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JUDUL **ENZYMATIC-ENHANCED PRODUCTION OF GAHARU OIL: EFFECTS OF ENZYME LOADING AND DURATION TIME**

SESI PENGAJIAN: **2007/2008**

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ENZYMATIC-ENHANCED PRODUCTION OF GAHARU OIL: EFFECTS OF ENZYME LOADING AND DURATION TIME

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering

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MAY 2008

I declare that this thesis entitled "Enzymatic-Enhanced Production of Gaharu Oil: Effects of Enzyme Loading and Duration Time" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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To my family,

Thanks for your love and support.

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ABSTRACT

Gaharu, a resinous wood that occurs in trees is belong to *Aquilaria* genus. It contains more than 12 chemical components that can be extracted that produce aromatic smells. To enhance oil availability and extractability, the effect of enzyme to substrate ratio and pretreatment time were studied. There are two main processes involved: enzymatic pre-treatment to break down the cell wall of gaharu, and extraction of the gaharu oil. The gaharu woods undergo drying and grinding processes before enzymatic hydrolysis. Gaharu were enzymatically hydrolyzed using Celluclast 1.5 L and the extraction was carried out using hydro-distillation. The extracted oil were then collected and analyzed by calculating the percentage of gaharu oil yield. Prolonged pretreatment time during enzymatic pretreatment process significantly increased the extraction rate of oil from gaharu. The longest pretreatment time, 9 hours gives the yield of 0.1275%. The higher enzyme to gaharu ratio, the higher yield of extracted oil has been achieved. With the highest ratio, 8 ml enzyme/ 400 g gaharu, the yield of extracted gaharu oil is 0.08375%.

ABSTRAK

Gaharu, ialah sejenis kayu resinus dari keluarga *Aquilaria.* Ia mengandungi lebih daripada 12 jenis komponen kimia yang boleh diekstrak untuk menghasilkan wangian. Untuk mempertingkatkan kebolehan pengekstrakan pati minyak gaharu, faktor nisbah enzim kepada gaharu dan masa rawatan awal berenzim telah dikaji. Dua proses yang penting terlibat ialah proses rawatan awal berenzim untuk memecahkan dinding sel pada kayu gaharu dan diikuti dengan proses pengekstrakan minyak gaharu. Kayu gaharu melalui proses pengeringan dan dikisar sebelum menjalani proses hidrolisis berenzim. Celluclast 1.5 L ditambah ke dalam gaharu dan proses pengekstrakan dijalankan dengan menggunakan penyulingan hidro. Selepas 72 jam penyulingan, minyak gaharu yang diekstrak dari hasil sulingan dan dianalisis berdasarkan peratusan hasil minyak. Masa bagi rawatan awal berenzim yang lebih lama meningkatkan minyak yang diekstrak. Dengan 9 jam rawatan awal berenzim, minyak yang didapati adalah sebanyak 0.1275%. Selain itu, nisbah enzim kepada gaharu yang lagi tinggi, memberikan lebih banyak hasil minyak gaharu. Nisbah 8 ml enzim/ 400 g gaharu menghasilkan sebanyak 0.08375% minyak gaharu.

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LIST OF SYMBOLS

g	-	gram
ml	-	mililiter
L	-	liter
kg	-	kilogram
°C	-	°Celcius
%	-	percent
rpm	-	rotation per minute
w/v	-	weight per volume
w/w	-	weight per weight
min	-	minute
hr	-	hour

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CHAPTER 1

INTRODUCTION

1.1 Background

Agar wood or Gaharu is a resinous wood that sometimes occurs in trees belong to the *Aquilaria* genus, *Thymelaeceae* family. Agar word producing species are found from India eastwards throughout Southeast Asia (Indonesia, Thailand, Cambodia, Laos, Vietnam, and Malaysia). *Aquilaria* is a fast-growing, archaic tropical forest tree. There are different names for agar wood such as ch'en hsiang, eagle wood, jin-koh, oud and others. There are 15 species of *Aquilaria*. In Malaysia, there are five species of *Aquilaria* which are *Aquilaria Malaccensis, Aquilaria Microcorpa, Aquilaria HIrta, Aquilaria Rostrata* and *Aquilaria Becanana.* Agar wood contains more than 12 chemical components that can be extracted. The resinous wood or oil extracted from the inside of *Aquilaria sp.* trees is extremely valuable for the use of religion cultural activities as well as an important ingredient in many traditional medicines and also used as perfume component. Agar wood extracts bring high prices range from a few dollars per kilo for the lowest quality to over 3000 US dollars for top quality oil and resinous wood (Cheksum *et al.,* 2002).

