more alcoholic compounds. Meanwhile, cinnamon oil extracted via the maceration method contains more sesquiterpene compounds. These differences may be caused by the different solvent used in the respective methods. Carboxylic acids and ketones are easily soluble in water, hence they can be extracted well using the hydrodistillation method. The sesquiterpene compounds are practically insoluble in water and consequently more available when extracted using n-hexane via the maceration and Soxhlet extraction.

Table 3 Effect of fermentation with *Aspergillus awamori* on the composition of cinnamon oil for different extraction methods

Compound	Fermentation time (day)											
	0			3			6			9		
	HD ¹	S ²	M ³	HD ¹	S ²	M ³	HD ¹	S ²	M ³	HD ¹	S ²	M ³
Cinnamaldehyde	80, 73	93, 19	73, 74	60, 78	74, 11	68, 75	53, 63	73, 38	63, 27	76, 05	79, 68	73, 5
trans-Cinnamic acid	n.d	n.d	n.d	33, 19		4,8 9	30, 48		n.d	18, 64		n.d
Benzaldehyde	0,5 6	n.d	n.d	1,9 5		1,3 7	4,6 2		n.d	1,8 2		n.d
3-Phenylprop-2-enoic acid	15, 44	n.d	n.d	n.d	n.d	n.d	n.d	n.d	16, 32	0,1 9	1,6 2	n.d
Benzenacetaldehyde	0,6	n.d	n.d	0,9 1	n.d	n.d	1,5 7		1,2 5	0,8 1		n.d
Acetophenone	n.d	n.d	n.d	n.d	n.d	n.d	0,5 1		n.d	n.d	n.d	n.d
3-Phenylfuran	n.d	n.d	n.d	n.d	n.d	n.d	1,1 1		n.d	n.d	n.d	n.d
Chalcone	n.d	n.d	n.d	n.d	n.d	n.d	0,2 3		n.d	n.d	n.d	n.d
1,4-diphenyl-1,4-	n.d	n.d	n.d	n.d	n.d	n.d	2,6 9		n.d	n.d	n.d	n.d

Butanedione													0,5
α -Cubebene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	5
δ -Cadinene													0,3
	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	8
Cinnamaldehyde dimethyl acetal			21,		20,	12,		24,	5,4			13,	23,
	n.d	n.d	6	n.d	94	81	n.d	95	8	n.d	59	0	
Caryophyllene oxide								0,1				0,2	0,2
	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1	n.d	n.d	2	5	
Benzaldehyde dimethyl acetate			3,1								3,2		
	n.d	n.d	2	n.d	n.d	3,6	n.d	n.d	7	n.d	n.d	n.d	
Benzenepropanol					0,5	n.d		n.d	n.d	n.d	n.d	n.d	n.d
	n.d	n.d	n.d	n.d	0,3								
3-Phenyl-2-propen-1-ol					7	n.d		n.d	n.d	n.d	n.d	n.d	n.d
	n.d	n.d	n.d	n.d									
α -Terpineol					n.d	n.d		n.d	0,2	n.d	n.d	n.d	n.d
	n.d	n.d	n.d	n.d									
Others	2,6	6,8	1,5	3,1	4,0	8,5	5,1	1,3	8,5		4,8	2,3	
	7	1	4	7	8	8	6	6	9	3,2	9	2	

