



**UNIVERSITI PUTRA MALAYSIA**

**ULTRASOUND-ASSISTED EXTRACTION OF PHOSPHOLIPIDS FROM  
PALM-PRESSED FIBER**

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**ULTRASOUND-ASSISTED EXTRACTION OF PHOSPHOLIPIDS FROM  
PALM-PRESSED FIBER**

**By**

**CHUA SEONG CHEA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

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**Chairman: Associate Professor Tan Chin Ping, PhD**

**Faculty: Food Science and Technology**

The extraction of phospholipids (PL) from palm-pressed fiber (PPF) using ultrasound technology was evaluated and quantified using high performance liquid chromatography coupled with an evaporative light scattering detector (HPLC-ELSD). The PL sample was treated with diol solid phase extraction (SPE) to achieve better separation and higher PL recovery by removing neutral lipids. The types of PL that were considered in the recovery study included phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), and lyso-phosphatidylcholine (LPC). A central composite design (CCD) was employed to study the effect of ultrasound-assisted extraction (UAE) conditions, namely amplitude (20%-90%), cycle (0.2-1.0 W/s), and sonication time (5-30 min), on the PL extraction yield from PPF. The optimum parameters for PL extraction using the ultrasound system



were 20% amplitude, 0.2 W/s cycle, and a sonication time of 30 min. Under these optimum conditions, the response values obtained for overall extraction efficiency and individual extraction yield of PE and PC were 110 mg/g, 12570, and 5426 mg/kg, respectively. In the purification of PL, it was observed that acetone can remove oil from the extract. Ethanol was used to fractionate PC and PI, as both PC and PI have different solubility in this solvent. In general, PC is more soluble in ethanol than PI. Therefore, the ethanol extract was the PC-enriched fraction and the ethanol precipitate was the PI-enriched fraction. The sample to ethanol ratio significantly ( $p < 0.05$ ) affected the purity of PC in the PC-enriched fraction. The best ratio of sample/ethanol (g/mL) was 1:20, which resulted in the highest percentage of the PC fraction, with the highest PC level in the PC fraction and the highest PI level in the PI fraction. The PC-enriched fraction contained 45% PC, 2% PE and 5% PI based on the total extract content, whereas the PI-enriched fraction contained 13% PC, 8% PE, and 60% PI.



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sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGEKSTRAKAN FOSFOLIPID DARIPADA SABUT KELAPA SAWIT  
HEMPIT DENGAN BANTUAN ULTRASONIK**

Oleh

**CHUA SEONG CHEA**

**Jun 2009**

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Pengekstrakan fosfolipid daripada sabut kelapa sawit hempit menggunakan teknologi ultrasonik telah dikaji dan penkuantitian telah dilakukan dengan kromatografi cecair berprestasi tinggi yang dilengkapi dengan pengesan pengewapan serakan cahaya. Pengekstrak fasa pepejal diol telah digunakan untuk menyingkirkan lipid neutral supaya pemisahan yang lebih baik dan pemulihan fosfolipid yang lebih tinggi daripada sampel dapat dihasilkan. Dalam kajian pemulihan, jenis fosfolipid yang dikaji adalah *phosphatidylethanolamine* (PE), *phosphatidylglycerol* (PG), *phosphatidylcholine* (PC) dan *lyso-phosphatidylcholine* (LPC). Reka bentuk komposit tengah telah digunakan untuk mengaji kesan parameter-parameter ultrasonik seperti amplitud (20 – 90 %), kitaran akuastik (kesan ultrasonik 0.2 – 1.0 W/s) dan masa sonikasi (5 – 30 minit) ke atas hasil pengekstrakan fosfolipid daripada sabut kelapa sawit. Parameter yang optimum untuk