The main extract of the *Aquilaria* wood contained sesquiterpene namely alfa-agarofuran, (-)-10 epi-gama-eudesmol and oxo-agarospirol that produce the aromatic smell of the *Aquilaria* incense wood (Cheksum *et al.,* 2002). In order to facilitate its extraction from seeds, it is necessary to degrade the cell walls to increase the permeability for oil (Olsen, 1988). Oil extraction can also be favoured upon partial hydrolysis of the cell walls by means of appropriate enzymes (Domínguez *et al.,*

1996*a*). Enzyme treatment with carbohydrases and proteases was reported to enhance the oil extractability of seeds (Lanzani *et al.,* 1975; Fullbrook, 1983; Domínguez *et. al.,* 1994). The oil extraction yields can be improved if an enzymatic treatment is applied during the mixing step (Fullbrook, 1983; Marek *et al.,* 1990; Tano-Debrah and Ohta, 1995*a*,*b*; Sengupta and Battacharyya, 1996; Tano-Debrah *et al.,* 1996). The cell wall degradation caused by the enzymes increases the permeability to the oil through the seed membranes. The use of several enzymes as cellulases, hemicellulases, pectinases, amylases, proteases has been reported (Lanzani *et al.,* 1975; Bhatnagar and Johari, 1987; Badr and Sitohy, 1992), and it is believed that the multiple activity complexes and enzyme mixtures being especially effective, due to their synergistic action on the demolition of cell walls (Düsterhöft *et al.,* 1993).

1.2 Problem Statement

The traditional way to extract the gaharu oil by soaking the gaharu powder in pure water takes months in order to extract the oil from the gaharu and the percentage of the yield is low. These are the reasons why this research is carried out in order to speed up the extraction of the gaharu oil. To overcome the low percentage of yield and long period to extract the oil, enzymatic hydrolysis, a pre-treatment process, is apply before the gaharu is being distillate. The results from the previous research shows that the yield of extraction gaharu oil using enzyme as pre-treatment (enzymatic hydrolysis) give the highest results compared with extraction without enzyme pre-treatment. Enzymatic hydrolysis need mild operational conditions (pH 4.8 & temperature 45 - 50^{o}C) favour production of high quality products such as oil that need no further refining and detoxified meal (Lanzani *et al.,* 1991; Ohlson, 1992). Enzymatic hydrolysis produces better yields than acid-catalyzed hydrolysis (Pan *et al.,* 2005). Domínguez *et al.,* (1994) indicated enzymatic hydrolysis to be a promising field in today's biotechnological applications which has potential to enhance oil recovery from the oilseeds in shorter time with increased capacity of the equipments. This research will be carried out in order to extract the highest yield of essential oil at optimum conditions of enzymatic hydrolysis which are duration time and enzyme loading. Increasing the dosage of cellulases in the process, to a certain

extent, can enhance the yield and rate of the hydrolysis, but would significantly increase the cost of the process (Bhatnagar, 1987). At the same time, the enzyme's characteristic also will be affected by pH, temperature and substrate concentration (Cantwell *et al.,* 1988; Durand *et al.,* 1988; Orpin, 1988). By studying the effects of the enzymatic pre-treatment, the yield of oil extracted will be greatly enhanced and the optimum condition for the hydrolysis process will be determined.

1.3 Scope of Research Work

In order to enhance the production of gaharu oil, two sequential steps processes are involved that are enzymatic hydrolysis followed by extraction using hydro distillation. In this research, effect of time during enzymatic pretreatment and enzyme loading are studied. Other factors that affect the enzymatic hydrolysis include substrate concentration, enzyme activity and reaction conditions (temperature, pH, shaking rate and others) were maintained at specific conditions.

1.4 Objectives

The objectives of this study are:

i. To study the effect of duration time (hour) on gaharu essential oil extraction with enzymatic hydrolysis.

ii. To study the effect of enzyme loading (ml enzyme/ g gaharu) on gaharu essential oil extraction with enzymatic hydrolysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Not all *Aquilaria* trees produce agar wood. It is estimated that only approximately 10% of wild *Aquilaria* produce resin. According to Chakrabarty *et al.,* (1994), infected trees produce resin from the age of 20 years onwards. Important chemical components and the number of components that contribute to the characteristic of aroma gaharu will determine the quality of the gaharu essential oil in all samples for every process of extraction (Bhat, 2000). There are three major components in gaharu which are agarospirol, jinkoh-eremol and khusenol which ensure every extraction process is successful (Shimada *et al.,* 1982).

2.2 Extraction

Extraction is a separation process to separate solute or removed undesirable solute component from the solid where the solid is contacted with a liquid phase. Fragrance extraction is processes which involve extracting aromatic compounds from the raw material using various methods such as distillation, solvent extraction and expression. The first and the most common method due to their simple construction, low cost and easy operation used for essential oil extraction is hydro-distillation (Gilbert and Martin*.,* 2002).

Like other biorenewable solvents (alcohols, supercritical fluids), the use of water as the most economical extracting agent is gaining interest, especially with the aim of replacing the use of toxic solvents (Shoemaker, 1981; Johnson and Lusas, 1983). Aqueous extraction is advantageous over conventional pressing and hexane extraction methods since the solvent is neither toxic nor presents any risk of fires and explosion. The operation is more flexible with safer startup and shutdown in the absence of flammable solvents, favouring less initial capital investment and operation costs and also offering the possibility of processing different temporal crops (Domínguez *et al.,* 1996*a*). The mild operation conditions favour production of high quality products such as oil, that need no further refining and detoxified meal (Lanzani *et al*., 1991; Ohlson, 1992).

