EFFECT OF ULTRASOUND ON ENZYMATIC EXTRACTION OF *ZINGIBER OFFICINALE*

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EFFECT OF ULTRASOUND ON ENZYMATIC EXTRACTION OF ZINGIBER OFFICINALE

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SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature

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STUDENT DECLARATION

I hereby declare that this thesis entitled “Effect of Ultrasound on Enzymatic Extraction of Zingiber officinale” 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.

Signature

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DEDICATION

In The Name of Allah, Most Gracious, Most Merciful

Special dedication of my grateful feelings to my parents, Mr. Zainal Bin Kadir & Mrs. Habibah Binti Saidon for their prayers and encouragement. Lastly not to forget, for my family members who always stand beside me.

For all your love, care, support, and believe in me.

Thank you so much.

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ABSTRACT

The production of essential oils from natural sources is highly profitable nowadays. The herbaceous perennial of ginger has been used as spices and is common condiment in variety of foods and beverages. Ginger is scientifically known as Zingiber officinale. This study is focused to extract the essential oil of ginger by using the technique of enzymatic extraction assisted with ultrasound in order to maximize the percentage of oil yield. Enzymatic extraction since past few decades had been acclaimed as the alternative process to produce the essential oil of herbs and oilseeds which more economically and environmental friendly than usual conventional extraction. This research has been carried out by using enzyme pectinase with different concentration from 1%, 1.5%, 2% and 3% for enzymatic extraction. The ultrasound procedure was performed by 10% and 20% duty cycle. As result indicates that by using enzymatic extraction method assisted with ultrasound, the high and good quality production of ginger oil was obtained. Pectinase concentration of 2% (v/w) yielded the highest percentage of oil recovered which is 66% and with 10% duty cycle of ultrasound, the yield was increased to 73.7% compared to solvent extraction yielded about 39% oil. As a conclusion, apart from being effective and as an alternative to conventional method, enzymatic extraction assisted with ultrasound also discovers the potential reduction of solvent consumption.

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ABSTRAK

Pengeluaran pati minyak daripada sumber semula jadi mempunyai pelbagai keuntungan dan kegunaan pada masa kini. Herba halia telah digunakan sebagai rempah dan perasa dalam pelbagai makanan dan minuman. Nama saintifik bagi halia adalah *Zingiber officinale*. Kajian ini adalah mengenai pengekstrakan pati minyak halia dengan menggunakan teknik pengekstrakan dengan menggunakan enzim dan dibantu oleh ultrabunyi untuk peningkatan penghasilan minyak. Melalui teknik pengekstrakan enzim, sejak beberapa dekad yang lalu telah diiktiraf sebagai proses alternatif untuk menghasilkan pati minyak herba yang lebih ekonomi dan mesra alam daripada pengekstrakan konvensional. Kajian ini telah dijalankan melalui penggunaan enzim *pectinase* dengan kepekatan yang berbeza iaitu 1%, 1.5%, 2% dan 3% untuk pengekstrakan enzim. Prosedur ultrabunyi telah dilakukan dengan menggunakan 10% dan 20% kitaran. Kaedah pengekstrakan menggunakan enzim dibantu dengan ultrabunyi, telah menghasilkan pengeluaran pati minyak halia yang berkualiti tinggi dan baik. Kepekatan enzim *pectinase* sebanyak 2% telah menghasilkan peratusan tertinggi minyak iaitu sebanyak 66% dan dengan kitaran 10% ultrabunyi, penghasilan minyak telah meningkat kepada 73.7% berbanding dengan pengekstrakan konvensional di mana penghasilan minyak adalah sebanyak 39%. Sebagai kesimpulan, selain daripada alternatif dan berkesan, kaedah pengekstrakan dengan menggunakan enzim dan dibantu oleh ultrabunyi juga dapat mengurangkan kadar penggunaan pelarut dalam pengesktrakan konvensional.

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LIST OF SYMBOLS/ABBREVIATIONS

NSAID	Non-Steroidal Anti-Inflammatory Drug
UAE	Ultrasound-Assisted Extraction
A	Area
P	Power
°C	Degree Celcius
G	Gram
mL	Millileter
h	Hour
kHz	KiloHertz
s	Second
W	Watt
W cm^{-2}	Watt per Centimeter Squared
®	Registered Sign
%	Percentage
α	Alpha

CHAPTER 1

INTRODUCTION

1.1 Overview of Ginger

Ginger is scientifically known as Zingiber *officinale*. The name was given by an English botanist, William Roscoe (1753-1831) in an 1807 publication. The species Zingiber officinale is said to be native from tropical climates, particularly Southeast Asia. Major world producers of ginger include Fiji, India, Jamaica, Nigeria, Sierra Leone, and China. These days broadly cultivated in India, China, Africa, Jamaica, Mexico and Hawaii. Ginger is consumed worldwide as spices and is a common condiment in variety of foods and beverages. Ginger also produce two primary extracts consist of essential oil and oleoresin. Essential oil basically gives the ginger its own unique aroma. The commercial ginger oil is normally extracted from dried rhizomes (Wohlmuth *et. al*., 2006).

1.2 Enzymatic Extraction

Recently, aqueous enzymatic extraction is found to be the alternative environmental friendly processes based on separation of oil and protein from oilseed, fruits, and herbs. By using specific enzyme to the extraction process, it may overcome the low extraction yields (Rosenthal *et. al*., 2001). Enzymes hydrolyze the structural polysaccharides forming the cell wall of oilseeds. Enzymes are used in extraction processes in order to assist the release of oil bodies. This kind of extraction offers many benefits, for instance, lower the investment cost and energy requirements rather than the conventional extraction (Rosenthal, et. al., 1996).

1.3 Ultrasound

Ultrasound has been proved to give the great impact in extraction processes. For example, it can improve the extraction yield, reduce the use of solvent, and thus to minimize the power consumption and reducing the extraction duration. The efficiency of extraction increase by ultrasound is because of the propagation of ultrasound pressure waves, and results in cavitations phenomena (Vilkhu et. al., 2008). According to Sulaiman (2011), ultrasound affects the enzyme-catalyzed reactions including formation and dissociation of the enzyme-substrate complex. Moreover, ultrasound influenced substrate and product inhibition characteristics of an enzyme.

1.4 Problem Statement

Enzymatic extraction since past few decades had been acclaimed by researcher as the alternative process to produce the essential oil and oleoresin of herbs and oilseeds which more economically and environmental friendly than usual conventional extraction. In addition, enzymatic extraction is to replace the solvent consumption in herbs extraction. The use of ultrasound is to gain enhancement and speed up the process of fine herbal extract, in this case is ginger. This research is going to be conduct to define the potential of ultrasound in order to enhance the performance of enzymatic extraction of ginger.