mengekstrak fosfolipid dengan menggunakan sistem ultrasonik adalah 20 % amplitud, 0.2 W/s kitaran akustik dan masa sonikasi selama 30 minit. Di bawah keadaan sistem ultrasonik yang optimum, jumlah fosfolipid keseluruhan yang diperolehi adalah 110 mg/g dan hasil ekstrakan individu bagi PE and PC adalah masing-masing 12570 dan 5426 mg/kg. Etanol telah digunakan untuk memisahkan PC dan PI kerana kedua-dua fosfolipid mempunyai kelarutan yang berlainan dalam etanol. PC adalah lebih larut dalam etanol berbanding dengan PI. Oleh itu, larutan etanol adalah bahagian yang kaya dengan PC dan sebaliknya mendapan etanol adalah bahagian yang kaya dengan PI. Empat nisbah sampel kepada etanol 1:10, 1:20, 1:30 dan 1:40 telah digunakan dalam kajian ini. Nisbah sampel kepada etanol telah mempengaruhi ketulenan PC dalam bahagian etanol yang kaya dengan PC secara bererti ( $p < 0.05$ ). Nisbah sampel kepada etanol (g/mL) yang terbaik adalah 1:20. Nisbah ini telah memberi kandungan PC yang tertinggi dalam bahagian PC dan kandungan PI yang tertinggi dalam bahagian PI. Bahagian yang kaya dengan PC mengandungi 45 % PC, 2 % PE dan 5 % PI berdasarkan kandungan keseluruhan fosfolipid, manakala bahagian kaya dengan PI mengandungi 13% PC, 8% PR dan 60% PI.

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I certify that an Examination Committee has met on **18<sup>th</sup> June 2009** to conduct the final examination of Chua Seong Chea on her Master of Science thesis entitled “Ultrasound Assisted Phospholipid Extraction from Palm-pressed Fiber” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science degree.

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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Date: 11 August 2009



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## LIST OF ABBREVIATIONS

DEAE	Diethylaminoethyl
DPG	Diphosphatidylglycerol
ELSD	Evaporative Light-Scattering Detector
FELDA	Federal Land Development Authority
FFB	Fresh Fruits Bunches
GL	Glycolipids
HLB	Hydrophilic-Lipophilic Balance
HPLC	High-Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
LPC	Lyso-phosphatidylcholine
MPOB	Malaysia Palm Oil Board
NAPE	N-Acyl-phosphatidylethanolamine
O/W	Oil in Water
PA	Phosphatidic Acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PGL	Phytoglycolipids
PI	Phosphatidylinositol
PS	Phosphatidylserine
PL	Phospholipids
PORIM	Palm Oil Research Institute of Malaysia



PPF	Palm-Pressed Fiber
RI	Refractive Index
RSM	Response Surface Methodology
SPE	Solid Phase Extraction
SPM	Sphingomyelin
TLC	Thin Layer Chromatography
UV	Ultraviolet
W/O	Water in Oil



# CHAPTER 1

## INTRODUCTION

Palm oil is well known for its versatility and its technically superior properties than other plant oils. It can be used in a wide range of products ranging from food, oleochemical, and biofuel industries as a main source of sustainable and renewable raw material. Furthermore, palm oil is the cheapest oil to produce and has the highest yield per hectare, i.e. 3.68 tons of palm oil per hectare of crop (Ng, 1972). Therefore, over the past four decades, the palm oil industry has been the driving force of the world's oil and fat economy. Due to their economic advantage, oil palm plantations are expanding with increased venture from investors to boost the output from oil palm to outpace the average world growth in oils and fats.

Malaysia is currently the number one palm oil producer in the world and according to forecasts will maintain its lead position over the next decade by producing 18 million tons, or 42% of the world palm oil, in 2020 (Basiron and Simeh, 2005). The number of land planted with this crop in Malaysia has increased from 54000 hectares in the 1960s to 4.3 million hectares of oil palm plantations yield 17.7 million tones of crude palm oil (MPOC, 2009)

Palm oil contains many valuable components (phytonutrients) that are beneficial for health maintenance (Choo *et al.*, 2002). The minor constituents in palm oil, making up approximately 1% of its total content, include



carotenoids, tocopherols, sterols, triterpene alcohol, phospholipids, glycolipids, terpenic and aliphatic hydrocarbons, and other trace impurities (Goh *et al.*, 1985). The phospholipids, especially phosphatidylcholine, are known for their emulsifying properties. Phosphatidylcholine also has a synergistic antioxidant effect with tocopherols (Choo *et al.*, 2002). According to George and Arumughan (1991), the exocarp (outer skin) of the palm fruit contains higher levels of phospholipids than its fleshy mesocarp. Palm oil is extracted mechanically, so a high level of phospholipids content is expected to remain in the wet-pressed fiber compared to solvent-extracted oils from seeds, phospholipids are lipid bilayers which are only can be extracted using solvent, however small amount of phospholipids might be leached out during the mechanical pressing.

