การวิเคราะห์เชิงคุณภาพและเชิงปริมาณของกรดไขมันและสเตอรอยด์ใน เพรียงทราย *Perinereis* sp.

นางสาว มณีรัตน์ ลิ้มสุวัฒนาธำรง

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QUALITATIVE AND QUANTITATIVE ANALYSES OF FATTY ACID AND STEROID IN SANDWORMS *Perinereis* sp.

Miss Maneerat Limsuwatthanathamrong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
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Thesis Title	QUALITATIVE AND QUANTITATIVE ANALYSES OF FATTY ACID AND STEROID IN SANDWORMS *Perinereis* sp.
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มณีรัตน์ ลิ้มสุวัฒนาธำรง : การวิเคราะห์เชิงคุณภาพและเชิงปริมาณของกรดไขมันและสเตอรอยด์ในเพรียงทราย *Perinereis* sp. (QUALITATIVE AND QUANTITATIVE ANALYSES OF FATTY ACID AND STEROID IN SANDWORMS *Perinereis* sp.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.นาตยา งามโรจนวณิชย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร.ศรินทิพ สุกใส, 92 หน้า.

จากงานวิจัยนี้ พบว่า ปริมาณไขมันในเพรียงทราย *Perinereis nuntia* จากฟาร์มเลี้ยงที่ช่วงอายุ 8 เดือน(20.96±0.73%) มีปริมาณสูงกว่าในเพรียงทรายที่ช่วงอายุ 2, 4 และ 6 เดือน (17.35±0.08%) ในขณะที่ปริมาณไขมันในเพรียงทรายสายพันธุ์ดังกล่าวที่จับจากธรรมชาติมีปริมาณเฉลี่ย13.43±2.83% สำหรับการวิเคราะห์ชนิดของกรดไขมัน พบว่าชนิดของกรดไขมันที่พบมากที่สุดในเพรียงทรายจากฟาร์มเลี้ยงคือ C16:0, C18:1 *n*-9 และ C18:2 *n*-6 และในเพรียงทรายจากธรรมชาติ คือ C16:0, C18:1 *n*-7 และ C18:0 เมื่อพิจารณาอัตราส่วนของกรดไขมันชนิด SFA : PUFA : MUFA ในเพรียงทรายจากฟาร์มเลี้ยงพบว่ามีอัตราส่วนเป็น 1.3 : 1.0 : 1.0 และในเพรียงทรายที่จับจากธรรมชาติในช่วงฤดูร้อนและฤดูหนาวเป็น 4.0 : 2.2 : 1.0 และ 2.3 : 1.5 : 1.0 ตามลำดับ สำหรับอัตราส่วนของกรดไขมันชนิด ARA : EPA : DHA และกรดไขมันในกลุ่ม *n*-3 : *n*-6 ในเพรียงทรายจากฟาร์มเลี้ยงมีค่าเป็น 1.6 : 1.3 : 1.0 และ 1.0 : 3.0 ตามลำดับ ซึ่งอัตราส่วนที่ได้ค่อนข้างคงที่ในช่วงอายุ 4, 6 และ 8 เดือน ในขณะที่อัตราส่วนดังกล่าวที่พบในเพรียงทรายจากธรรมชาติมีค่าที่แตกต่างกันไป สำหรับผลรวมของกรดไขมันในเพรียงทรายจากฟาร์มเลี้ยงอายุ 6 เดือนสายพันธุ์ *P. aibuhitensis* มีปริมาณสูงกว่าในเพรียงทรายสายพันธุ์ *P. nuntia* และ *P. quatrefagesi* อัตราส่วนของกรดไขมันชนิด SFA : PUFA : MUFA และกรดไขมันในกลุ่ม *n*-3 : *n*-6 ในเพรียงทรายสายพันธุ์ *P. aibuhitensis* และ *P. quatrefagesi* จากฟาร์มเลี้ยง พบว่ามีค่าใกล้เคียงกับสายพันธุ์ *P. nuntia* เมื่อทำการวิเคราะห์องค์ประกอบของลิปิดที่พบในเพรียงทรายทั้ง 3 สายพันธุ์พบว่า องค์ประกอบที่พบมากที่สุดคือ ฟอสโฟลิปิด ชนิดฟอสฟาติดิลคอลีน (PC) สำหรับ ปริมาณของคอเลสเตอรอล ในเพรียงทรายสายพันธุ์ *P. nuntia* จากฟาร์มเลี้ยง (60 มก./ 100 กรัมน้ำหนักเปียก) มีค่าน้อยกว่าเพรียงทรายจากธรรมชาติ (81 มก./ 100 กรัมน้ำหนักเปียก) ในขณะที่ปริมาณดังกล่าวในเพรียงทรายสายพันธุ์อื่นมีค่าต่างกันเพียงเล็กน้อย

ภาควิชา เคมี ลายมือชื่อนิสิต มณีรัตน์ ลิ้มสุวัฒนาธำรง

สาขาวิชา เคมี ลายมือชื่อ.ที่ปรึกษาวิทยานิพนธ์หลัก นาตยา งามโรจนวณิชย์

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MANEERAT LIMSUWATTHANATHAMRONG : QUALITATIVE AND QUANTITATIVE ANALYSES OF FATTY ACID AND STEROID IN SANDWORMS *Perinereis* sp. THESIS ADVISOR : ASSOC. PROF. NATTAYA NGAMROJANAVANICH, Ph.D., THESIS CO-ADVISOR : SARINTIP SOOKSAI, Ph.D., 92 pp.

The percentage of fat of *Perinereis nuntia* farm-raised sandworm aged 8 months (20.96±0.73%) was higher than those of aged 2, 4 and 6 months (17.35±0.08%), while that of *P. nuntia* wild-caught sandworm was 13.43±2.83%. The most abundant fatty acids of the farm-raised sandworm were C16:0, C18:1 *n*-9 and C18:2 *n*-6 while those of the wild-caught were C16:0, C18:1 *n*-7 and C18:0. The ratio of SFA : MUFA : PUFA of all of the farm-raised was 1.3 : 1.0 : 1.0 while the ratio of those of the wild-caught in summer and winter season were 4.0 : 2.2 : 1.0 and 2.3 : 1.5 : 1.0, respectively. The ratio of ARA : EPA : DHA and *n*-3 : *n*-6 fatty acid of the farm-raised sandworm were 1.6 : 1.3 : 1.0 and 1.0 : 3.0, to stand in aged 4, 6 and 8 months, whereas ratio of those of the wild-caught were different. Total fatty acid in age 6 months of *P. aibuhitensis* was higher than that of *P. nuntia* and *P. quatrefagesi.* The ratio of SFA : MUFA : PUFA and *n*-3 : *n*-6 fatty acid of *P. aibuhitensis* and *P. quatrefagesi* of farm-raised were similar to those of *P. nuntia*. The major component of the lipid was phospholipids, especially phosphatidylcholine (PC). Cholesterol of *P. nuntia* farm-raised sandworm was (60 mg/ 100g ww) less than that of the wild-caught (81 mg/ 100g ww), while in difference species was slightly different.