1.5 Objective of Research

The main objective of this research based on:

i) To obtain the best enzyme (pectinase) concentration used for enzymatic extraction of Zingiber *officinale.*

ii) To study the effect of ultrasound with various concentration of enzyme (pectinase) and various sonication regimen for extraction.

1.6 Research Question

i) What is the best characterization of enzyme used in enzymatic extraction of ginger oil?

ii) What is the best sonication regimen of ultrasound to produce good yield of ginger oil by enzymatic extraction?

1.7 Scope of Research

To achieve the objective of the study, the scope of research work is consists of as the following:

i) To determine the best enzyme concentration used in enzymatic extraction of Zingiber *officinale*.

ii) To define the best possible sonication regimen of ultrasound in order to produce good yield of Zingiber *officinale*.

1.8 Significant and Contribution of Research

Ginger oil has many benefits to human and also environment. The aqueous enzymatic extraction is an alternative to produce ginger oil which is more environmental friendly. Furthermore, this kind of extraction will also eliminate the solvent consumption

in usual conventional extraction. Hence, potential savings in the operational cost and reduce the energy consumption. This process also has been proved in yielding a good quality of oil. By using ultrasound-assisted, the duration of extraction also can be reduced and also will generate more good products.

CHAPTER 2

LITERATURE REVIEW

This chapter consists of three subtopics that discuss about previous study related to the extraction of Zingiber officinale. Three subtopics consists of background information of Zingiber officinale, a range of extraction method used as well as ultrasound-assisted extraction that will help in providing information for this current research.

2.1 Background Information of *Zingiber officinale*

Ginger, the rhizome of Zingiber officinale, from the Zingiberaceae family is one of the extensively used spices and is a common condiment for various foods and beverages. The Zingiberaceae is a tropical family consists of several species, which are known to produce essential oils, mainly in their seeds and rhizomes. The name of Zingiber officinale was given by an English botanist, William Roscoe (1753-1831) in

an 1807 publication. The name of Z. officinale comes from the Sanskrit word which by means is “horn-shaped” and it is said to be native from tropical climates, particularly Southeast Asia.

Nowadays, it is extensively been cultivated in most tropical countries for example in Australia, China, West Africa, Brazil, Japan and the West Indies (Ukeh *et. al.*, 2009). Ginger is an herbaceous perennial with upright stems and narrow medium green leaves arrayed in two ranks on each stem. Skinner (2003) found that the plant may grow to about 1.2 m tall with leaves about 1.9 cm wide and 17.8 cm long. The rhizomes are aromatic, thick lobed, branched and scaly structures with a spicy lemon-like scent. It is well known that ginger rhizomes contain both aromatic and pungent components (Singh, *et. al*., 2008).

Figure 1.0 Ginger Plant

2.1.1 Active Constituents of Ginger

The unique flavor properties of ginger come from the combination of pungency and aroma. The pungency is provided by non-volatile phenolic compounds, while the essential oil gives the ginger its own unique aroma. Ginger produce two primary extracts which are oleoresin and essential oil. Solvent extraction usually by using acetone or ethanol will yield the oleoresin which is containing both essential oil and the phenolic compounds, mainly for the pungency of ginger. The major pungent compounds in ginger, from studies of the lipophilic rhizome extracts, have generated potentially active gingerols (Govindarajan, 1982).

Ginger oleoresin is used extensively as a flavoring agent in the food and beverage industries. Commercial ginger oil is normally extracted from dried rhizomes. Typical ginger oil is characterized by the high content of by a high content of sesquiterpene hydrocarbons, mainly zingiberene (Wohlmuth *et. al.*, 2006). The West African entirely unscraped ginger is reported to have the highest essential oil content and the most pungent flavor (Ukeh, *et. al.*, 2009).

2.1.2 Usage of Ginger

Ginger is consumed worldwide as spices and is a common condiment in variety of foods and beverages. Ginger also been used in cosmetic industries. Ginger oil is useful in order to overcome any symptoms of food poisoning. It has antiseptic

and carminative properties that are useful in curing and relieving stomach problems. In addition, it is used to improve the symptoms of nausea in pregnancy. The study by Philips *et. al*. (1993) shows that ginger reduced nausea and vomiting.

Furthermore, ginger extract has been studied as an alternative to non-steroidal anti-inflammatory drug (NSAID) therapy for arthritic conditions. This ginger rhizome is warming, thus it stimulates digestion, respiration, blood circulation and the nervous system. Ginger may be helpful in reduction of cholesterol levels and prevention of blood clotting and reduces the incidences of heart strokes. Ginger also used as an expectorant and relieves the symptoms of colds, cough and flu but not recommended for individuals with digestive ulcers, high fevers or inflammed skin conditions. Recently, ginger has been pronounced as an antioxidant, anti-inflammatory, antithrombotic and anticancer agent, also effective in reducing the symptoms of arthritis in humans (Lantz, *et. al*., 2007).

2.2 Extraction

Extraction is the process to remove one or more solutes from a liquid by removing the solute into a second liquid phase, for which the solute has a higher affinity (Ibrahim, 2006). Extraction depends on the disparity in both solute solubility and density of the two phases. Extraction always involves two steps. First, the solvent is contacted with the solid to be treated so as to transfer the solute to the solvent. Second, the separation or washing of the solution from the residual solid.

Liquid always adheres to the solid which must be washed to prevent either the loss of solution or the contamination loss of the solids, if these are the desired material. The complete process includes the separate recovery of the solute and solvent and can be done by evaporation or distillation. The advantages of extraction is it can be performed at ambient temperature. Thus, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules.

2.2.1 Solid-Liquid Extraction

Solid-liquid extraction or leaching is the process of removing a solute from a solid phase, the solid is contacted with a liquid phase for instance by using liquid solvent. The solid and liquid phases are in contact and the solute can diffuse from the solid to the liquid phase, resulting in a separation of the components originally in the solid.

Solid-liquid extraction process can be considered in three parts involving diffusion of the solvent through the pores of the solid, the diffused solvent dissolves the solutes, and transfer of the solution from porous solid to the main bulk of the solution. Leaching is widely used in chemical industries and extraction of sugar from sugar beets, oil from oil bearing seeds, production of a concentrated solution of a valuable solid material are typical industrial examples of leaching. In leaching, washing is the process when an undesirable component is removed from a solid with water (Geankoplis, 2003).