Note: ¹Hydrodistillation ²Soxhlet; ³Maceration; n.d : not detected

3.4 Estimation of Cinnamon Oil using a Mathematical Model

Extraction of cinnamon oil is highly influenced by the diffusion rate caused by a concentration gradient. In this study, the diffusion rate constant and diffusion coefficient for different extraction methods were determined using Fick's Second Law and the results are shown in Table 4. The diffusion rate constant varies from $9 \times 10^{-6} \text{ s}^{-1}$ to $1.49 \times 10^{-4} \text{ s}^{-1}$ whereas the diffusion coefficient varies from $1.42 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ to $2.26 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$. The results show that the diffusivity rate of the hydrodistillation and Soxhlet methods are greater than the maceration method. This could have been caused by the application of heat for the hydrodistillation and Soxhlet methods, while the maceration method was operated at room temperature. In general, the rate of diffusion is influenced by the movement of molecules. Hence, application of heat will increase the movement of the molecules, thus increasing the diffusion rate [40]. The diffusion rate constant and diffusion coefficient can be used to develop a model to estimate the cinnamon oil yield and the results are shown in Figure 3. The figure illustrates that at any time greater than 0, the model can predict the cinnamon oil yield reasonably well for hydrodistillation, Soxhlet and maceration.

Table 4 Diffusion rate constant and diffusion coefficient for extraction of cinnamon oil using different methods

Method	Diffusion rate constant (s^{-1})	Diffusion coefficient (m^2s^{-1})
Hydrodistillation	1.49×10^{-4}	2.36×10^{-11}
Maceration	9.00×10^{-6}	1.42×10^{-12}
Soxhlet	9.15×10^{-5}	1.44×10^{-11}