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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CONTENTS

Page

Page

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Table Page

Table Page

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

Figure Page

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF ABBREVIATIONS

µm	Micrometre(s)
µL	Microlitre(s)
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
ARA	Arachidonic acid (C20:4 *n*-6)
°C	degree Celsius
Cxx:y *n*-z	xx means number of carbon, y means number of double bonds on fatty acid chain, z means position of the first double bond that count from CH_3 -end of fatty acid chain. Example: C18:2 *n*-6 means this fatty acid have 18 carbon atoms, contains 2 double bonds and the first double bond counts from CH_3 - end of fatty acid chain is at carbon 6.
DHA	Docosahexaenoic acid (C22:6 *n*-3)
EPA	Eicosapentaenoic acid (C20:5 *n*-3)
ESI	Electrospray ionization
FA	Fatty acid
FAME	Fatty acid methyl ester
g	Gram(s)
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HUFA	Highlyunsaturated fatty acid (Fatty acid which have more than 3 double bonds)
id	Diameter
kV	Kilovolt(s)
L	Litre(s)
LA	Linoleic acid
LC-FA	Long chain-fatty acid (Fatty acid which have 16 to 18 carbons)
m	Metre(s)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
MS	Mass spectrometry
MUFA	Monounsaturated fatty acid (Fatty acid that has one double bond)
m/z	Mass per charge
N	Normal
n-x	x means position of the first double bond that count from CH_3 -end of fatty acid chain.
PUFA	Polyunsaturated fatty acid (Fatty acid that have more than one double bonds)
SFA	Saturated fatty acid (Fatty acid that don't have double bond)
sp	Species
VLC-FA	Very long chain fatty acid (Fatty acid which have more than 18 carbons)
v/v	Volume by volume
v/v/v/v	Volume by volume by volume by volume
ww	Wet weight(s)

ABBREVIATIONS OF PHOSPHOLIPIDS ;

DPG	Diphosphatidylglycerol
LPC	Lysophosphatidylcholine
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PS	Phosphatidylserine
SM	Sphingomylin

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

Polychaete, *Perinereis* sp., is used extensively for shrimp broodstock to obtain better maturation and oocyte, sperm production especially when worms are in a reproductive stage [1] and for prawn broodstock diet due to their qualities in enhancing prawn reproductive performances [2, 3]. The common polychaetes used for shrimp hatcheries in Thailand are sandworm (*Perinereis* sp.) and mudworm (*Marphysa* sp.) [4]. Broodstock fed sandworm yielded a higher number of egg with a better hatching rate compared to other commercially available diets [5]. The outstanding nutritional composition such as high protein and highly unsaturated fatty acids (HUFAs) of polychaetes are responsible for their qualities in enhancing prawn reproductive system.

Lipids are an important role in the physiology of most animals. They are an essential source of nutrients and energy reserves during periods of poor feeding and negative energy balance, such as hibernation (mammals), sexual development and breeding (starfish, many vertebrates) and migration (birds, fish and insects) [6]. Sterols, pigments, phospholipids, free fatty acid and thirteen different hydrocarbons were found in the lipid extracts of whole earthworms, but neither triglycerides nor diglycerides nor monoglycerides were detected, suggested that earthworms possibly have other unidentified types of storage lipids (nonpolar or neutral lipids) [7]. During periods of good feeding and sexual immaturity, several marine invertebrates, including polychaetes, accumulate stores of lipid which are depleted during maturation and/or starvation [7, 8, 9, 10].

Fatty acid is very important for marines, for example fatty acid profile in life feed especially in polychaete such as bloodworms and sandworms have been used successfully as a feed source to induce maturation in penaeid shrimp [1, 3]. Such success results partly from their HUFAs component, particularly arachidonic acid (ARA) content [4]. One of the reasons that made the difference is that sandworm

contains a high content of lipid with profile of polyunsaturated fatty acids (PUFAs) suitable for gonadogenesis of marine shrimp. Fatty acid compositions of marine polychaete, *Nereis diversicolor* in Portugal, were determined and was found that major fatty acid were C16:0, C18:1, C18:2 and C20:5. The fatty acids compositions were 23.37% saturated fatty acids and 65.38% unsaturated fatty acids as when average the all year [11].

Fatty acid compositions in *N. diversicolor* fed with different diet were investigated [12]. The wild-caught *N. diversicolor* was caught and grew them in laboratory and fed with 6 different experimental diets. The fatty acid compositions of all experiments were determined. The results showed that *N. diversicolor* was fed with different diets but the major fatty acids in all tests were C16:0, C18:1, C18:2 and C20:5. It means that the majority of fatty acids in marine polychaete were not effect by diet.

The wild-caught sandworm for broodstock feed was collected from natural populations on sediment shore and they may be a carrier of pathogens to shrimp broodstock [13]. Furthermore, the worm collected activities aggravated environment due to an over-harvesting and destruction of worm habitat and wild-caught worms were depleting. For these reasons, Chunhabundit (1991) [14] collected sandworm (*Perinereis nuntia* Savigny) from natural habitat and cultivated them with semi-sterile technique and developed to a farming scale in order to reduce the impact of over-harvesting and pathogen-carrier. However, farm-raised sandworm fed with shrimp feed in a commercial farming system differed from wild-caught sandworm which fed by scavenging. In other marine organism such as fish, farmed and wild animals were different in nutritional values depending on a variety of factors including age, diet and environment and many researchers try to adjust nutritional value of farmed animals [15, 16]. However the information on lipid compositions and fatty acid values of wild-caught compared to the farm-raised sandworm (*Perinereis* sp.) was not available. Therefore, the purpose of this study was to determine the lipid and fatty acid values of farm-raised sandworm aged 2, 4, 6 and 8 months and wild-caught sandworm was caught in summer and winter. In this study, *Perinereis* sp. was determined for 3 species; *P. nuntia*, *P. aibuhitensis* and *P. quatrefagesi*. The data will

be used as a guideline for quality control of farm-raised sandworm as live feed for aquatic animals.

Objectives

The objective of this research was to analyze as qualitative and quantitative of fatty acid and steroid in sandworms (*Perinereis* sp.).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

THEORETICAL

2.1 Sandworm *Perinereis* sp.

The biological classification of the sandworm is as following;

Kingdom : Animalia

Phylum : Annelida

Class : Polychaeta

Order : Phyllodocida

Family : Nereidae

Genus : Perinereis

Polychaetes ('Poly' = many; 'chaeta' = hairs) are the largest groups of annelids and majority are marine polychaetes. More than 8,000 species are described in this class. Most of them live in soft or rocky environment on sea floor, abundant from the intertidal zone to depths of over 5,000 meter and distribute around tropical Indo-Pacific. They may be, but rarely, found in freshwater and terrestrial (humid), or parasitic. Most polychaetes fed by sediment swallowing of microphytobenthios, mainly benthic diatoms. Polychaetes in family Nereidae were contained 42 genera and about 500 species [17].

2.1.1 General Morphology of Sandworm

The body of sandworm is covered by a flexible external cuticle to make it softbody. The shape is long flat like and multi segmented. It can be divided into three parts; head, trunk and tail. Head part contains 2 pairs of antennas, 4 tentacular cirri and 1 pair of complex eyes. It has a complex brain which divides into three divisions; a forbrain, midbrain and hindbrain. In trunk part, segment is limited by septa from

neighboring segments. Each segment carries parapodia and chaetae and contains digestive, vascular, muscular, nervous system and pair of nephridia for excretion. The parapodia is the flatlike projective on both sides of segment for locomotion and gas exchange. It has a closed circulation system with hemoglobin. The close circulation system consists of medial, dorsal and ventral longitudinal vessels, linked by smaller vessels, capillary beds and gut lacunae. The last part, tail; the posterior section of the body which contains simple terminal anus [18].

2.1.2 Reproductive of Sandworm

They are separate sexes, breeding only once in their lifecycle before dying. When male and female sandworms reach maturity, hormonal changes cause their bodies to change (Figure 2.1). Their digestive systems break down, to enable large numbers of eggs and sperm to be produced, and most species develop large eyes and swimming legs in preparation for leaving their burrows to spawn at the water surface. Eggs and sperm shed into coelom and leave the body through the nephridia. After the external fertilization, a ciliated free-swimming larval is developed. A combination of a temperature and lunar cycle stimulates the release of pheromones and gametes from all the mature worms in the population. Spawning during spring tides probably ensures the maximum dispersion of fertilized eggs in the water [19].