2.2.2 Solvent Extraction

Based on the research found by Rosenthal *et. al.* (1996), continuous solvent extraction system has been developed after the World War I, and it has been proved that the system was excellent for processing oleaginous materials with very low oil content. In these days, the modern solvent extraction process has evolved. The solvent used is hexane. The process basically by successive countercurrent washes with hexane of the previously cracked, flaked, ground, or pressed of oleaginous material for example soybean and oilseed. Then, the extracted meal is carried by a sealed conveyor for solvent recovery in enclosed vessels by using jacket or steam. Hexane is removed from the oil in rising film evaporators and finally, by vacuum distillation. For extraction using hexane as a solvent, it is possible to achieve oil yields in excess of 95% with a solvent recovery of over 95%.

Solvent extraction resulting in low extraction yields, requires long extraction times and the final product often contains traces of organic solvents, which decrease the product quality. Solvent extraction underwent the severe heat treatment during the oil extraction. The process will affect the quality of oil. Moreover, further chemical treatments are needed after the extraction to make it useful for human consumption.

High demands for oil without any chemical treatment are discovered recently. Solvent extraction may yield low oil recovery which may be overcome by utilizing selected enzymes (Latif and Anwar, 2011). Thus, the development of an effective and selective method for oil extraction is important and the potential alternative to this conventional extraction methods is enzyme-based extraction.

2.2.3 Aqueous Enzymatic Extraction

Enzyme-based extraction is a potential alternative to conventional solvent-based extraction methods. Aqueous enzymatic extraction is one of the alternative eco-friendly processes based on simultaneous separation of oil and protein from oilseed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid, and aqueous phases. According to Rosenthal *et. al.* (2001), the low extraction yields of aqueous processes can be defeat by using enzymes that hydrolyze the structural polysaccharides forming the cell wall of oilseeds. The presence of certain enzymes during extraction boosts oil recovery by breaking cell walls and oil bodies.

Enzymes are ideal catalysts to assist in the extraction of natural origin. Enzymes have the ability to degrade or disrupt cell walls and membranes, thus will help in releasing and more efficient extraction of bioactives, oils and protein from plants. Because of the need for eco-friendly extraction technologies nowadays, enzyme-assisted extraction has gaining more attention in worldwide. To degrade some cell walls, improve juice extractability and increase the oil yield, enzymes such as pectinases, cellulases and hemicellulases are widely used. Components such as phenolic compounds will released by the disruption of the cell wall, therefore improving product quality (Puri, *et. al*., 2012).

Pectic enzymes have been used in food processing for the extraction of oils, extraction, clarification and concentration of fruit juices, extraction of pectin and also flavours and pigments from plant materials. For oil extraction, enzymes which is most used for oil extraction are cellulase, α-amylase and pectinase. According to Rosenthal, *et. al.* (1996), enzymes pectinase and cellulase are the most

effective for increasing oil yields of olives. While for avocado, the better extraction yields by using α-amylase alone and resulting 75% of the original content of oil in comparison to 65% with the combination of polygalacturonase, α-amylase and protease. By using mixture of cellulase and pectinase, an increase to 30% in the sunflower aqueous extraction yield is discovered by Dominguez, *et. al.* (1995). It is a very effective approach for oil extraction of coconut, soybean, and corn germ, yield the oil recovery in the range of 90–98% and good quality protein meal (Sharma, *et. al.*, 2002). The past studies about the enzymatic aqueous extraction for different oil-bearing materials in comparison to control are shown in Table 2.1.

Table 2.1 Enzymatic aqueous extraction for different oil-bearing materials in comparison to control (without enzyme)

Material	Enzyme	Concentration	Oil Yield (%)	Reference
Rapeseed	Control Pectinase (Pectinase Ultra-SP)	- 2%	53.9 71.4	Deng, *et. al.* (1992)
Avocado	Control α-Amylase + cellulase	- 1%	2.0 67.0	Buenrostro and Lopez-Munguia (1986)
Sunflower	Control Cellulase (CGA)	- 3%	30.0 44.0	Lanzani, *et. al.* (1975)
Soybean	Control Protease (Sigma)	- 0.2%	62.0 86.0	Yoon, *et. al.* (1991)
Peanut	Control Protease (pepsin-Merck)	- 3%	72.0 78.0	Lanzani, *et. al.* (1975)

Aqueous enzymatic oil extraction offers many benefits compared to conventional extraction. The process will eliminate the solvent consumption which reportedly may also lower the investment cost and energy requirements. Furthermore, it allows simultaneous recovery of oil and protein from most oilseeds and the process yields oil of good quality complying with Codex specifications (Rosenthal, *et. al.*, 1996). Recent studies by Puri, *et. al*. (2012) shown that, the enzyme-assisted extraction method will achieve high extraction yield for compounds for instance oils, natural pigments, flavour and medicinal compounds. Furthermore, this kind of extraction will resulting in faster extraction and higher recovery when compared to non-enzymatic methods.

2.3 Ultrasound-Assisted Extraction

Ultrasound has been widely used as an assisting extraction from plant material since few past decades. Ultrasound technology has been proved wherein offer many advantages in extraction, for instance, increasing the extraction yield, reducing solvent usage, economizing power consumption and reduce the duration of extraction. The efficiency of extraction increase by ultrasound is because of the propagation of ultrasound pressure waves, and results in cavitation phenomena (Vilkhu *et. al.*, 2008). The improvement of extraction process can be made with the disruption of cell walls and the release of cellular materials (Patist & Bates, 2008). Moreover, ultrasound-assisted extraction (UAE) may performed at a lower temperature, thus, avoiding thermal damage to the extracts and minimize the loss of

bioactive compounds (Zhang *et. al.*, 2008). From the past literature, some publications have included continuous ultrasonic process development and pilot-scale applications. The published extraction applications consist of herbal, oil, protein and bioactive from plant materials are shown in Table 2.2.