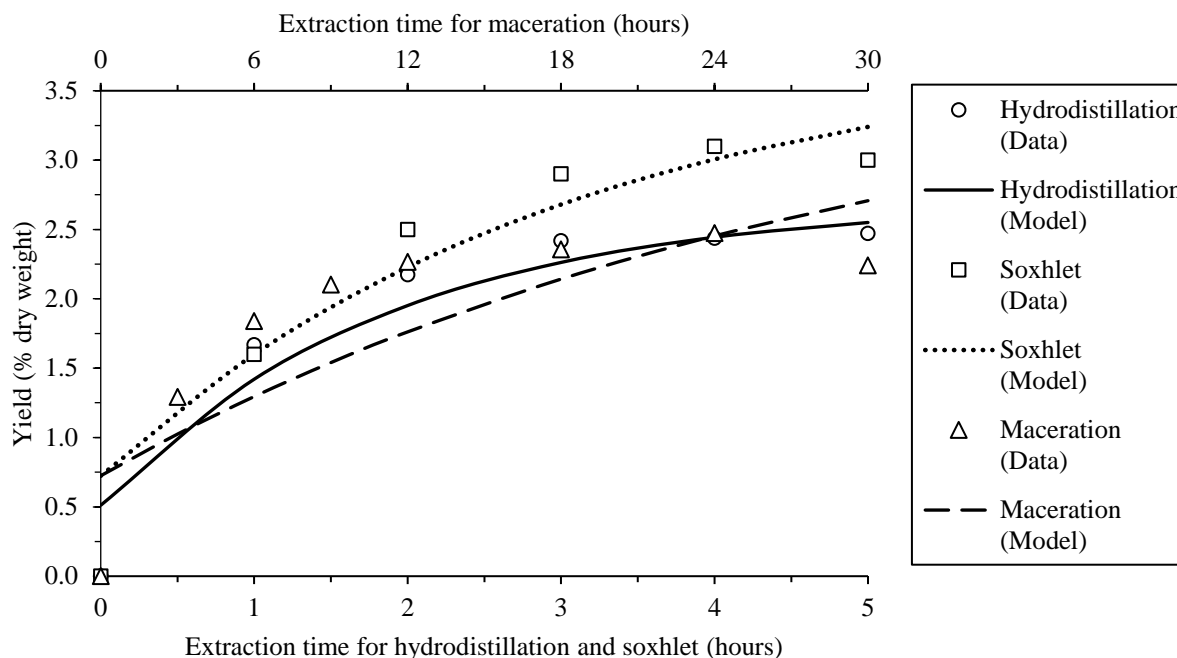


Figure 3 Comparison of experimental and modelled cinnamon oil yield over time

Conclusion

Solid-state fermentation of cinnamon bark using *Aspergillus awamori* able to decrease the lignocellulose content and increased the cinnamon oil yield. The biggest decrease of cellulose, hemicellulose, and lignin occurred after 9 days of fermentation: 12.37%, 16.55%, and 39.95%, respectively. The yield of cinnamon oil after 9 days of fermentation using the hydrodistillation, maceration and Soxhlet methods were 2.09%, 2.47%, and 3.01%, respectively. The cinnamon oil primarily composed of cinnamaldehyde, and the concentration varies with fermentation time and extraction methods. A mathematical model has been successfully to determine the diffusion coefficient of cinnamon oil during the extraction process and can be used predict the cinnamon oil yield. The results indicate that a higher diffusion coefficient of $2.36 \times 10^{-11} m^2/s$ was obtained for the hydrodistillation method, followed by Soxhlet ($1.44 \times 10^{-11} m^2/s$) and maceration ($1.42 \times 10^{-12} m^2/s$). The mathematical model also can predict the cinnamon oil yield for all extraction methods reasonably well.

Acknowledgement

The authors would like to thank the Central Tea Plantation and Kina Gambung for providing the cinnamon bark necessary for this research. The authors would also like to thank PPM KK ATB-ITB for financing this study.

References

- [1] Wong, Y. C., Ahmad-Mudzaqqir, M. Y., & Wan-Nurdiyana, W. A., 2014, "Extraction of essential oil from cinnamon (*Cinnamomum zeylanicum*), "Oriental journal of chemistry.,30(1), 37-47.
- [2] Chen, P., Sun, J., & Ford, P., 2014, "Differentiation of the four major species of cinnamons (*C. burmannii*, *C. verum*, *C. cassia*, and *C. loureiroi*) using a flow injection mass spectrometric (FIMS) fingerprinting method, "Journal of agricultural and food chemistry., 62(12), pp. 2516-2521.
- [3] Ferry, Y., 2013, "Prospek pengembangankayumanis (*Cinnamomum burmannii* L) di Indonesia, "Balai Penelitian Tanaman Industri dan Penyegar. Sukabumi.
- [4] Food and Agriculture Organization. 2019. FAOSTAT. Retrieved on 31 October 2021 from <http://www.fao.org/faostat/en/>
- [5] Dirjenbun., 2008, "Kebijakan Pembangunan Komoditi Kayu Manis,"Seminar AgribisnisKayumanis Nasional 27/03 di Auditorium Deptan, Jakarta. Direktorat Jenderal Perkebunan Departemen Pertanian
- [6] Paranagama, P. A., Wimalasena, S., Jayatilake, G. S., Jayawardena, A. L.Senanayake, U. M., & Mubarak, A. M., 2001, "A comparison of essential oil constituents of bark, leaf, root and fruit of cinnamon (*Cinnamomum zeylanicum* Blum) grown in Sri Lanka,"Journal of the National Science Foundation of Sri Lanka., 29(3&4), pp. 147-153
- [7] Rao, V. P., & Pandey, D., 2007, "Extraction of essential oil and its applications, "Biotechnology thesis., National Institute of Technology Rourkela., Rourkela, India.
- [8] Haddi, K., Faroni, L. R., & Oliveira, E. E., 2017, "Cinnamon oil, "Green Pesticides Handbook: Essential Oils for Pest Control., pp. 118-150.
- [9] Fajar, A., Ammar, G. A., Hamzah, M., Manurung, R., & Abduh, M. Y., 2019, "Effect of tree age on the yield, productivity, and chemical composition of essential oil from *Cinnamomum burmannii*, "Current Research on Biosciences and Biotechnology., 1(1), pp. 17-22
- [10] Younis, A., Mehdi, A., & Riaz, A., 2011, "Supercritical carbon dioxide extraction and gas chromatography analysis of *Jasminum sambac* essential oil,"Pakistan Journal of Botany., 43, pp.163-168.
- [11] Jeyaratnam, N., Nour, A. H., Kanthasamy, R., Nour, A. H., Yuvaraj, A. R., & Akindoyo, J. O., 2016, "Essential oil from *Cinnamomum cassia* bark through hydrodistillation and advanced microwave assisted hydrodistillation,"Industrial Crops and Products., 92, pp. 57-66.
- [12] Geng, S., Cui, Z., Huang, X., Chen, Y., Xu, D., & Xiong, P., 2011, "Variations in essential oil yield and composition during *Cinnamomum cassia* bark growth, "Industrial Crops and Products., 33(1), pp. 248-252.
- [13] Setyowati, F. P., Utami, R., Khasanah, L. U., & Manuhara, G. J., 2018, "Optimization of two-stage cinnamon bark (*Cinnamomum burmannii*) oleoresin maceration extraction process with ethanol solvent using response surface methodology (RSM),"AIP Conference Proceedings., 2014(1), 20072, pp. 1- 8
- [14] Baydar, H., Schulz, H., Krüger, H., Erbas, S., & Kineci, S., 2008, "Influences of fermentation time, hydro-distillation time and fractions on essential oil composition of Damask Rose (*Rosa damascena* Mill.), "Journal of Essential Oil Bearing Plants., 11(3), pp. 224-232.
- [15] Campbell, N., Reece, J., & Mitchell, L., 2005, "A tour of the cell, "Biology., pp. 1-1390.