The considerations of choosing distillation to extract the essential oil are based on the sensitivity of the essential oils to the action of heat and water, the volatility of the essential oil and the water solubility of the essential oil (Mishra *et al.,* 2005). The quality of the oil extracted by hydro-distillation relies on several factors that are pressure, temperature and time (Dron *et al.,* 1997). These factors extensively influenced the quality of the product. Essential oils on a molecular level possess a delicate bond. If any of these factors are not properly met, these bonds will break up and the 'essence' of the plants will not preserved (Dron *et al.,* 1997).

By using hydro-distillation method, the plant material is fully submerged in water and placed in a distilling flask. Heat is applied to the flask. Once the water reaches its boiling point and become steam, it extracts the oils from the plant and evaporates. The steam is then chilled in a condenser and the resulting distillate product is collected. The essential oil normally float on top of the distilled water component which called hydrosol. The oil and water are then separated because of its different density by using oil separator (Mishra *et al.,* 2005).

Reverchon *et al.,* (1992) stated that using hydro-distillation protect the oils since the surrounding water acts as a barrier to prevent it from overheating. Lanzani *et al.,* (1983) reported 63% removal of polyphenolics from whole sunflower seeds during the oil extraction with water as extracting agent and 77% from dehulled seeds.

2.3 Enzymatic Hydrolysis

Lignocellulose is the major structural component of woody that strengthens woody plant cells. In lignocellulosic materials cellulose, a liner polymer of glucose is associated with hemicellulose and surrounded by lignin seal (Coughlan, 1992). Lignin, a complex three-dimensional polyaromatic matrix prevents enzymes from accessing some regions of the cellulose polymers. Crystallinity of the cellulose further impedes enzymatic hydrolysis. Pretreatment of lignocellulosic materials to remove lignin and hemicellulose can significantly enhance the hydrolysis of cellulose. Optimization of the cellulase enzymes and the enzyme loading can also improve the hydrolysis (Sun and Cheng, 2001). The performance for the treated samples being 12% higher than for untreated samples (Domínguez *et al.,* 1996*b*).

Hydrolysis of cellulosic materials has been intensively investigated since the 1970s. Alkaline (Chahal, 1992) and acid (Nguyen, 1993; Converse and Grethlein, 1991) hydrolysis methods have been used to degrade lignocellulose. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperature and pH which necessitate the use of expensive equipment. Also, unspecific side reactions occur which yield non-specific by-products (Nguyen, 1993). Utility cost of enzymatic hydrolysis is lower than acid or alkaline hydrolysis.

Enzymatic hydrolysis not only give a high yield of pure glucose, low environmental impact, economize energy on account of the relatively mild reaction conditions (pH 4.8 and temperature 45 – 50^0C), but also avoid using toxic and corrosive chemicals (Duff and Murray, 1996). Enzymatic hydrolysis does not produce sugar degradation products that are inhibitory to fermentative micro-organisms (Hsu, 1996). Domínguez *et al.,* (1994) indicated enzymatic hydrolysis to be a promising field in today's biotechnological applications which has potential to enhance oil recovery from the oilseeds in shorter time with increased capacity of the equipments.

The enzymatic treatment was successfully performed either during aqueous processing for oil and protein extraction (Fullbrook, 1984; Marek *et al.,* 1990) or during conventional oil extraction by pressing (Sosulski and Sosulski, 1990). Ramos *et al.,* (1993) reported that the enzyme mixture of the commercial Celluclast and Novozyme preparation was successfully recycled for five consecutive steps. The efficiency of cellulose hydrolysis decreased gradually with each recycling step.

Enzymatic hydrolysis of cellulose consists of three steps: adsorption of cellulase enzymes onto the surface of the cellulose, the biodegradation of cellulose to fermentable sugars, and desorption of celllulase. Cellulase activity decreases during the hydrolysis. The irreversible adsorption of cellulase on cellulose is partially responsible for this deactivation (Converse *et al*., 1988).

The cellulase enzymatic complex offered by many commercial enzyme products consist of four major enzymes referred to endoglucanase, exoglucanase, cellobiohydrolase and β-glucosidase. Endoglucanase permits “random” scission of cellulose chains yielding glucose, cellobiose and cellotriose and attacks the regions of low crystallinity in the cellulose fiber, creating free chain-ends. Exoglucanase and cellobiohydrolase degrades the molecule further by removing cellobiose units from the free chain-ends. Meanwhile β-glucosidase which also known as cellobiase hydrolyzes cellobiose to produce glucose, with possible activity with respect to cellulodextrins (Coughlan and Ljungdahl, 1988).