Table 2.2 List of ultrasound-assisted extraction from past reviews on various food components

Product	Ultrasound Process	Extraction (Solvent)	Performance	Reference
Ginger	Batch, 20 kHz	Supercritical, carbon dioxide	Increased 30% of yield, extraction time reduction	(Balachandran *et. al.*, 2006)
Almond oils	Batch, 20 kHz	Supercritical, carbon dioxide	Increased 30% of yield, extraction time reduction	(Riera *et. al.*, 2004)
Soy isoflavones	Batch, 24 kHz	Water and solvent	15% increasing in extraction efficiency	(Rostagno *et. al.*, 2003)
Pyrethrines from flowers	Batch, 20 and 40 kHz	Hexane	Increasing yield at 40°C, comparison with 66°C	(Romdhane & Gourdan, 2002)

Ultrasound has been recognised as a potential application in industrial extraction involving wide range for herbal extracts. In the edible oil industry, UAE has been recognised for application to improve efficiency and reduce extraction time (Babaei, *et. al.*, 2006). This potential was based on UAE increasing the oil from soybeans; carvone and limonene from caraway seeds. For caraway seeds and

soybean flakes in comparison with conventional extraction, the disruption of cell walls provided more evidence for the mechanical effects of ultrasound thus alleviating the release of their contents (Vilkhu, *et. al*., 2008). In the case of ginseng saponins, UAE resulted in 3-times faster than the traditional extraction method which is using reflux of boiling solvents in a soxhlet extractor. Research by Wu, *et. al.* (2001) found that for thermally unstable compounds, it is more favorable when the UAE technique was achieved at lower temperatures.

Two different methods to the application of ultrasound have been explored and related to the frequency and the power of the ultrasonic vibration. Primarily, the method consists of ultrasound with high frequency and low intensity, and focused on the quality observing of products or processes. The applications related to the measurement of ultrasound velocity, the reduction of the signal, or the analysis of frequency spectra and no influence on the process or product (Esclapez *et. al.*, 2011). The other one is ultrasound with low frequency and high power has been applied to enhance the process or products. Some of the processes are the extraction of natural products (Vilkhu *et. al.*, 2008), microbial and enzyme inactivation (Velickovic *et. al.*, 2008) and fermentation processes (Riener, 2010).

In ultrasound, the lower the frequency, the larger the cavitation bubble. Lower frequencies of high power ultrasound around 20 kHz will be more efficient in extraction processes and attain more bubble implosions. The ultrasound's frequency may effect the cavitation bubble size and also to its influence on the external and internal resistances to mass transfer.

Recently, Ma *et. al.*, (2008) found that the best frequency for the same applied power, for the extraction of hesperidin from peengan peel was 60 kHz,

instead of 20 or 100 kHz. In comparison, the extraction yields of salvianolic acid B from *Salvia miltiorrhiza* root with temperature 30°C and extracted for 25 minutes with power of 100 W under varies of ultrasound frequencies which are 28, 25 and 100 kHz in a cleaning bath (Dong *et.al.*, 2010). The extraction yield with highest frequency was lower than those with lower frequencies.

CHAPTER 3

METHODOLOGY

3.1 Materials and Chemicals

The mature and healthy rhizomes of ginger were purchased from a local market. The solvents used which is ethanol and n-hexane was of analytical grade. Commercial enzyme used in the study, pectinase (Pectinex Ultra® SPL with activity of ≥ 3800 units/mL) in aqueous solution was obtained from Sigma-Aldrich (Selangor, Malaysia).

3.2 Ginger Oil Extractions

3.2.1 Solvent Extraction

Ginger rhizomes were properly washed and sliced into uniform size. Then, all ginger slices were dried for 1 day in a dryer with constant temperature of 60°C. Dried

ginger slices were ground into fine powder using a laboratory grinder. The ground ginger (25 g) was placed in a Soxhlet extractor apparatus fixed with a 250 mL round-bottom flask and a water condenser as in Figure 3.1. The conventional extraction was performed for 24 hours with 200 mL of ethanol. After extraction has been performed, ethanol was removed by using vacuum rotary evaporator at 80°C. The recovered oil was measured in g by using analytical balance. Then, the recovered oil was stored under refrigeration (4°C) for further analysis.

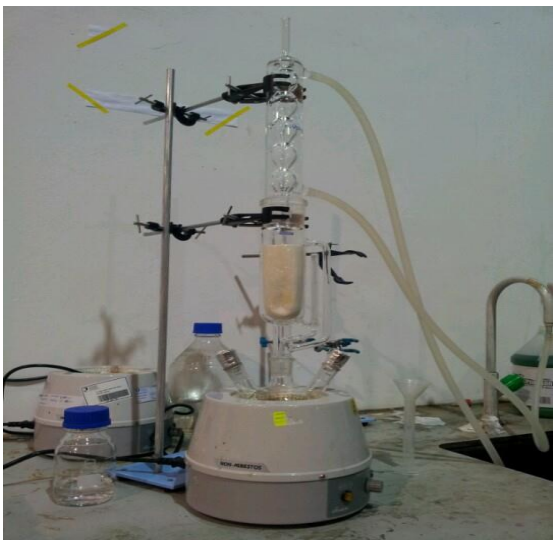

Figure 3.1 Soxhlet apparatus for solvent extraction

3.2.2 Enzymatic Extraction

The same amount of ground ginger (25g) was mixed with 250 mL of distilled water in a flask or beaker. According to Rosenthal *et. al*. (1996), it is important to use large quantities of water to achieve the highest extraction rates and yields. The mixture

was boiled for 5 minutes and then was allowed to cool down to room temperature. The pH was then adjusted to the optimal value of pectinase (pH 4.5 – 5.0) by using 0.5 N NaOH or 0.5 N HCl aqueous solutions. Then, various enzyme pectinase concentrations were added (1%, 1.5%, 2% and 3% v/w) and the mixture was incubated at 50°C (optimal temperature for pectinase) for 24 h with 120 rpm constant shaking in an incubator shaker as in Figure 3.2. After incubation was performed, the aqueous layer from the mixture (Figure 3.3) was taken and was boiled to 100°C for 5 minutes to destroy the activity of enzyme. Then, 1.5 volume of n-hexane based on ginger weight was added, and the mixture was stirred for 15 min to demulsify the oil-water emulsion (Sengupta & Bhattacharyya, 1996). It was then centrifuged for 15 minutes at 10 000 rpm and 15°C to separate the emulsion and residue. The upper layer after centrifugation was oil in hexane. The oil was separated by using micropipette. By using rotary evaporator to distillate hexane, yielded the oil. The oil recovered is then weighed.