Figure 2.1 Farm-raised sandworm in the beginning stage of reproductive cell synthesis

2.1.3 Important of Sandworm

Sandworm is an important part of the invertebrate community of intertidal mud flat, providing a source of food for wading birds and fish. They are also used as live bait for anglers and live feed for marine culture especially shrimp farm. At least 6 *Perinereis* species are harvested commercially for these propose, including *P. brevicirris* in Taiwan and *P. nuntia* in Thailand [20]. Wild-caught sandworm used for shrimp hatcheries in Thailand is collected by digging from sediment shore line and farm-raised sandworm is collected from the commercial sandworm farms. Moreover, many bioactive components were identified from sandworm including collagens, glycerophospholipids, eicosapentaenoic acid, fibrinolytic enzyme and antimicrobial peptide [21].

2.2 Lipid

2.2.1 Introduction of Lipid

The word “lipid” has long been used to denote a chemically heterogeneous group of substances. Lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (such as ether, chloroform, acetone or benzene) and general insolubility in water. Lipids are hydrophobic because the molecules consist of long, 16 or more carbon, hydrocarbon backbones with only a small amount of oxygen containing groups. Although the term *lipid* is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di- and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet. The lipids of physiological importance for humans have many functions: they serve as energy source, as well as storage for energy in the form of fat cells. Lipids serve many functions in organisms, are the major components of waxes, pigments, steroid hormones and cell membranes. These membranes in association with

carbohydrates and proteins regulate the flow of water, ions and other molecules into and out of the cells. Lipids rarely exist in an organism in the free state but are more usually combined with proteins or carbohydrates as lipoproteins or lipopolysaccharides. Hormone steroids and prostaglandins are chemical messengers between body tissues. Vitamins A, D, E and K are lipid soluble and regulate critical biological processes, and other lipids add in vitamin absorption and transportation. Lipids act as a shock absorber to protect vital organs and insulate the body from temperature extremes [22, 23, 24].

2.2.2 Categories of Lipids

2.2.2.1 Triacylglycerols, Triglycerides

Triacylglycerols, triglycerides (neutral fats) are synthesized from two different classes of molecules: glycerol (a type of alcohol with a hydroxyl group on each of its three carbons) and three fatty acid molecules are attached to the glycerol through an ester bond between the carboxyl group of the fatty acids and the three alcohol groups of a glycerol molecule (Figure 2.2). Since there are three fatty acids attached, these are known as triglycerides. Triglycerides are make up adipose tissue in human and other mammals bodies and functions as long term energy storage, insulates and protects [25, 26].

ศูนย์วิทยทรัพยากร
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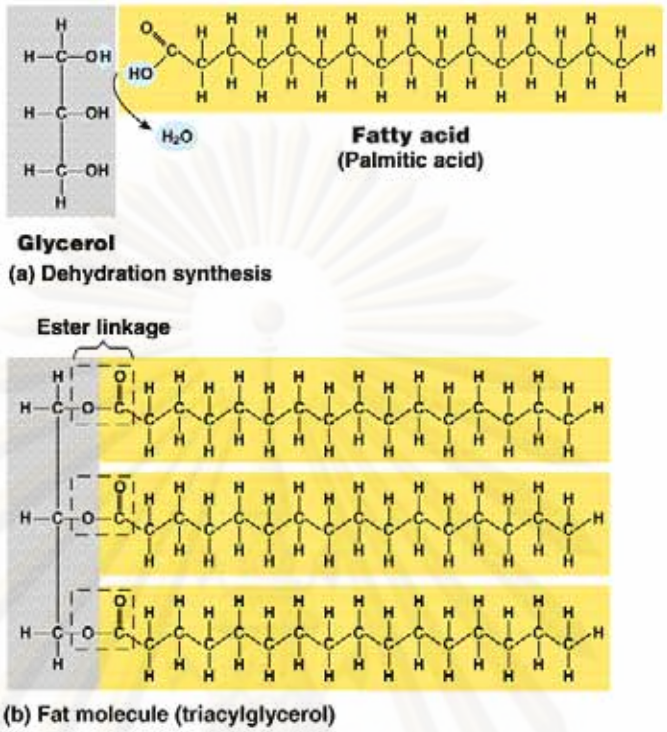

Figure 2.2 The synthesis of a triglyceride (a) Dehydration synthesis and (b) Fat molecule (triacylglycerol)

2.2.2.2 Phospholipids

Phospholipids contain only two fatty acids attached to a glycerol head. The third alcohol of the glycerol forms an ester bond through reaction with phosphoric acid (Figure 2.3). This is an example of a condensation reaction between an acid and an alcohol with the release of water. As a triprotic acid (it has three acidic functions on the phosphorus atom) the phosphate group attached to the glycerol has the potential to form ester links with a variety of other molecules such as carbohydrates, choline, inositol and amino acids. The phosphate group along with the glycerol group makes the head of the phospholipid hydrophilic, whereas the fatty acid tail is hydrophobic. Thus phospholipids are amphipathic: a molecule with a polar terminal and a hydrophobic terminal. When phospholipids are in an aqueous solution they will self assemble into micelles or bilayers, structures that exclude water molecules from the hydrophobic tails while keeping the hydrophilic head in contact with the aqueous solution [27, 28].

Phospholipids serve a major function in the cells of all organisms: they form the phospholipid membranes that surround the cell and intracellular organelles such as the mitochondria. The cell membrane is a fluid, semi-permeable bilayer that separates the cell's contents from the environment. The membrane is fluid at physiological temperatures and allows cells to change shape due to physical constraints or changing cellular volumes. The phospholipid membrane allows free diffusion of some small molecules such as oxygen, carbon dioxide, and small hydrocarbons, but not charged ions, polar molecules or other larger molecules such as glucose. This semi-permeable nature of the membrane allows the cell to maintain the composition of the cytoplasm independent of the external environment. The phosphate heads are hydrophilic (attracted to water) and interface with the aqueous environment of the extracellular fluid outside the cell and the intracellular fluid inside the cell. The lipid tails are predominantly long fatty acid chains that form a stable layer in the water-free environment between the outer and inner layer of phosphate heads. Cholesterol molecules give additional stability [28] (Figure 2.4).