Oil extraction with enzymes as processing aids has been extensively reported for fruits (Buenrosto and López-Munguía, 1986; Cintra *et al.,* 1986; Alba *et al.,* 1987; Christenson and Olsen, 1988). Oil extraction yields from seeds became greatly enhanced when the enzymatic treatment was performed during the aqueous oil extraction (Olsen 1988; Laiho *et al.,* 1990; Marek *et al.,* 1990; Badr and Sithohy, 1992). Several investigators have been successful in using enzymatic hydrolysis process to achieve enhanced release of extractable oil from melon seed, sunflower seed, crushed soybean, cottonseed, castor seed, canola seed, soybrokens, soygrits and soyflakes (Bargale *et al*., 2000; Bhatnagar and Johri, 1987; Fullbrook, 1983; Kashyap *et al.,* 1997; Kashyap *et al*., 2006; Smith *et al.,* 1993; Sosulski *et al.,* 1988).

2.3.1 Effect of enzyme/ substrate ratio

The effects of enzyme loading were investigated by adding different amounts of Celluclast-1.5L to the hydrolysis system (Wen *et al.,*, 2003). For cellulases, water activity plays an important role in swelling, expanding the structure of fibre, increasing the surface area accessible to cellulolytic enzymes and also facilitating the diffusion of enzymes and the inhibiting products formed (Fan *et al*., 1987). The oil yield improvement is dependent on cell wall rupture (Domínguez *et al.,* 1996*b*).

Maximum oil extraction yield with higher enzyme/ substrate ratio (Domínguez *et al.,* 1996*a*). Cell wall degradation increase when the quantity of enzyme used increase, this lead to higher oil extracted (Badr and Sitohy, 1992). The degree of cell walls enzymatic attack was found to be dependent on enzyme/ susbtrate ratio and treatment-extraction time (Domínguez *et al.,* 1996*a*).

Figure 2.1 shows the results carried out by Ming *et al.*, (2007). The optimal enzyme dosage was 20 FPU/ g substrate. Further increase in enzyme dosage did not produce a corresponding increase in the hydrolysis yield.

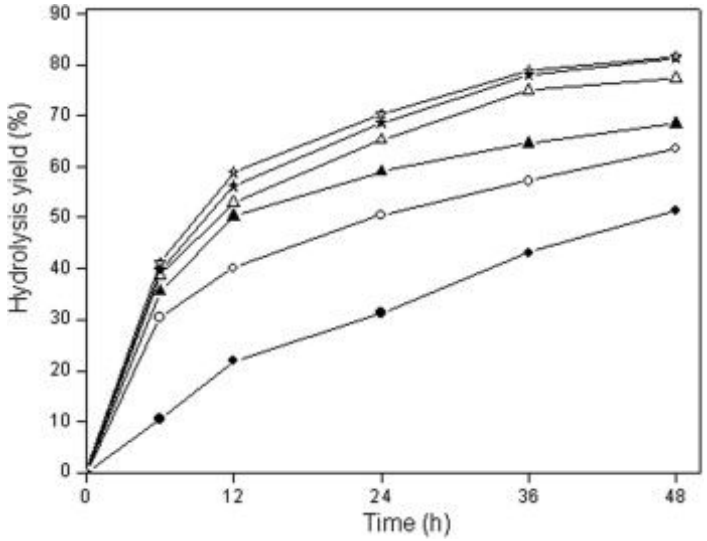

Figure 2.1 Effects of enzyme dosage (presented as filter paper activity per gram of substrate, FPU/g substrate) on the enzymatic hydrolysis. (•) 7 FPU/g substrate; (○) 10 FPU/g substrate; (▲) 13 FPU/g substrate; (△)17 FPU/g substrate; (★) 20 FPU/g substrate; (☆) 23 FPU/g substrate. (Ming *et al.*, 2007)

2.3.2 Effect of length of pretreatment time

Prolonged treatment times and high enzyme/ substrate ratio are beneficial (Domínguez *et al.,* 1996*a*). From the experimental carried out by Domínguez *et al.,* (1996*a*), with the increase of treatment time range 2.5-7.5 hours, oil extraction yield increased slightly from 75% - 77%. Meanwhile, the performance for the treated samples being 12% higher than for untreated samples.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This research involves two major processes which are enzymatic treatment and followed by hydro-distillation. Six steps are involved which are drying, grinding, prepare buffer solution, enzymatic pre-treatment, extraction and analysis. Enzyme cellulase from *Trichoderma* reesei (ATCC 26921) also known as Celluclast 1.5 L is used in the enzymatic treatment. Figure 3.1 shows the methods carry out in a flow chart.