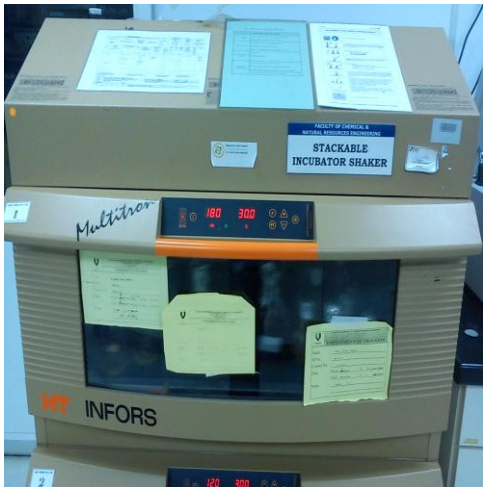

Figure 3.2 Incubator shaker

Figure 3.3 The aqueous layer of mixture after incubation period

3.3 Ultrasound Procedure

The mixture of enzyme and ground ginger was incubated for 24 h to obtain oil. The aqueous layer of mixture was taken and with enzyme was still active, the ultrasound procedure was applied as in Figure 3.4. The amplitude setting of sonotrode was set at position 2 to get the power input of 15 W and intensity of 7.14 W cm^{-2}. The sonication intensity was determined by using the following equation:

$$I = P/A$$

where A was the area of sonotrode tip which is 2.1 cm^2. The duty cycle of sonication were represented to the energy imparted to the sample. The duty cycle determine the

time of sonication was on. A 10% duty cycle (1 s sonication, 9 s rest period) and 20% duty cycle (2 s sonication, 8 s rest period) was applied for 1 minute each to the sample.

Figure 3.4 Ultrasound procedure

CHAPTER 4

RESULT AND DISCUSSION

This chapter discuss on the results of the experiment that was done in order to study the effect of ultrasound on enzymatic extraction of *Zingiber officinale*. Enzymatic extraction was conducted by using a range of enzyme pectinase concentration which are 1%, 1.5%, 2% and 3% (v/w) respectively, and incubation time of 24 hours and temperature constant at 50°C. For ultrasound procedure, different sonication regimens were used consists of 10% duty cycle and 20% duty cycle. The frequency, power and intensity of ultrasound were constant at 20 kHz, 15 W and 11.77 W cm^{-2} respectively.

4.1 Solvent Extraction

Solvent extraction method was conducted by using Sohxlet apparatus. The mixture of oil, water and solvent collected in a round bottom flask was taken every 4 hours and further recovered. The total percentage oil recovered for solvent extraction of *Zingiber officinale* for 24 hours was about 39%. Initially, the oil yield was increased with increasing time until the optimum time of 16 hours was reached. Generally, the longer the time of extraction, the higher the yield because a longer extraction time will desire large contact between material and solvent (Supardan, et. al.,2011). The colour of recovered oil was varied from dark amber to pale yellow and had specific ginger flavour. Figure 4.1 below shown the ginger oil yield (%) versus with time (h) for solvent extraction.

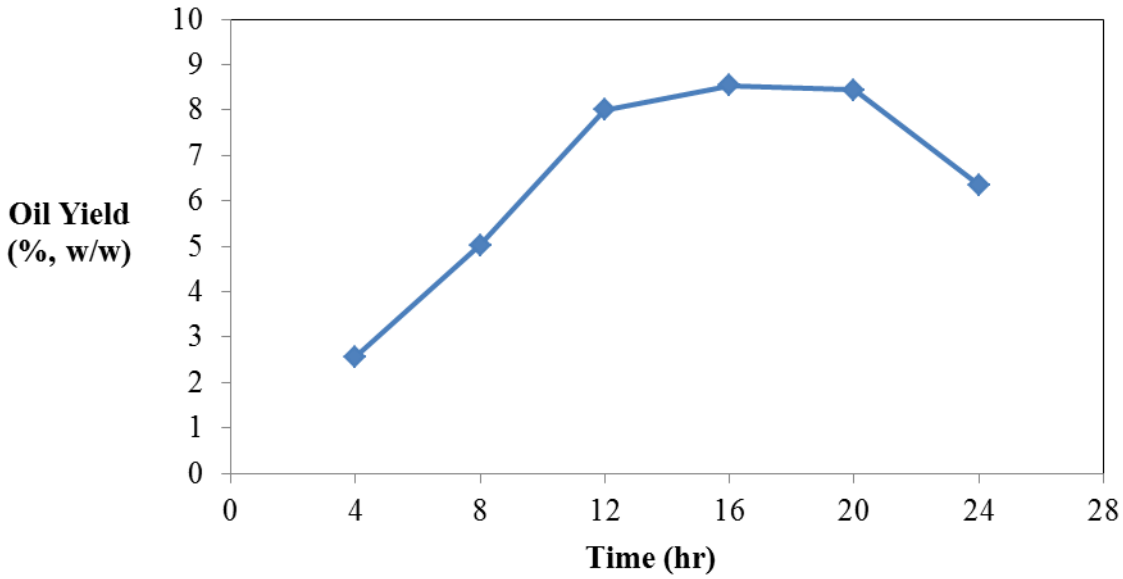

Figure 4.1 Recovered oil yield for solvent extraction

4.2 Enzymatic Extraction

4.2.1 Effect of Enzyme Concentration

Enzymatic extraction was performed by the presence of enzyme in order to disrupt the cell wall and oil bodies during the extraction. The enzyme used was pectinase (Sigma Aldrich). The effect of enzyme concentration on enzymatic extraction was conducted in order to get the optimum concentration of enzyme. The concentration of enzyme pectinase was varied from 1%, 1.5%, 2% and 3% of ginger weight. The incubation time and temperature were constant at 24 hours and 50°C. The temperature was chosen as the optimum temperature for enzyme pectinase to active. From the observation, the highest peak of ginger oil yield was 66% in Figure 4.2 and proved that the maximum concentration of pectinase being used was at 2%.