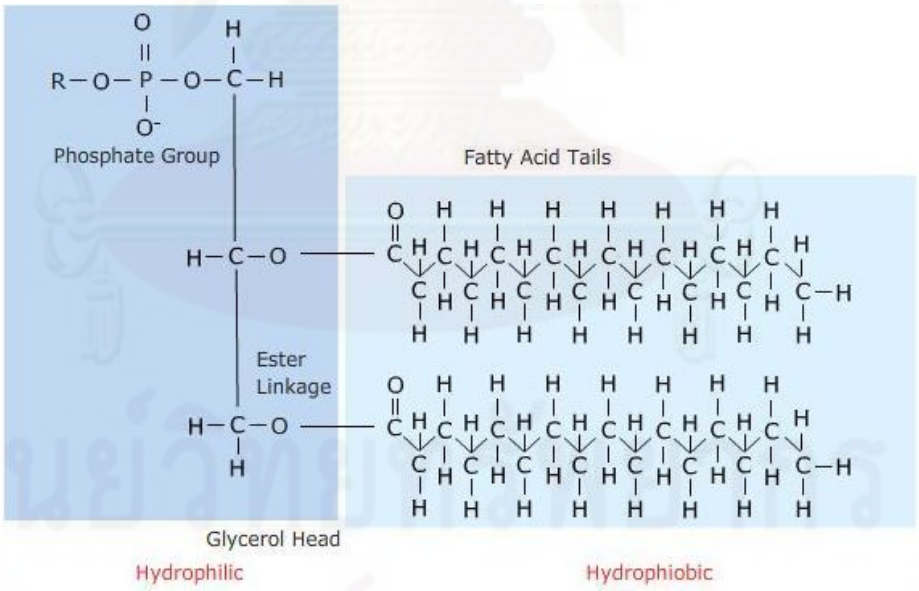

Figure 2.3 Structure of a phospholipid

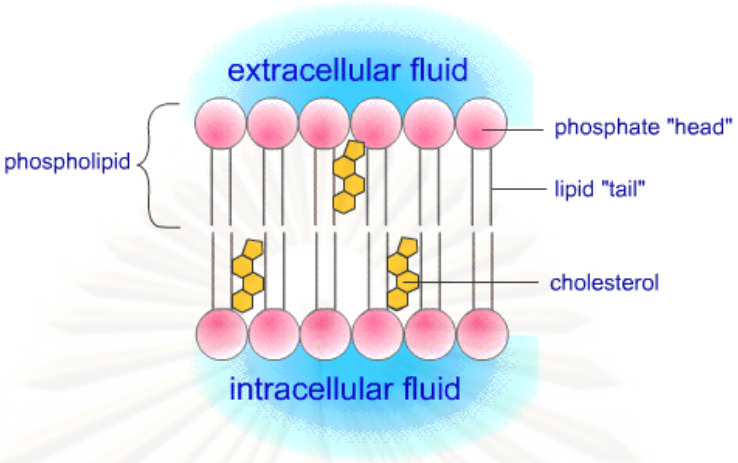

Figure 2.4 The phospholipid membrane

Phospholipids are synthesized by esterification of an alcohol to the phosphate of phosphatidic acid (1, 2-diacylglycerol 3-phosphate). Most phospholipids have a saturated fatty acid on C-1 and an unsaturated fatty acid on C-2 of the glycerol backbone. The most commonly added alcohols (serine, ethanolamine and choline) also contain nitrogen that may be positively charged, whereas, glycerol and inositol do not. The major classifications of phospholipids are listed in Table 2.1.

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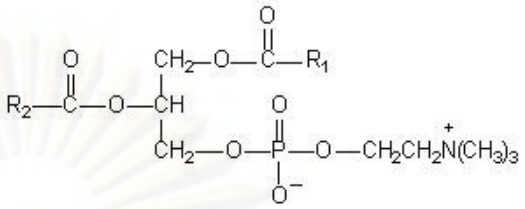
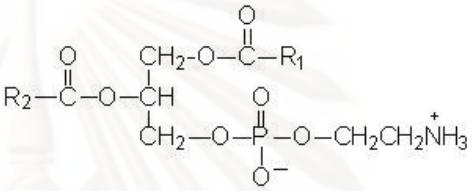
Table 2.1 The major classifications of phospholipids

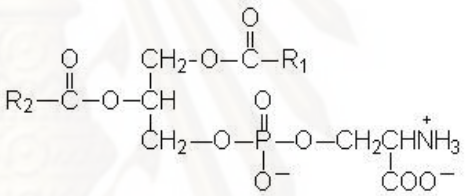
Phosphatidylcholine (PC)



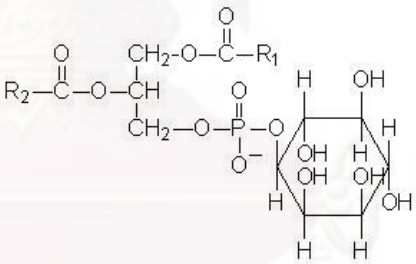
Phosphatidylethanolamine (PE)



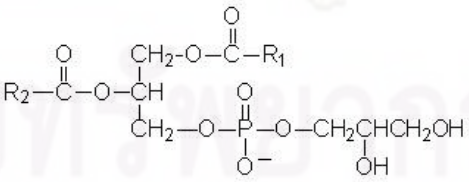
Phosphatidylserine (PS)



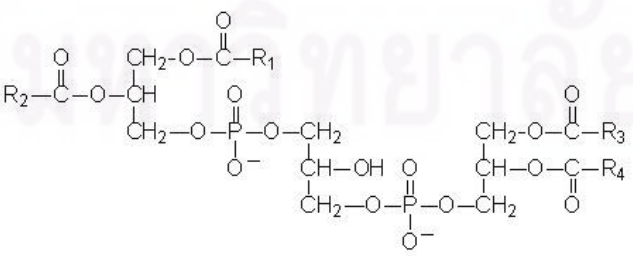
Phosphatidylinositol (PI)



Phosphatidylglycerol (PG)



Diphosphatidylglycerol (DPG)



Phosphatidylcholine (once given the trivial name 'lecithin', **PC**) is a class of phospholipid which incorporates choline as a headgroup. PC is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is obviously the key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer-leaflet of the plasma membrane. PC is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL. On the other hand, it is less often found in bacterial membranes, perhaps 10% of species. It is a neutral or zwitterionic phospholipid over a pH range from strongly acid to strongly alkaline.

Phosphatidylethanolamine (cephalin, sometimes abbreviated **PE**) is usually the second most abundant phospholipid in animal and plant lipids and it is frequently the main lipid component of microbial membranes. It can amount to 20% of liver phospholipids and as much as 45% of those of brain; higher proportions are found in mitochondria than in other organelles. As such, it is obviously a key building block of membrane bilayers. Cephalin is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neural tissue, and in spinal cord. Whereas lecithin is the principal phospholipid in animals, cephalin is the principal one in bacteria. Its role in nature is not certain.

Phosphatidylserine (abbreviated **Ptd-L-Ser** or **PS**) is a phospholipid component, usually kept on the inner-leaflet, the cytosolic side, of cell membranes by an enzyme called flippase. When a cell undergoes apoptotic cell death PS is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. PS is an essential component in all our cells; specifically, it is a major component of the cell membrane. The cell membrane is a kind of "skin" that surrounds living cells. Besides keeping cells intact, this membrane performs vital functions such as moving nutrients into cells and pumping waste products out of them.

Phosphatidylinositol (abbreviated PtdIns, or **PI**) is a negatively charged phospholipid and a minor component in the cytosolic side of eukaryotic cell membranes.

Phosphatidylglycerol (abbreviated **PG**) the general structure of PG consists of a L-glycerol 3-phosphate backbone ester-bonded to either saturated or unsaturated fatty acids on carbons 1 and 2. The head group substituent glycerol is bonded through a phosphomonoester. It is the precursor of surfactant and its presence in the amniotic fluid of the newborn indicates fetal lung maturity. PG is found in almost all bacterial types. For example, *Escherichia coli*, a widely studied organism, has up to 20% of PG in its membranes (PE makes up much of the rest with a little cardiolipin).

Diphosphatidylglycerol (Cardiolipin, sometimes abbreviated **DPG**) is the trivial but universally used name for a lipid that should be correctly termed 'diphosphatidylglycerol' or more precisely 1,3-*bis*(*sn*-3'-phosphatidyl)-*sn*-glycerol. It is a unique phospholipid with in essence a dimeric structure, having four acyl groups and potentially carrying two negative charges. It is found almost exclusively in certain membranes of bacteria (plasma membrane and hydrogenosomes) and of mitochondria, i.e. those whose function is to generate an electrochemical potential for substrate transport and ATP synthesis. The trivial name 'cardiolipin' is derived from the fact that it was first found in animal hearts, where it is especially abundant, but it can be found in mitochondria of all animal tissues and indeed of the eukaryotic kingdom. For example, it amounts to about 10% of the phospholipids of bovine heart muscle, and 20% of the phospholipids of the mitochondrial membrane in this organ. It is a minor component of human plasma lipoproteins, although it is the most abundant anionic lipid [29].