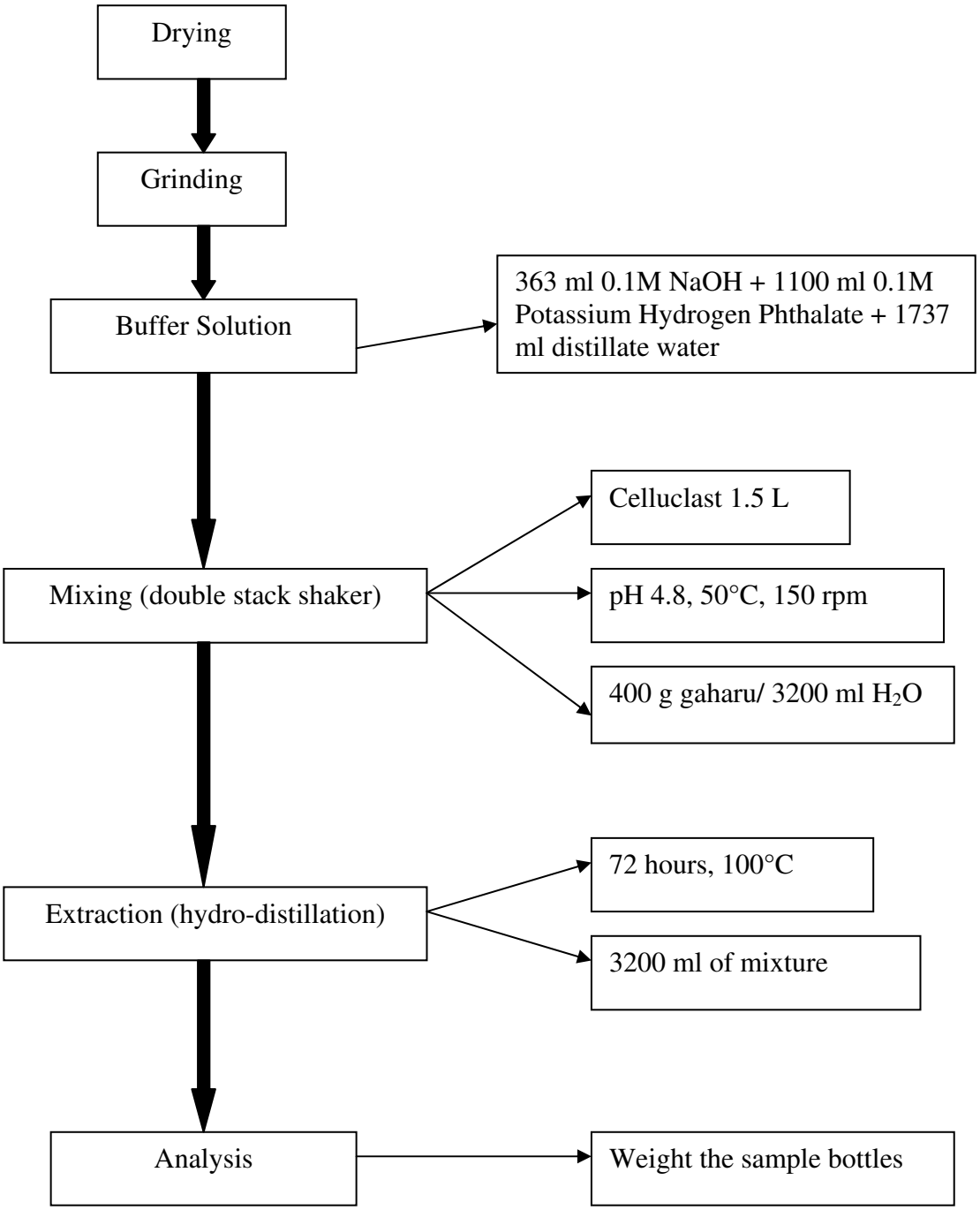

Figure 3.1 Flow chart of the overall process

3.2 Drying Process

Gaharu wood is cut into smaller pieces to increase the surface area for drying process. The gaharu has to undergo this process in order to avoid any blockage in grinding process. The gaharu wood is dry by using tray dryer type Guntt Hamburg CE130.

3.3 Grinding Process

Grinder Disk Mill FFC23 types is use to grind the pieces of gaharu wood into sawdust with the size of 1 mm. In extraction process, the rate of extraction will increase when the area of contact between the solvent and solid is high. Therefore, the higher surface area of gaharu sawdust, more gaharu essential oil can be extracted.

3.4 Buffer Solution Preparation

363 ml 0.1M natrium hydroxide, NaOH were mixed together with 1100 ml 0.1 M potassium hydrogen phthalate. Then 1737 ml of distillate water were added into the buffer solution.

3.5 Enzymatic Pre-treatment

Gaharu are in sawdust form. 400 g of gaharu is soak with 3200 ml of buffer solution at pH 4.8. The mixing of gaharu sawdust and buffer solution takes place in the fume hood. This is to minimize the sawdust flying around in the air. The mixture is poured into a 5000 ml flask. The Celluclast 1.5 L is added when the mixture of gaharu and buffer solution is poured into half of the flask. The range of the ratio

enzyme substrate and duration time are tabulated in Table 3.1. The flasks are put into the stackable incubator shaker with temperature 50^{o}C. 50^{o}C is the optimum to preserve the quality of products and to favour both the activity and stability of enzymes and shaking rate is 150 rpm (Domínguez *et al.,* 1996*a*).