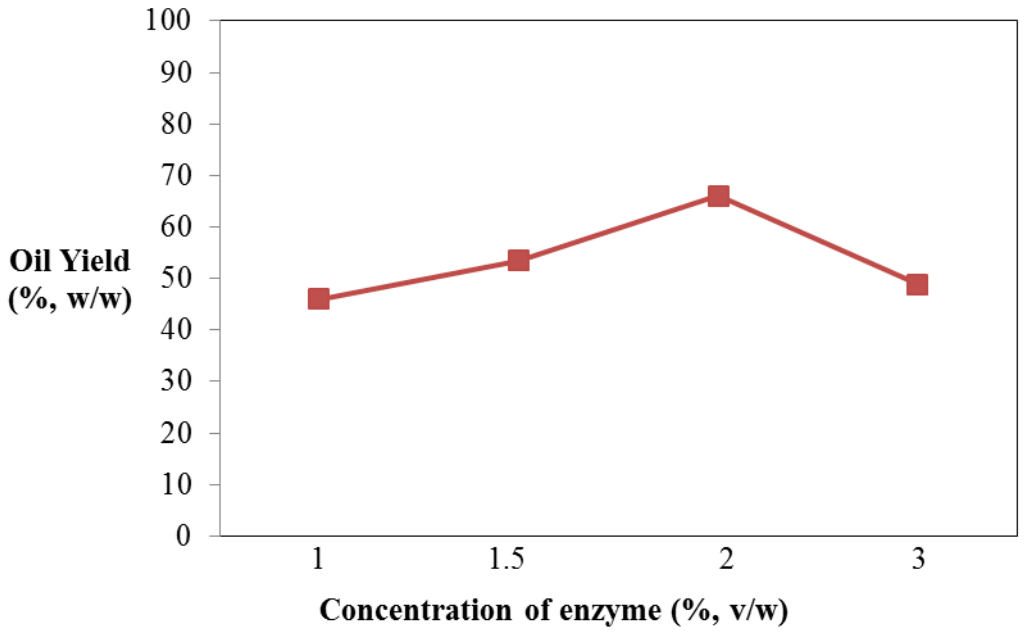

Figure 4.2 Effect of Enzyme Concentration on enzymatic extraction of *Zingiber officinale*

The rate of reaction increasing when enzyme concentration is increasing because there are more presence of enzyme to aid break down the substrate. However, the condition will become equilibrium when the substrate acts as a limiting factor, which by means that there are not enough substrate to be broken down compared to amount of enzyme (Silverthon, 2004). Thus, this may explained better the reaction of enzyme concentration of 3% was slow compared to other concentrations as stated, respectively.

4.2.2 Enzymatic Extraction in Comparison with Solvent Extraction

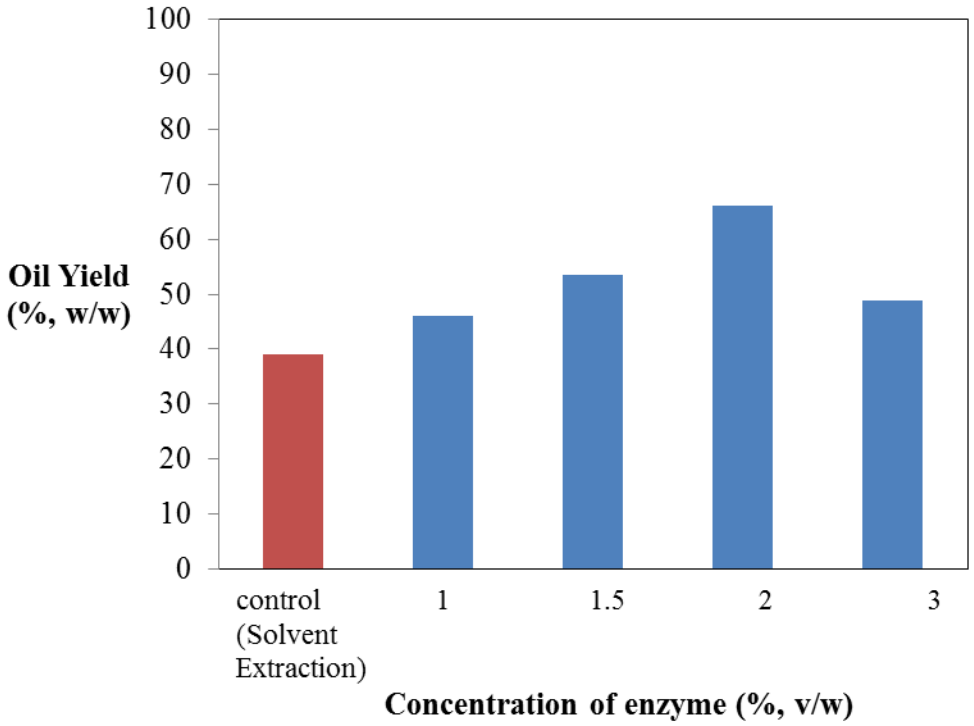

Figure 4.3 Effect of enzyme concentration on enzymatic extraction of *Zingiber officinale* and in comparison with control (solvent extraction).

Figure 4.3 above shown that the effect of enzyme concentration was compared to solvent extraction. As can be seen, the percentage of oil yield obtained by solvent extraction is lower than the yield obtained by enzymatic extraction by using a range of concentration. The yield was about 39% compared to 66% yield by enzyme concentration of 2% (v/w). This statement was supported by a research according to Rosenthal *et. al.* (2001), the low extraction yields can be defeat by using enzymes that hydrolyze the structural polysaccharides forming the cell wall of oilseeds.

4.3 Enzymatic Extraction and Ultrasound-Assisted Extraction

4.3.1 Effect of Different Sonication Regimens of Ultrasound on Enzymatic Extraction

Ultrasound procedure was conducted as an assisting extraction in order to obtain maximum percentage of yield and reducing the consumption of solvent. Ultrasound procedure was conducted by using 10% duty cycle and 20% duty cyle. The power, intensity and frequency for ultrasound was constant at 15 W, 11.77 W cm^{-2} and 20 kHz. Without inactivation of enzyme, the ultrasound procedure was performed. The incubation time was 24 hours. From the observation, 10% duty cycle of ultrasound produced more percentage of yield compared to 20% duty cycle of ultrasound as shown in Figure 4.4.