- [16]Rahardjo, Y. S., Weber, F. J., Le Comte, E. P., Tramper, J., &Rinzema, A.,2002, "Contribution of aerial hyphae of *Aspergillus oryzae* to respiration in a model solid-state fermentation system, "Biotechnology and Bioengineering., 78(5), pp. 539-544.
- [17]Su, Y., Yu, X., Sun, Y., Wang, G., Chen, H., & Chen, G. ,2018, "Evaluation of screened lignin-degrading fungi for the biological pretreatment of corn stover, "Scientific reports., 8(1), pp. 1-11.
- [18]Katara, G., Hemvani, N., Chitnis, S., Chitnis, V., &Chitnis, D. S., 2008, "Surface disinfection by exposure to germicidal UV light, "Indian journal of medical microbiology., 26(3), 241-242.
- [19]Sautour, M., Dantigny, P., Guilhem, M. C., &Bensoussan, M., 2003, "Influence of inoculum preparation on the growth of *Penicillium chrysogenum*,"Journal of applied microbiology., 95(5), pp.1034-1038.
- [20]Mayhati, A., Patong, R., Djide, M. N., &Taba, D., 2013, "Biodegradation of lignin from corn cob by using a mixture of *Phanerochaete chrysosporium*, *Lentinus edodes* and *Pleurotus ostreatus*,"International Journal of Scientific & Technology Research., 11, pp. 79-82.
- [21]Geng, S., Zhou, W., Yuan, Q., Cai, D., & Zeng, Y., 2011," EEG non-linear feature extraction using correlation dimension and Hurst exponent," Neurological research., 33(9), pp. 908-912.
- [22]Cassel, E., Vargas, R. M. F., Martinez, N., Lorenzo, D., &Dellacassa, E., 2009, "Steam distillation modeling for essential oil extraction process, "Industrial crops and products., 29(1), pp. 171-176.
- [23]Katiyar, R., 2017, "Modeling and simulation of *Mentha arvensis* L. essential oil extraction by water-steam distillation process, "International Research Journal of Engineering and Technology., 4(6), pp. 2793-2798.
- [24]Chan, C. H., Yusoff, R., &Ngoh, G. C., 2014, "Modeling and kinetics study of conventional and assisted batch solvent extraction, "Chemical engineering research and design, 92(6),pp. 1169-1186.
- [25]Jahongir, H., Miansong, Z., Amankeldi, I., Yu, Z., &Changheng, L., 2019, "The influence of particle size on supercritical extraction of dog rose (*Rosa canina*) seed oil," Journal of King Saud University-Engineering Sciences., 31(2), pp.140-143.
- [26]Paibon, W., Yimnoi, C. A., Tembap, N., Boonlue, W., Jampachaisri, K., Nuengchamnon, N., &Ingkaninan, K., 2011, "Comparison and evaluation of volatile oils from three different extraction methods for some Thai fragrant flowers, "International journal of cosmetic science., 33(2), pp. 150-156
- [27]Stoilova, I., Krastanov, A., & Bui, H., 2008, "Biodegradation of mixed phenolic compounds by a microbial association of *Aspergillus awamori* and *Thermoascus aurantiacus*," Electron. J. Environ. Agric. Food Chem., 7, pp. 2625-2633.
- [28]El Monssef, R. A. A., Hassan, E. A., & Ramadan, E. M., 2016, "Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment, "Annals of Agricultural Sciences., 61(1), pp. 145-154.
- [29]Umsza-Guez, M. A., Díaz, A. B., Ory, I. D., Blandino, A., Gomes, E., & Caro, I., 2011, "Xylanase production by *Aspergillus awamori* under solid state fermentation conditions on tomato pomace, "Brazilian Journal of Microbiology., 42(4), pp. 1585-1597.
- [30]Ajijolakewu, A. K., Leh, C. P., Abdullah, W. N. W., & Lee, C.. 2017, "Optimization of production conditions for xylanase production by newly isolated strain *Aspergillus niger* through solid state fermentation of oil palm EFB, "Biocatalysis and Agricultural Biotechnology, 11, pp. 239-247.
- [31]Eaton, R. A., & Hale, M. D., 1993, "Wood: decay, pests and protection, "Chapman and Hall Ltd.