2.2.2.3 Sterol Lipids, Steroids

The important classes of lipid called steroids are actually metabolic derivatives of terpenes, but they are customarily treated as a separate group. Steroids may be recognized by their tetracyclic skeleton, consisting of three fused six-membered and one five-membered ring, as shown in the Figure 2.5. The four rings are designated A, B, C & D as noted, and the peculiar numbering of the ring carbon atoms (shown in number) is the result of an earlier misassignment of the structure. The substituents designated by R are often alkyl groups, but may also have functionality. The R group at the A:B ring fusion is most commonly methyl or

hydrogen, that at the C:D fusion is usually methyl. The substituent at C-17 varies considerably, and is usually larger than methyl if it is not a functional group. The most common locations of functional groups are C-3, C-4, C-7, C-11, C-12 & C-17. Ring A is sometimes aromatic. Since a number of tetracyclic triterpenes also have this tetracyclic structure, it cannot be considered a unique identifier. The central core of this molecule, consisting of four fused rings, is shared by all steroids, including estrogen (estradiol), progesterone, corticosteroids such as cortisol (cortisone), aldosterone, testosterone, and vitamin D. In the various types of steroids, various other groups/molecules are attached around the edges. Steroids are widely distributed in animals, where they are associated with a number of physiological processes [29, 30].

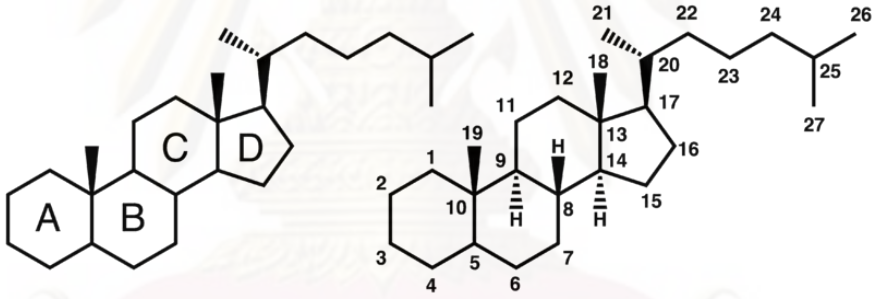


Figure 2.5 The structure of steroid

The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signaling molecules. The eighteen-carbon (C18) steroids include the estrogen family whereas the C19 steroids comprise the androgens such as testosterone and androsterone. The C21 subclass includes the progestogens as well as the glucocorticoids and mineralocorticoids. The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure. Other examples of sterol are the bile acids and their conjugates, which in mammals are oxidized derivatives of cholesterol and are synthesized in the liver. The plant equivalents are the phytosterols, such as β-sitosterol, stigmasterol, and brassicasterol; the latter compound is also used as a biomarker for algal growth. The predominant sterol in fungal cell membranes is ergosterol [31].

Sterol lipids, such as cholesterol (cholest-5-en-3β-ol) and its derivatives, are an important component of cell membrane in animals and functions to moderate membrane fluidity because it restricts the motion of the fatty acid tails, along with the glycerophospholipids and sphingomyelins. Cholesterol has vital structural roles in membranes and in lipid metabolism in general. It is a biosynthetic precursor of bile acids, vitamin D and steroid hormones (glucocorticoids, oestrogens, progesterones, androgens and aldosterone) (Figure 2.6). In addition, it contributes to the development and working of the central nervous system, and it has major functions in signal transduction and sperm development. It is found in covalent linkage to specific membrane proteins or proteolipids (‘hedgehog’ proteins), which have vital functions in embryonic development. However, because plasma cholesterol levels are a major contributory factor to atherogenesis, media coverage has created what has been termed a ‘cholesterophobia’ in the population at large [32, 33].

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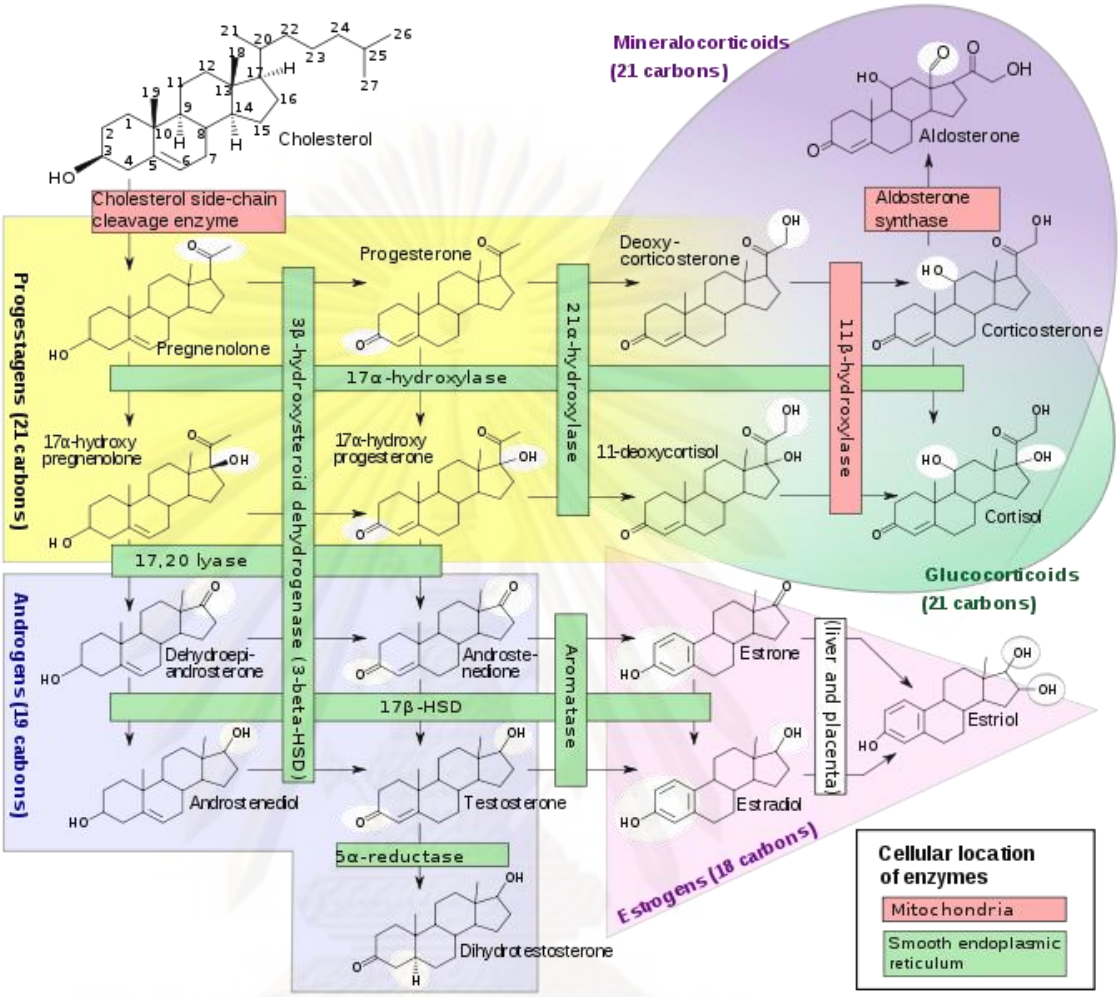


Figure 2.6 Steroidogenesis, using cholesterol as building material

2.2.2.4 Fatty Acids

Fatty acids are long-chain hydrocarbon molecules (tail), making it hydrophobic, containing a carboxylic acid moiety at one terminal (head) which is hydrophilic. Fatty acids are the main component of soap, where their tails are soluble in oily dirt and their heads are soluble in water to emulsify and wash away the oily dirt. However, when the head terminal is attached to glycerol to form a fat, that whole molecule is hydrophobic [34]. Fatty acids that contain no carbon-carbon double bonds are termed saturated fatty acids (SFA); those that contain double bonds are monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), those shown in Figure 2.7. The terms saturated, monounsaturated and polyunsaturated refer

to the number of hydrogens attached to the hydrocarbon tails of the fatty acids as compared to the number of double bonds between carbon atoms in the tail.