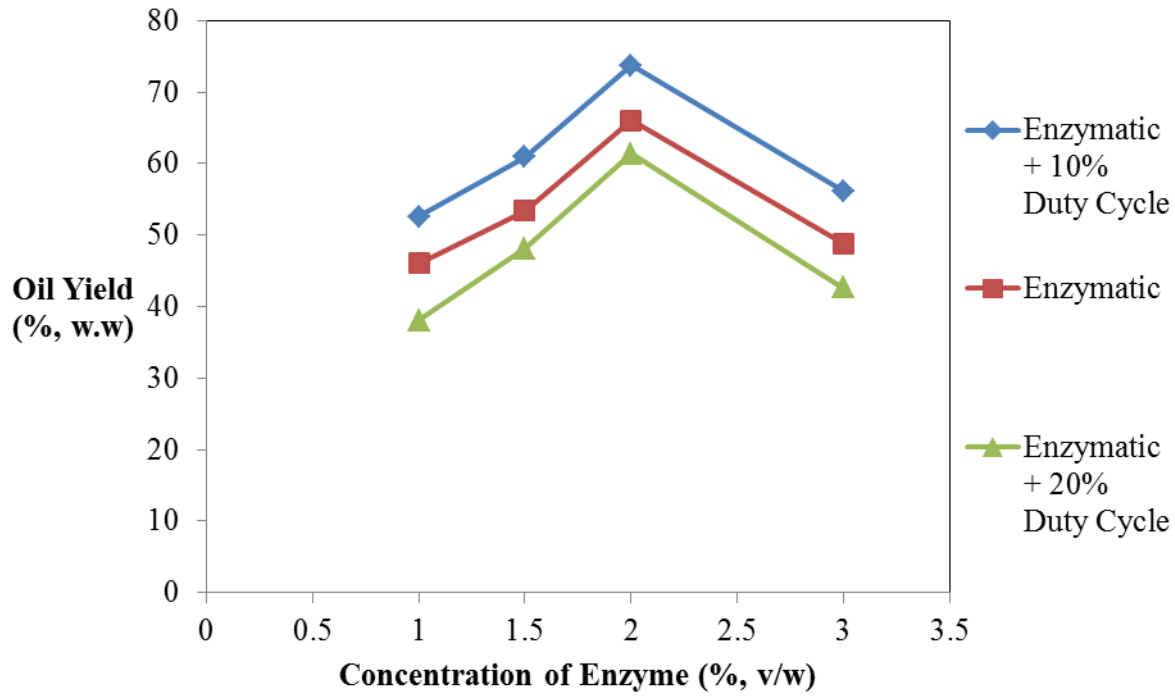

Figure 4.4 Effect of different sonication regimens of ultrasound on enzymatic extraction consists of 10% duty cycle and 20% duty cycle

From figure 4.4, the highest percentage yield (73.7%) was obtained from enzymatic extraction with enzyme concentration of 2% and assisted with 10% duty cycle ultrasound compared to 20% duty cycle and other enzyme concentration, respectively. For 20% duty cycle of ultrasound, the highest yield obtained from enzyme concentration of 2% which is 61.4%. These total percentages of yield were after incubation of 24 hours. According to Vilkhu *et. al.* (2008) and Patist & Bates (2008), the efficiency of extraction increase by ultrasound is because of the ultrasound pressure waves, and results in cavitation phenomena and improvement of extraction can be made by the disruption of cell walls and release of cellular materials.

In ultrasound, the lower the frequency, the larger the cavitation bubble. Lower frequencies of high power ultrasound around 20 kHz will be more efficient in

extraction processes, attain more bubble implosions and influence on the external and internal resistances to mass transfer.

4.3.2 Enzymatic Extraction and Ultrasound-Assisted Extraction in Comparison with Solvent Extraction

Based on the best enzyme concentration and sonication regimens which are 2% (v/w) and 10% duty cycle respectively, the comparison were made between solvent extraction (control), enzymatic extraction and also enzymatic extraction assisted with ultrasound. Based on Figure 4.5, the enzymatic extraction assisted with ultrasound obtain the highest yield which is 73.7% followed by 66% yield by enzymatic extraction in 24 hours. As can be seen, the solvent extraction produced lowest yield in 24 hours which is 39%.

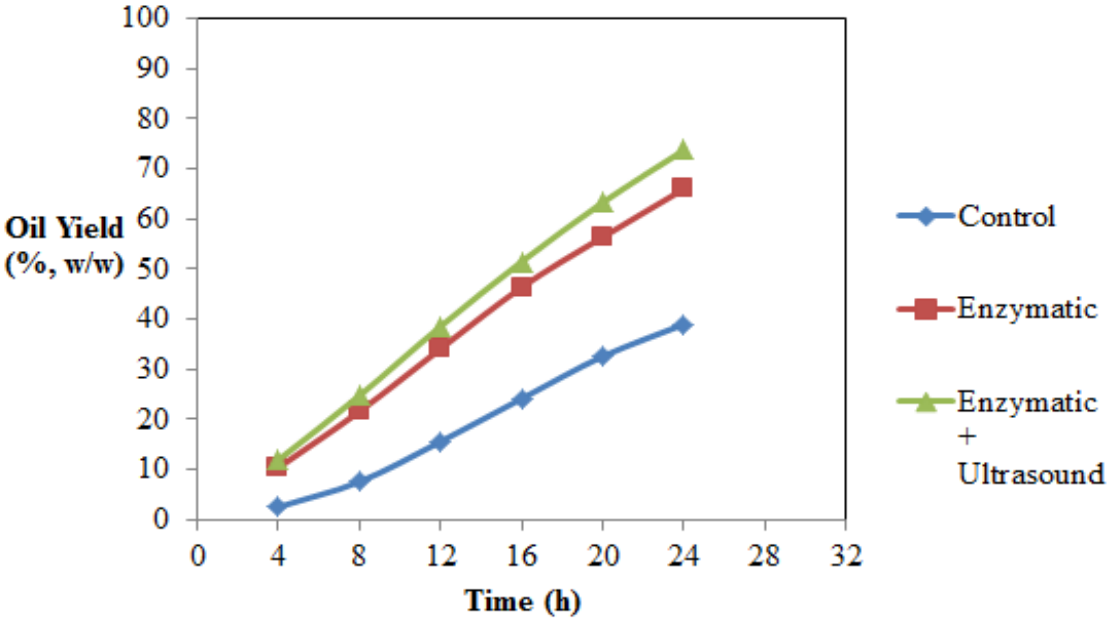

Figure 4.5 Effect of ultrasound on enzymatic extraction of *Zingiber officinale* in comparison with control (solvent extraction).

As acclaimed by Rosenthal *et. al.* (1996), in aqueous enzymatic extraction, enzymes are used to facilitate oil releasing from oil bodies enmeshed in protein and cellulosic or hemicellulosic networks. Ultrasonication is a tool to accelerate many chemcial and physical processes and also has been used to increase the oil yield during aqueous oil extraction in some cases (Szentmihalyi *et.al.*, 2002). Considering that the cost of enzyme will caused aqueous enzymatic extraction less acceptable approach economically, ultrasonication may be a good technique to be tried along with the process.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The main objective of this research to study the effect of ultrasound on enzymatic extraction of Zingiber officinale was achieved. The optimum temperature and pH for pectinase were at 50°C and pH 4.5 – 5.0 respectively. Result from solvent extraction act as a control in this study obtained 39% of oil yield. The study resulted the highest percentage of oil yield for enzymatic extraction was obtained by enzyme concentration of 2% (v/w) which is 66% (w/w) yield. By applying ultrasound, the extraction yield was increased. The highest yield 73.7% (w/w) obtained by 10% duty cycle of ultrasound combined with 2% (v/w) enzyme concentration. Therefore, it can be concluded that the combination of ultrasound-assisted extraction with enzymatic extraction can be an alternative approach in replacing the conventional method which used high heat and highest solvent consumption for the extraction.