- [32] Muhammad, B. A. O. A. A., & Okiki, P. A., 2016, "Cellulase Production by Fungi Isolated from OdoAremu Dumpsite in Ado-Ekiti, Nigeria" *Advances in Life Science and Technology.*, 46, pp.46-51.
- [33] Malherbe, S., & Cloete, T. E., 2002, "Lignocellulose biodegradation: fundamentals and application, " *Reviews in Environmental Science and Biotechnology.*, 1(2), pp. 105-114
- [34] Chávez-González, M. L., López-López, L. I., Rodríguez-Herrera, R., Contreras-Esquivel, J. C., & Aguilar, C. N., 2016, "Enzyme-assisted extraction of citrus essential oil, " *Chemical Papers.*, 70(4), pp. 412-417.
- [35] De Castro, M. L., & Garcia-Ayuso, L. E., 1998, "Soxhlet extraction of solid materials: an outdated technique with a promising innovative future, " *Analytica chimica acta.*, 369(1-2), pp. 1-10.
- [36] Patel, P. N., Patel, K. M., Chaudhary, D. S., Parmar, K. G., Patel, H. A., Kansagra, C. D., & Sen, D. J., 2011, "Extraction of herbal aroma oils from solid surface, " *International Journal of Comprehensive Pharmacy.*, 9(2), pp. 1-10.
- [37] Suryani, E., Nurmansyah, N. F. N., Purwiyanti, S., & Rostiana, O., 2018, "The Growth, Productivity and Quality of Fifteen Accessions of Ceylon Cinnamon at Medium Elevation of Solok, West Sumatera, " *Buletin Penelitian Tanaman Rempah dan Obat.*, 28(2), pp. 105-112.
- [38] Parthasarathy, V., Chempakam, B., & Zachariah, T., 2008, "Chemistry of spices, " Wallingford, UK: CABI Pub
- [39] Moghaddam, M., & Mehdizadeh, L., 2017, " Chemistry of Essential Oils and Factors Influencing Their Constituents, " *Soft Chemistry and Food Fermentation.*, pp. 379–419.
- [40] Jones, H. G., 2013, "Plants and microclimate: a quantitative approach to environmental plant physiology, " Cambridge university press