Table 3.1 : Optimization of enzymatic pretreatment

Variable	Experiment	Time of treatment (hr)	Ratio sample water (w/v)	Ratio enzyme substrate (v/w)	Result % Yield
Time	B1	1	1:8	1.0:100	
	B2	3	1:8	1.0:100	
	B3	6	1:8	1.0:100	
	B4	9	1:8	1.0:100	
Ratio enzyme substrate (v/w)	D1	3	1:8	0.5:100	
	D2	3	1:8	1.0:100	
	D3	3	1:8	1.5:100	
	D4	3	1:8	2.0:100	

3.6 Extraction Process

After the enzymatic pretreatment, the mixture is then transfer to the distilling flask to continue the extraction process. Hexane is added at the separating funnel together with water in order to separate the oil well. The apparatus are cover with aluminum foil where there is no heat loss occurs. The temperature of the extraction process is 100^{o}C, 3200 ml of mixture, and is carried out for 72 hours. The diagram of the hydro-distillation is shown in Figure 3.2.

Figure 3.2 Hydro-distillation

3.7 Analysis

After the extraction process, the condensate, which contains mixture of water and essential oil, is collected in a receiving flask. To separate the small amount of water from the oil, sodium sulphate is added to absorb the water. Hexane is added to absorb the oil trapped along the apparatus. At the receiving flask, the layer of essential oil is decanted and collected to sample bottles. The sample bottles were put in the fume hood for the vaporized of hexane from the oil. Each of the sample bottles are weight before and after collecting the oil. The weight of the bottles was recorded until the mass of the bottle and oil were constant. The percentage of gaharu oil yield is calculated as bellow:

$$\text{Oil Yield (\%)} = \frac{\text{Weight of Oil Extracted (g)}}{\text{Dry Weight Sample (g)}} \times 100 \qquad (3.1)$$

CHAPTER 4

RESULT AND DISCUSSION

In this research, two major parameters have been studied which are pre-treatment time and enzyme ratio. Four experiments were carried out for each parameter. For each of the experiment carried out, the extraction process is 72 hours and at 100°C.

4.1 Effect of Pre-treatment Time

Table 4.1 shows the percentage yield extracted oil for each of the experiment. For the pretreatment time during the pretreatment process, it varies for 1, 3, 6 and 9 hours. It is very obvious that 9 hours of treatment time give the highest yield among others with 0.1275% of extracted oil. With only one hour treatment, it gives the lowest percentage of yield that is only 0.03875. The oil extracted for 3 and 6 hours are 0.06675% and 0.09850%. The degree of cell walls enzymatic attack was found to be dependent on treatment time (Domínguez *et al.,* 1996*a*). Domínguez *et al.,* (1996*a*) also mentioned prolonged treatment times are beneficial to extract more essential oil. Figure 4.1 illustrates the yield of gaharu essential oil versus the time of pretreatment plotted based on the data from Table 4.1. In Figure 4.1, it is clear that the amount of gaharu essential oil extracted using hydro-distillation show an increasing trend of yield with pre-treatment time.

Based on the research by Domínguez *et al.,* (1996*a*), prolonged treatment times are beneficial.

Table 4.1 : Percentage yield for Pre-treatment time

Variable	Experiment	Time of treatment (hr)	Ratio sample water (w/v)	Ratio enzyme substrate (v/w)	Extraction Time, t (day)	Results % Yield
Time	B1	1	1:8	1.0:100	3	0.03875
	B2	3	1:8	1.0:100	3	0.06675
	B3	6	1:8	1.0:100	3	0.09850
	B4	9	1:8	1.0:100	3	0.1275