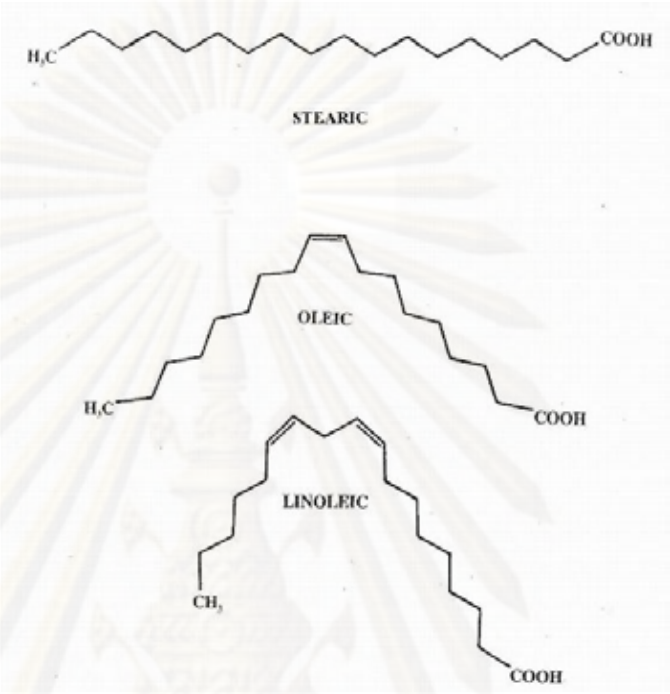


Figure 2.7 Saturated fatty acid, SFA (Stearic acid; C18:0) (above), Monounsaturated fatty acid, MUFA (Oleic acid; C18:1 *n*-9) (middle) and Polyunsaturated fatty acid, PUFA (Linoleic acid; C18:2 *n*-6) (below)

The shortest descriptions of fatty acids include only the number of carbon atoms and double bonds in them (e.g., C18:0 or 18:0). C18:0 means that the carbon chain of the fatty acid consists of 18 carbon atoms, and there are no (zero) double bonds in it, whereas C18:1 describes an 18-carbon chain with one double bond in it. The site of unsaturation in a fatty acids is indicated by the symbol *n*- or ω- (omega-) and the number of the first carbon (counting from the terminal methyl carbon) of the double bond (e.g., palmitoleic acid is a 16-carbon fatty acid with one site of unsaturation between carbons 7 and 8, and is designated by C16:1 *n*-7). Table 2.2 is shown in the most naturally-occurring saturated, monounsaturated and polyunsaturated fatty acids [35].

Table 2.2 The most naturally-occurring saturated, monounsaturated and polyunsaturated fatty acids

Common name	Systematic name	Chemical structure	Numeric name
Saturated Fatty Acid (SFA)			
Lauric acid	Dodecanoic acid	$C_{12}H_{24}O_2$	C12:0
Myristic acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	C14:0
Pentadecylic acid	Pentadecanoic acid	$C_{15}H_{30}O_2$	C15:0
Palmitic acid	Hexadecanoic acid	$C_{16}H_{32}O_2$	C16:0
Stearic acid	Octadecanoic acid	$C_{18}H_{36}O_2$	C18:0
Arachidic acid	Eicosanoic acid	$C_{20}H_{40}O_2$	C20:0
Behenic acid	Docosanoic acid	$C_{22}H_{44}O_2$	C22:0
Monounsaturated Fatty Acid (MUFA)			
Myristoleic acid	9-tetradecenoic acid	$C_{14}H_{26}O_2$	C14:1 *n*-5
Palmitoleic acid	9-hexadecenoic acid	$C_{16}H_{30}O_2$	C16:1 *n*-7
Oleic acid	9-octadecenoic acid	$C_{18}H_{34}O_2$	C18:1 *n*-9
Vaccenic acid	11-octadecenoic acid	$C_{18}H_{34}O_2$	C18:1 *n*-7
Eicosenoic acid	11-eicosenoic acid	$C_{20}H_{38}O_2$	C20:1 *n*-9
Erucic acid	13-docosenoic acid	$C_{22}H_{42}O_2$	C22:1 *n*-9
Polyunsaturated Fatty Acid (PUFA)			
Omega 3 Family			
α-Linolenic acid (ALA)	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	C18:3 *n*-3
Stearidonic acid	6,9,12,15-octadecatetraenoic acid	$C_{18}H_{28}O_2$	C18:4 *n*-3
Eicosatrienoic acid (ETA)	11,14,17-eicosatrienoic acid	$C_{20}H_{34}O_2$	C20:3 *n*-3
Eicosatetraenoic acid	8,11,14,17-eicosatetraenoic acid	$C_{20}H_{32}O_2$	C20:4 *n*-3
Eicosapentaenoic acid (EPA)	5,8,11,14,17-eicosapentaenoic acid	$C_{20}H_{30}O_2$	C20:5 *n*-3
Docosapentaenoic acid (DPA)	7,10,13,16,19-docosapentaenoic acid	$C_{22}H_{34}O_2$	C22:5 *n*-3
Docosahexaenoic acid (DHA)	4,7,10,13,16,19-docosahexaenoic acid	$C_{22}H_{32}O_2$	C22:6 *n*-3
Omega 6 Family			
Linoleic acid (LA)	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	C18:2 *n*-6
γ-Linolenic acid (GLA)	6,9,12-octadecatrienoic acid	$C_{18}H_{30}O_2$	C18:3 *n*-6
Eicosadienoic acid	11,14-eicosadienoic acid	$C_{20}H_{36}O_2$	C20:2 *n*-6
Homo-γ-linolenic acid	8,11,14-eicosatrienoic acid	$C_{20}H_{34}O_2$	C20:3 *n*-6
Arachidonic acid (ARA)	5,8,11,14-eicosatetraenoic acid	$C_{20}H_{32}O_2$	C20:4 *n*-6
Docosadienoic acid	13,16-docosadienoic acid	$C_{22}H_{40}O_2$	C22:2 *n*-6
Adrenic acid	7,10,13,16-docosatetraenoic acid	$C_{22}H_{36}O_2$	C22:4 *n*-6
Docosapentaenoic acid (DPA)	4,7,10,13,16-docosapentaenoic acid	$C_{22}H_{34}O_2$	C22:5 *n-6*

Saturated fatty acids (SFA) The SFA molecules do not contain any double bonds or other functional groups along the hydrocarbon chain. The term "saturated" refers to hydrogen, in that all carbons (apart from the carboxylic acid [-COOH] group) contain as many hydrogens as possible. In other words, the terminal carbon contains 3 hydrogens (CH_3-), and each carbon within the chain contains 2 hydrogen atoms. Saturated fatty acids form straight chains and, as a result, can be packed together very tightly, allowing living organisms to store chemical energy very densely. The fatty tissues of animals contain large amounts of long-chain saturated fatty acids. In IUPAC nomenclature, fatty acids have an [-*oic* acid] suffix. In common nomenclature, the suffix is usually -*ic*.

Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are of similar form, except that one or more alkenyl functional groups exist along the chain, with each alkene substituting a single-bonded "-CH_2-CH_2-" part of the chain with a double-bonded "-CH=CH-" portion (that is, a carbon double-bonded to another carbon). The two next carbon atoms in the chain that are bound to either side of the double bond can occur in a *cis* or *trans* configuration.

cis configuration

> A *cis* configuration means that adjacent carbon atoms are on the same side of the double bond. The rigidity of the double bond freezes its conformation and, in the case of the *cis* isomer, causes the chain to bend and restricts the conformational freedom of the fatty acid. The more double bonds the chain has in the *cis* configuration, the less flexibility it has. When a chain has many *cis* bonds, it becomes quite curved in its most accessible conformations. For example, oleic acid, with one double bond, has a "kink" in it, whereas linoleic acid, with two double bonds, has a more pronounced bend. Alpha-linolenic acid, with three double bonds, favors a hooked shape. The effect of this is that, in restricted environments, such as when fatty acids are part of a phospholipids in a lipid bilayer, or triglycerides in lipid droplets, cis bonds limit the ability of fatty acids to be closely packed, and therefore could affect the melting temperature of the membrane or of the fat.

trans configuration

> A *trans* configuration, by contrast, means that the next two carbon atoms are bound to *opposite* sides of the double bond. As a result, they do not cause the chain to bend much, and their shape is similar to straight saturated fatty acids.

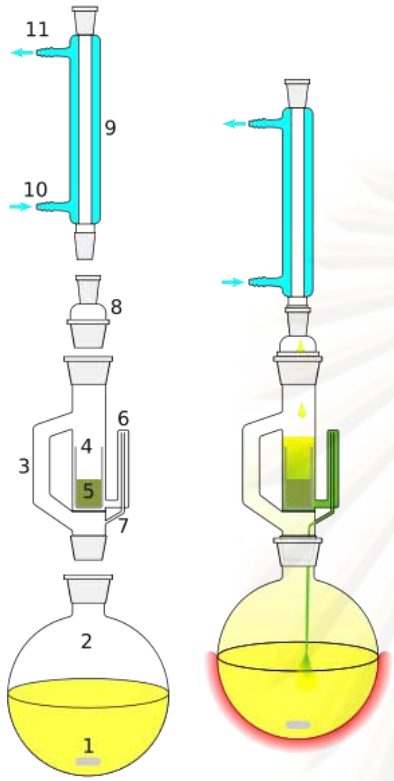
The differences in geometry between the various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes, and in the construction of biological structures (such as cell membranes).

The human body can produce all but two of the fatty acids it needs. These two, linoleic acid (LA) and alpha-linolenic acid (ALA), are widely distributed in plant oils. Since they cannot be made in the body from other substrates and must be supplied in food, they are called essential fatty acids. Hence linoleic acid and alpha-linolenic acid are essential fatty acids for humans. In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection. Essential fatty acids are polyunsaturated fatty acids and are the parent compounds of the omega-6 and omega-3 fatty acid series, respectively. In addition, fish oils contain the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Other marine oils, such as from seal, also contain significant amounts of docosapentaenoic acid (DPA), which is also an omega-3 fatty acid. Although the body to some extent can convert LA and ALA into these longer-chain omega-3 fatty acids, the omega-3 fatty acids found in marine oils help fulfill the requirement of essential fatty acids (and have been shown to have wholesome properties of their own) [36, 37, 38].

2.3 Separation Techniques

2.3.1 Soxhlet Extraction

A soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet [39]. It was originally designed for the extraction of a lipid from a solid material. However, a soxhlet extractor is not limited to the extraction of lipids. Typically, a soxhlet extraction is only required where the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The soxhlet extractor is placed onto a flask containing the extraction solvent. The soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded [40] (Figure 2.8).



1: Stirrer bar/ anti-bumping granules

2: Still pot (extraction pot) - still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber.

3: Distillation path

4: Soxhlet Thimble

5: Extraction solid (residue solid)

6: Syphon arm inlet

7: Syphon arm outlet

8: Expansion adapter

9: Condenser

10: Cooling water in

11: Cooling water out

Figure 2.8 The soxhlet extractor

2.3.2 Fatty Acid Methyl Ester

The technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time [41]. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters.

A fatty acid methyl ester (FAME) can be created by an alkali catalyzed reaction between fats or fatty acids and methanol (Figure 2.9). For the food chemist, determining the fatty acid composition of foods that contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids, each with a variety of carbon chain lengths can be identified by Gas Chromatography (GC). GC analysis of

fatty acids were specifically developed for use in the qualitative and/or quantitative identification of fatty acids, either as free fatty acids or as fatty acid methyl esters (FAMEs) [42, 43, 44].

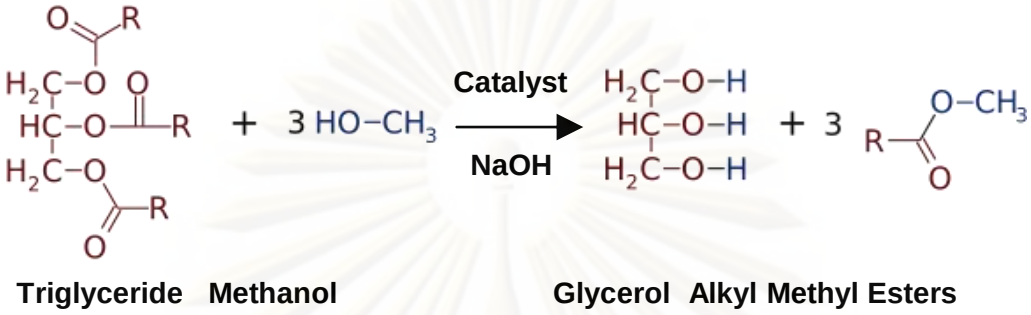


Figure 2.9 Transesterification of triglyceride with methanol

2.3.2.1 Acid-Catalysed Esterification and Transesterification (Boron trifluoride-methanol)

The Lewis acid, boron trifluoride, in the form of its coordination complex with methanol is a powerful acidic catalyst for the esterification of fatty acids. For example, esterification of free fatty acids was completed in two minutes with 12 to 14% boron trifluoride in methanol under reflux. Morrison and Smith [45] showed that the reagent could be used to transesterify most lipid classes (inert solvent must again be added to effect solution of simple lipids), although in general longer reaction times are necessary than with free fatty acids. Boron trifluoride can of course be used with other alcohols, and as examples ethyl, propyl and butyl [46, 47] esters have been prepared in this way.

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2.3.3 Gas Chromatography

Gas-liquid chromatography (GLC), or simply gas chromatography (GC), is a common type of chromatography used in organic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In microscale chemistry, GC can be used to prepare pure compounds from a mixture [48]. In gas chromatography, the *moving phase* (or "mobile phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a *gas chromatograph* (or "aerograph", "gas separator") (Figure 2.10).

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column [49, 50].

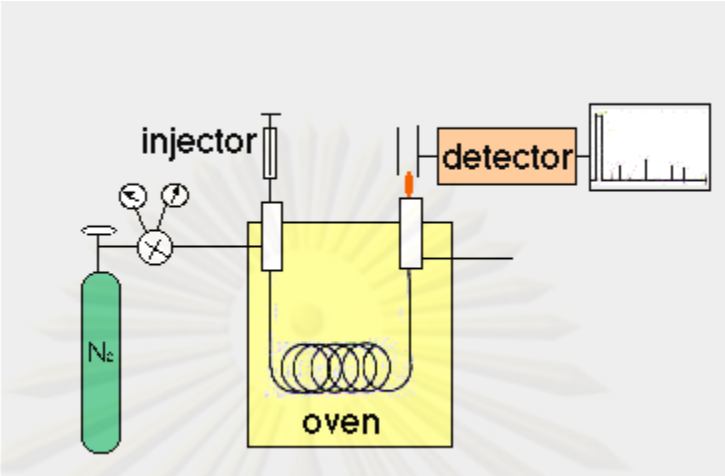

Figure 2.10 Diagram of gas chromatography

2.3.4 High-Performance Liquid Chromatography

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) (Figure 2.11) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

The sample to be analyzed is introduced in small volume to the stream of mobile phase. The analyte's motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time; the retention time under particular conditions is considered a reasonably unique identifying characteristic of a given analyte. The use of smaller particle size column packing (which creates higher backpressure) increases the linear velocity giving the

components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combination of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent [51, 52].