During oil processing, phospholipids are undesirable in the oil since they lead to oil discoloration during deodorization and steam distillation (Nzai and Proctor, 1998). However, phospholipids are one of the major groups of food emulsifiers. Due to their amphiphilic character and endogenous nature, phospholipids are also an important raw material in the modern pharmaceutical industry and are widely used in intravenous preparations for parenteral nutrition. Today, numerous innovative phospholipid-based drug carriers, such as nanoemulsions, liposomes, micelles, solid lipid nanoparticles, niosomes, and lipid microbubbles, are being developed in academic research.

Palm-pressed fiber is a by-product of palm oil milling and has been reported to contain greater amounts of phospholipids (46800 ppm) (Choo *et al.*, 2004) than palm oil (1000-2000 ppm) (Goh *et al.*, 1982). Pressed fiber is generated by the palm oil mills after extracting oil from fresh fruit bunches and it is often under-utilized commercially. Mills usually use the produced biomass, like fruit mesocarp fibers, as boiler fuel to generate power and produce steam for mill processes. The energy generated from burning shell and fiber is usually more than sufficient to supply the mill's electricity needs and surplus electricity is sometimes available for use in estate domestic housing or can even be sold to the local electricity grid (Redshhaw, 2003).

Extraction, identification, and purification of phospholipids from palm-pressed fiber is still an unexplored area of research. Since phospholipids can be obtained in large quantities like that obtained in palm-pressed fiber, purification of this valuable food or pharmaceutical ingredient possesses the potential of great revenue to the palm oil industry. Ultrasound has been used to assist with the extraction of bioactive components from plant material (Herrera and Castro, 2005, Fan *et al.*, 2007, Chen *et al.*, 2007 and Corrales *et al.*, 2008). It is still a new processing technique for the palm oil industry. Cavitation produced from the ultrasound system produces high energy around the solvent molecules and causes cell disruption for better penetration of the solvent into the cell (Maricela *et al.*, 2001). A systematic study of the efficiency of ultrasound-assisted phospholipids extraction from palm-pressed fibers and the physico-chemical properties of extracted

phospholipids will provide an essential step for the acceptance of this processing technique in the palm oil industry and will turn previously under-utilized products into profit.

Hence, the objectives of this study were to:

- i. evaluate the fractionation of phospholipids from ultrasound treated extract using aqueous ethanol.
- ii. evaluate the best solid phase extraction method for purifying phospholipids from palm-pressed fiber.
- iii. optimize the extraction method of phospholipids from palm-pressed fiber using ultrasonic technology.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Sonochemistry

Sonochemistry refers to the study of the effects of sound on chemical reactions (Mason, 1991 and Price, 1992). There are reports of chemistry being performed using audible or sub-audible (i.e. infrasound) sound frequencies, with the vast majority of sonochemical work using ultrasound. This is defined as sound having a frequency above that detectable by human hearing, the threshold of which is usually taken to be ~20 kHz, Figure 2.1. Ultrasound has been used for a variety of purposes that include communication with animals (dog whistles), the detection of flaws in concrete building, synthesis of the fine chemicals, and treatment of disease (Mason, 2003).