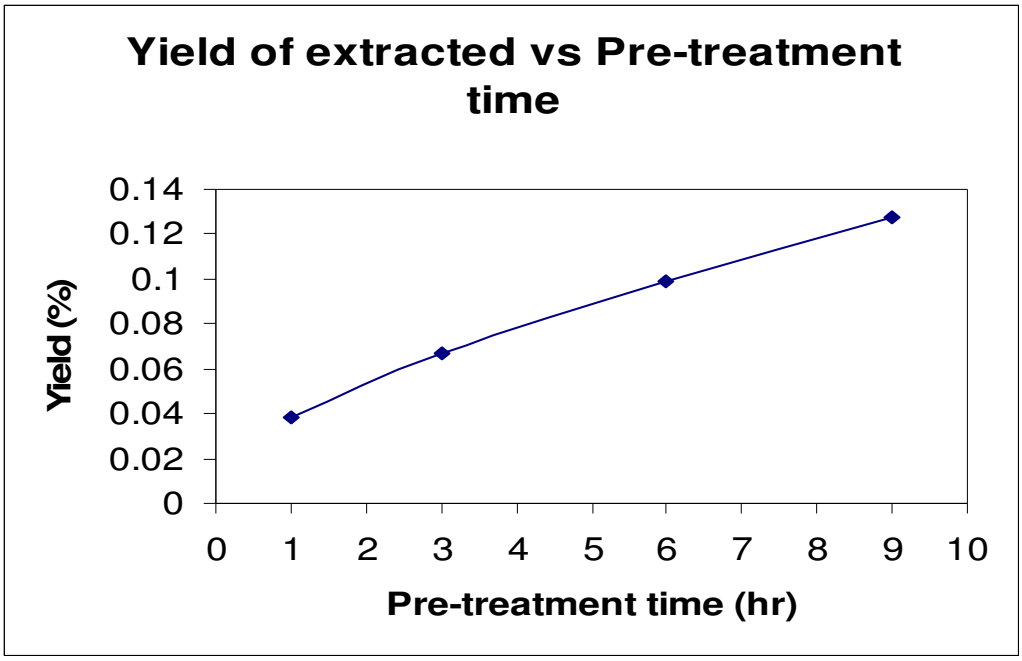

Figure 4.1 Yield of gaharu essential oil versus the time of pretreatment

4.2 Effect of Enzyme Ratio

The enzyme ratio varies according to Table 4.2 during the enzymatic pretreatment process. With 400 g of gaharu powder used in each of the experiment, the volume of the enzyme used is 2 ml, 4 ml, 6 ml and 8 ml. From Table 4.2, the highest yield extracted oil is 0.08375% with 2 : 100 enzyme/ substrate ratio. With ratio enzyme/ substrate, 0.5 : 100, gives the lowest yield with 0.05625%. The yield of extracted oil from each experiment gives approximately 0.01% increments. The oil

extracted from 1.0 : 100 and 1.5 : 100 are 0.06675% and 0.07650% respectively. Figure 4.2 is the graph plotted based on the data from Table 4.2. The degree of cell walls enzymatic attack was found to be dependent on enzyme ratio. Again, oil extractability of gaharu was progressively enhanced as the enzyme to substrate ratio increased.

From the research carried out by Domínguez *et al.,* (1996*a*), a high enzyme/ kernel ratio is beneficial. Badr and Sitohy (1992), research shows cell wall degradation increase when the quantity of enzyme used increase, this lead to higher oil extracted.

Table 4.2 : Percentage yield for Enzyme ratio

Variable	Experiment	Time of treatment (hr)	Ratio sample water (w/v)	Ratio enzyme substrate (v/w)	Extraction Time, t (day)	Results % Yield
Ratio enzyme substrate (v/w)	D1	3	1:8	0.5:100	3	0.05625
	D2	3	1:8	1.0:100	3	0.06675
	D3	3	1:8	1.5:100	3	0.07650
	D4	3	1:8	2.0:100	3	0.08375

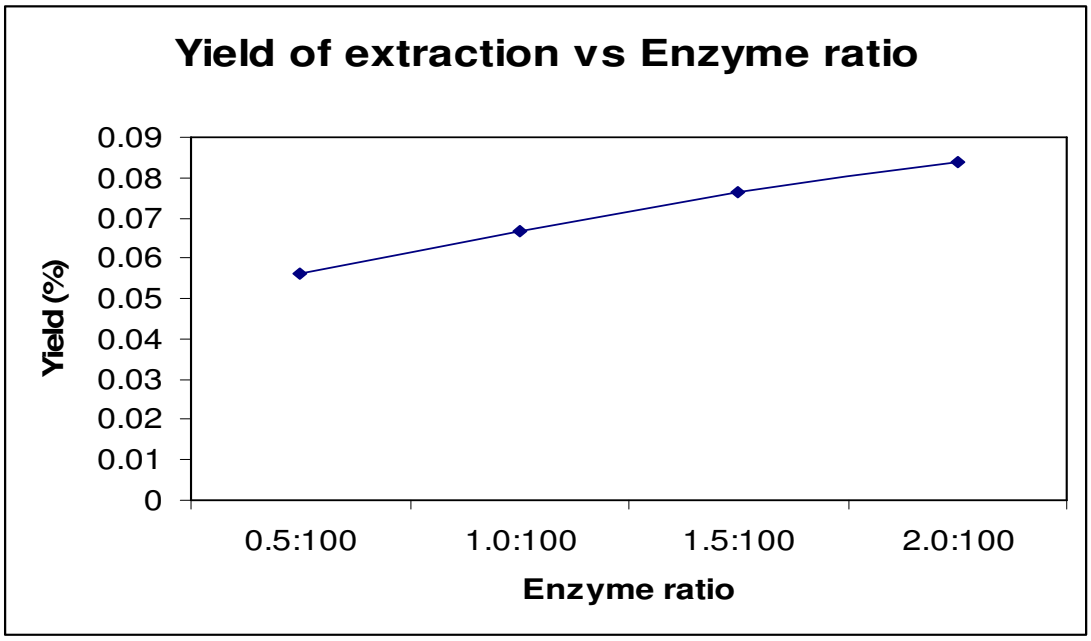

Figure 4.2 Yield of gaharu essential oil versus the enzyme ratio

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The objectives of this research have been fulfilled. The following conclusions were drawn:

1. Prolonged pretreatment time during enzymatic pretreatment process significantly increased the extraction rate of oil from gaharu. At optimum parameter value that is 9 hours of pretreatment, 0.1275% of extracted gaharu oil collected.
2. With higher enzyme to gaharu ratio, higher yield of extracted oil has been achieved. With 8 ml of enzyme, the yield of extracted gaharu oil is 0.08375%.