5.2 Recommendation

Several numbers of recommendations are being proposed in order to improvise this study. Parameters that have been studied in this research were effect of enzyme concentration and different sonication regimen towards enzymatic extraction of ginger. For the future study, addidtional parameter can be added for instance using different type of enzyme with various concentration and using a range of temperature. In addition, fixed on specific time of extraction also can be very useful for this study such as the extraction will be done for 30, 60, 90 and 120 minutes respectively. Furthermore, the enzyme also can be replaced by using immobilized pectinase enzyme. Thus, it will be very economically since enzyme nowadays is quite expensive and immobilized enzyme can be easily separated from the product. This research also can be improved by using statistic process control Analysis of Variances (ANOVA) in order to compare the significant parameters such as enzymes concentration versus ultrasound sonication regimens.

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APPENDIX A

Gantt Chart

Project Title (PSM): The Effect of Ultrasound on Enzymatic Extraction of *Zingiber officinale*										
Year	PSM I: 2012					PSM II: 2012-2013				
Month	Feb	Mac	Apr	May	Jun	Sep	Oct	Nov	Dec	Jan
Project Tasks / Weeks	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Identify project (problem to investigate) and scope of research	x									
Plan work schedule		x								
Review related literature		x x x x								
Determine methodology			x x							
Write proposal and abstract (summary of proposal)			x x							
Present and defend proposal in oral presentation				x						
Submit written research proposal and abstract					x					
Collect and analyse data					x					
Interpret results						x x x x	x			
Evaluate results**:**							x x	x		
Achieve research objectives/ milestones										
Draw conclusions and **suggest** recommendations								x x		
Revise and edit first draft of Introduction, Literature Review and Methodology (from proposal)								x	x	
Write first draft of Results & Discussions, Conclusions & Recommendations									x	
Revise and edit abstract (from proposal)									x x	x
Compile entire final report and revised abstract										x
Present and defend final report in oral presentation										x
Submit written final report and abstract										x

APPENDIX B

Table B.1 demonstrated the result collected from solvent extraction of *Zingiber officinale* for 24 hours.

Table B.1 Result of recovered oil from solvent extraction

Time (hour)	Yield (g)	Yield (%)
4	0.6431	2.5724
8	1.2571	5.0284
12	2.0032	8.0128
16	2.1371	8.5484
20	2.1102	8.4408
24	1.5883	6.3532

Table B.2, B.3, B.4 and B.5 illustrated the result obtained from enzymatic extraction of *Zingiber officinale* by using different concentration of enzyme pectinase for 24 hours.

Table B.2 Result of recovered oil from enzymatic extraction for enzyme pectinase concentration of 1%

Time (hour)	Yield (g)	Yield (%)
4	1.4531	5.8124
8	1.8024	7.2096
12	2.6124	10.4496
16	2.4623	9.8492
20	1.9222	7.6888
24	1.2502	5.0008

Table B.3 Result of recovered oil from enzymatic extraction for enzyme pectinase concentration of 1.5%

Time (hour)	Yield (g)	Yield (%)
4	1.9796	7.9184
8	2.1419	8.5676
12	2.7235	10.8940
16	2.5653	10.2612
20	2.1808	8.7232
24	1.7652	7.0608

Table B.3 Result of recovered oil from enzymatic extraction for enzyme pectinase concentration of 2%

Time (hour)	Yield (g)	Yield (%)
4	2.6091	10.4364
8	2.7989	11.1956
12	3.1267	12.5068
16	3.0678	12.2712
20	2.5007	10.0028
24	2.3987	9.5948

Table B.5 Result of recovered oil from enzymatic extraction for enzyme pectinase concentration of 3%

Time (hour)	Yield (g)	Yield (%)
4	1.5987	6.3948
8	1.8613	7.4452
12	2.6420	10.5680
16	2.4357	9.7428
20	2.1018	8.4072
24	1.5576	6.2304

Table B.6, B.7, B.8 and B.9 shows the result obtained from the combination of enzymatic extraction with ultrasound-assisted extraction (10% duty cycle) for different concentration of enzyme pectinase

Table B.6 Result of recovered oil from combination of enzyme pectinase concentration of 1% with 10% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	1.6243	6.4972
8	1.9509	7.8036
12	2.9421	11.7684
16	2.7203	10.8812
20	2.2763	9.1052
24	1.9602	7.8408

Table B.7 Result of recovered oil from combination of enzyme pectinase concentration of 1.5% with 10% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	2.2903	9.1612
8	2.4212	9.6848
12	3.0043	12.0172
16	2.9221	11.6884
20	2.4998	9.9992
24	2.0793	8.3172

Table B.8 Result of recovered oil from combination of enzyme pectinase concentration of 2% with 10% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	2.8978	11.5912
8	2.9082	11.6328
12	3.4571	13.8284
16	3.1097	12.4388
20	2.9603	11.8412
24	2.5987	10.3948

Table B.9 Result of recovered oil from combination of enzyme pectinase concentration of 3% with 10% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	1.8205	7.2820
8	2.3117	9.2468
12	2.8076	11.2304
16	2.6608	10.6432
20	2.3123	9.2492
24	1.8654	7.4616

Table B.10, B.11, B.12 and B.13 indicates the data collection about the combination of enzymatic extraction with ultrasound-assisted extraction (20% duty cycle) for different concentration of enzyme pectinase

Table B.10 Result of recovered oil from combination of enzyme pectinase concentration of 1% with 20% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	1.3312	5.3248
8	1.4587	5.8348
12	2.4098	9.6392
16	2.9676	9.1904
20	1.8170	7.2680
24	0.8039	3.2156

Table B.11 Result of recovered oil from combination of enzyme pectinase concentration of 1.5% with 20% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	1.3761	5.5044
8	2.0043	8.0172
12	2.4209	9.6836
16	2.3704	9.4816
20	1.9431	7.7724
24	1.1816	4.7264

Table B.12 Result of recovered oil from combination of enzyme pectinase concentration of 2% with 20% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	2.3734	9.4936
8	2.6254	10.5016
12	2.9601	11.8404
16	2.7678	11.0712
20	2.4287	9.7148
24	2.1987	8.7948

Table B.13 Result of recovered oil from combination of enzyme pectinase concentration of 3% with 20% duty cycle of ultrasound

Time (hour)	Yield (g)	Yield (%)
4	1.3223	5.2892
8	1.4871	5.9484
12	2.4591	9.8364
16	2.1689	8.6756
20	1.8723	7.4892
24	1.0607	4.2428