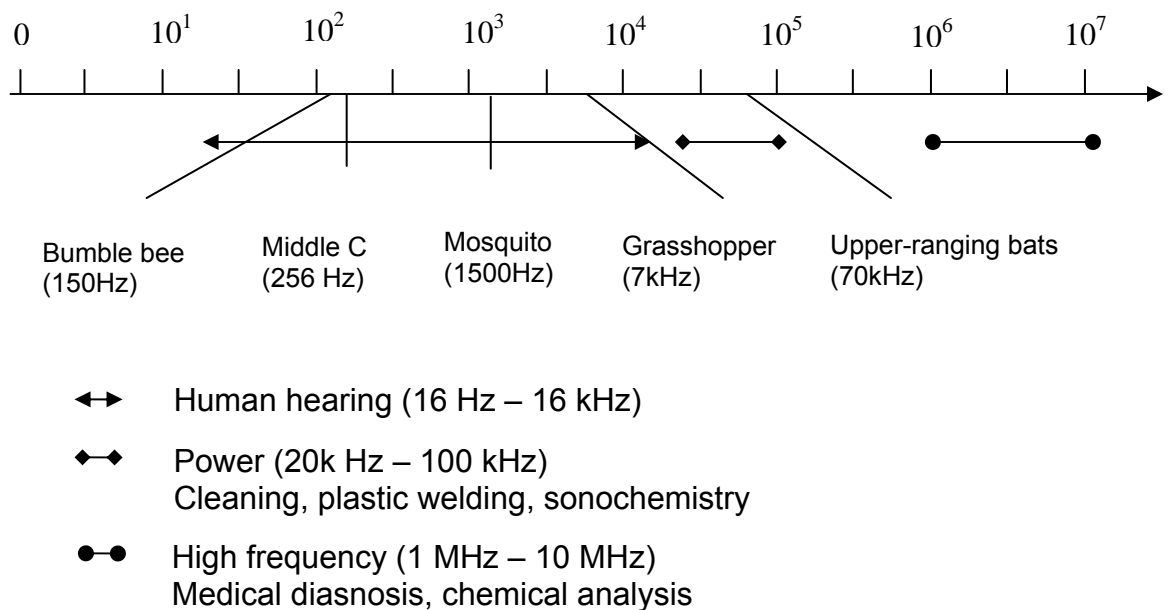
Ultrasound has been used since the 1930s, when, in the years preceding the Second World War, its use was investigated for a range of technologies including emulsification and surface cleaning. Power ultrasound has great potential for uses in a wide variety of processes in the chemical and allied industries. The applications of ultrasound are cleaning, sterilization, flotation, drying, degassing, defoaming, soldering, plastic welding, drilling, filtration, homogenization, emulsification, dissolution, deaggregation of powder, biological cell disruption, extraction, crystallization, electrochemistry, food



technology, nanotechnology, phase separation, sewage treatment, and use as a stimulus for chemical reactions (Mason, 1991 and Mason, 2003).

### 2.1.1 The power of sound

Sound is transmitted through a medium by inducing vibrational motion of the molecules through which it travels. This motion is similar to the ripples produced when a pebble is dropped into a pool of still water. The waves move, but the water molecules which constitute the wave revert to their normal positions after the wave has passed. An alternative representation is provided by the effect of a sudden twitch of the end of a horizontally stretched spring.



**Figure 2.1: Sound frequencies (Hz – cycles per second)**  
Source: Mason, 1991.



### 2.1.2 Cavitation

Cavitation is the production of microbubbles or formation of a void in a liquid when large negative pressure is applied to it. During the compression cycle, the average distance between the molecules decreased, whilst during rarefaction the distances increased. If a sufficiently large negative pressure is applied to the liquid, such that average distance between the molecules exceeds the critical molecular distance to hold the liquid intact, the liquid will break down and voids or cavities will be created (Mason and Lorimer, 2001). Cavitation bubbles will be formed according to the equation below,

$$P_c = P_h - P_a$$

Where  $P_c$ , negative pressure;  $P_h$ , ambient pressure for hydrostatic; and  $P_a$ , applied acoustic pressure. Ultrasound produces its chemical effects through the phenomenon of cavitation. In practice, this happens at pressures much lower than those required to overcome the tensile strength of a liquid since there are always minute dust particles or dissolved gases present that act as nucleating sites. During its growth, dissolved gases and/or solvent vapor may diffuse into the bubble. Once bubbles are formed, they can oscillate in size as the sound waves propagate and two distinct types of cavitation can be identified, although the situation is rarely as unambiguous as that defined below (Price, 1992).

#### a) Stable cavitation

Stable cavities exist for many acoustic cycles and may oscillate in resonance with the applied field, during which time they may grow