5.2 Recommendations

Gaharu oil extraction using enzymatic pretreatment followed by hydro distillation is a newly method discovered can improve the quantity of the extracted gaharu oil, thus, increase the demand of gaharu in local or global. To enhance the enzymatic production of gaharu oil by studying the effect of enzyme loading and duration time has contributed its advantages to the local entrepreneur, businessman and also university. From this research, several recommendations recommended in order to improve the quantity and quality of the extracted gaharu oil are as follow:

1. During the pretreatment process, activity of the enzyme should be study. This might contribute the types of enzymes can be considered to use in order to achieve a higher yield of extracted oil.
2. Cellulase mixture from Novozym and Celluclast can be considered to be used during the enzymatic pretreatment process. Based on Ovando *et al.,* (2005), Novozym were found as the most active and stable during hydration and hydrolysis of industrially. Besides, this mixture cellulase can be recycled during the hydrolysis process.
3. Other method of extraction process also can be used. Supercritical CO_2 extraction, steam distillation and others has claimed efficiently extract oil from plant material. The study of using different extraction utilities should be carried out.
4. A sample of standard and high quality of gaharu essential oil should be used to compare the quality of extracted gaharu oil by using enzymatic pretreatment process.
5. Product of gaharu essential oil should be analyzed using Gas Chromatography, GC to ensure the main component of gaharu is well extracted and to check whether the activity of enzyme caused any effect to the quality of the oil.

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APPENDIX A

Optimization of enzymatic pretreatment

Percentage yield for pretreatment time

Variable	Experiment	Time of treatment (hr)	Ratio sample water (w/v)	Ratio enzyme substrate (v/w)	Extraction Time, t (day)	Results % Yield
Time	B1	1	1:8	1.0:100	3	0.03875
	B2	3	1:8	1.0:100	3	0.06675
	B3	6	1:8	1.0:100	3	0.09850
	B4	9	1:8	1.0:100	3	0.1275

Percentage yield for Enzyme ratio

Variable	Experiment	Time of treatment (hr)	Ratio sample water (w/v)	Ratio enzyme substrate (v/w)	Extraction Time, t (day)	Results % Yield
Ratio enzyme substrate (v/w)	D1	3	1:8	0.5:100	3	0.05625
	D2	3	1:8	1.0:100	3	0.06675
	D3	3	1:8	1.5:100	3	0.07650
	D4	3	1:8	2.0:100	3	0.08375

APPENDIX B

Results on extraction process

Experiment B1

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	4 ml
Pretreatment time	=	1 hour
Extraction Process		
Date of experiment	=	23 January 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	4.641 g
Mass of bottle with oil	4.796 g
Weight of gaharu oil	0.155 g
Yield	0.03875 %

Experiment B2

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	4 ml
Pretreatment time	=	3 hours
Extraction Process		
Date of experiment	=	10 March 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	4.756 g
Mass of bottle with oil	5.023 g
Weight of gaharu oil	0.267 g
Yield	0.06675 %

Experiment B3

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	4 ml
Pretreatment time	=	6 hours
Extraction Process		
Date of experiment	=	10 March 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	4.746 g
Mass of bottle with oil	5.140 g
Weight of gaharu oil	0.394 g
Yield	0.09850 %

Experiment B4

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	4 ml
Pretreatment time	=	9 hours
Extraction Process		
Date of experiment	=	3 March 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	18.878 g
Mass of bottle with oil	19.388 g
Weight of gaharu oil	0.51 g
Yield	0.1275 %

Experiment D1

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	2 ml
Pretreatment time	=	3 hours
Extraction Process		
Date of experiment	=	14 January 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.
Oil collected		
Mass of empty bottle		4.769 g
Mass of bottle with oil		4.994 g
Weight of gaharu oil		0.225 g
Yield		0.05625 %

Experiment D2

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	4 ml
Pretreatment time	=	3 hours
Extraction Process		
Date of experiment	=	10 March 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.
Oil collected		
Mass of empty bottle		4.756 g
Mass of bottle with oil		5.023 g
Weight of gaharu oil		0.267 g
Yield		0.06675 %

Experiment D3

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	6 ml
Pretreatment time	=	3 hours
Extraction Process		
Date of experiment	=	18 January 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	4.740 g
Mass of bottle with oil	5.046 g
Weight of gaharu oil	0.306 g
Yield	0.07650 %

Experiment D4

Enzymatic Hydrolysis		
Amount of gaharu	=	400 g
Amount of buffer solution	=	3200 ml
Amount of enzyme	=	8 ml
Pretreatment time	=	3 hours
Extraction Process		
Date of experiment	=	23 January 2008
Setting temperature	=	100°C
Observation		Gaharu oil (green color) is on top of the water layer. Smell of gaharu in hydrosol, essential oil seem to dilute in water.

Oil collected	
Mass of empty bottle	4.746 g
Mass of bottle with oil	5.081 g
Weight of gaharu oil	0.335 g
Yield	0.08375 %