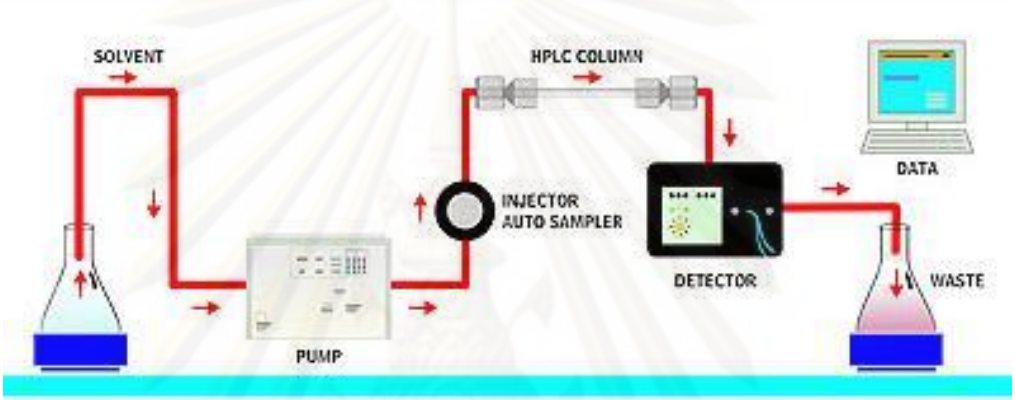

Figure 2.11 Diagram of high-performance liquid chromatography

2.4 Identification Technique

2.4.1 Mass Spectrometry

Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. It is also used for elucidating the chemical structures of molecules, such as phospholipids and other chemical compounds. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-per-charge ratios. In a typical MS procedure, a sample is loaded onto the MS instrument, and its compounds are ionized by different methods (e.g., by impacting them with an electron beam), resulting in the formation of charged particles (ions). Then the generated ions are calculated their mass in mass analyzer component and detector [53] (Figure 2.12).

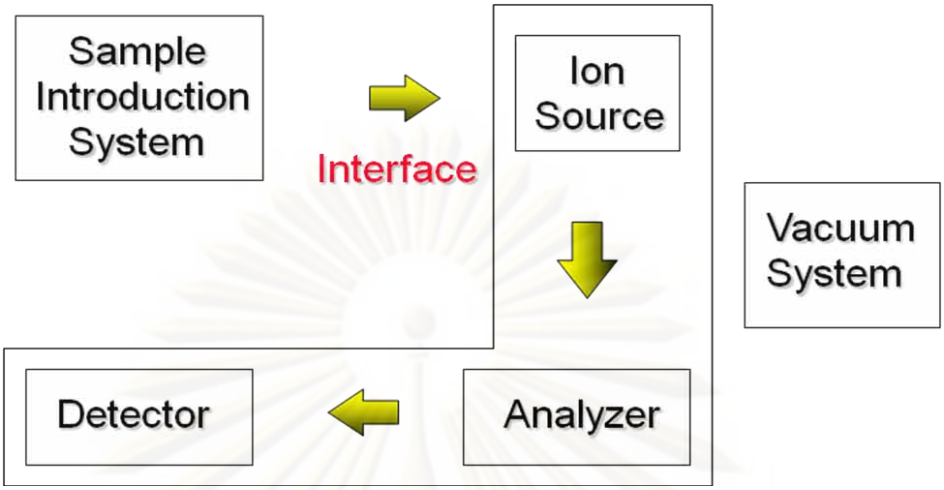

Figure 2.12 A full diagram of a mass spectrometer

2.4.1.1 Electrospray Ionization

Electrospray Ionization (ESI) is one of the Atmospheric Pressure Ionization (API) techniques and is well-suited to the analysis of polar molecules ranging from less than 100 Da to more than 1,000,000 Da in molecular mass [53].

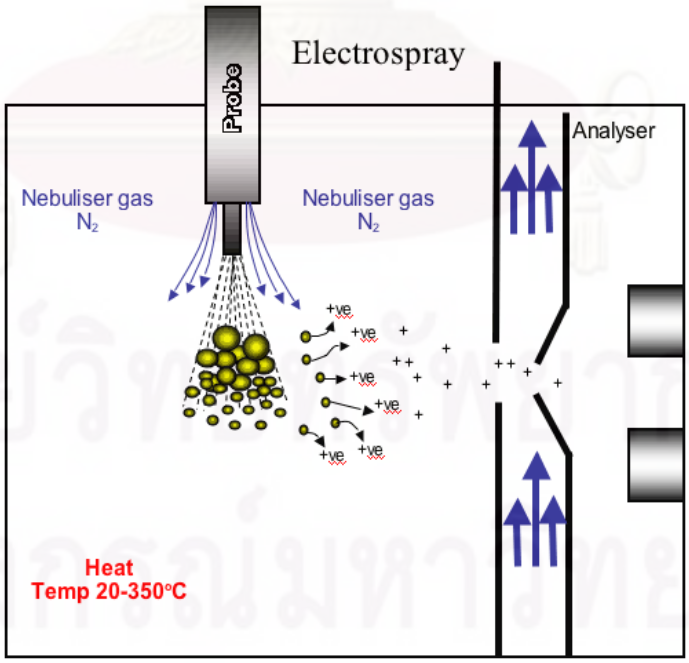

Figure 2.13 Electrospray ionization source

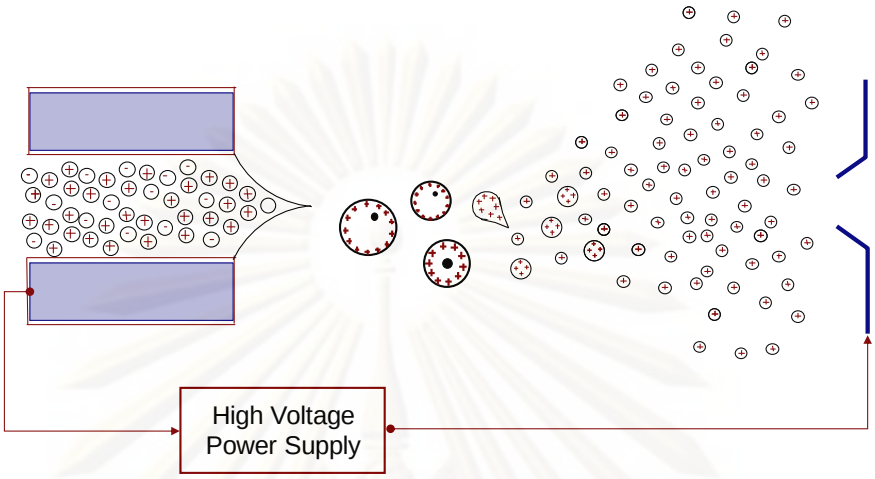

Figure 2.14 The electrospray ionization process

A fundamental challenge to the application of mass spectrometry to any class of analytes is the production of gas-phase ions of those species, and difficulties in producing gas-phase ions can prevent mass spectrometric analysis of certain classes of molecules. The first techniques that were applied, electron ionization and chemical ionization, are two step processes in which the analyte is vaporized with heat and ionization occurs once the analyte is in the gas phase.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2.5 Literature Review

The lipid content and composition of *Nereis (Hediste) diversicolor* O. F. Miillcr (Annelida, Polyehaeta, Nereidae) a mud-dwelling, intertidal errant polychaete in the Tagus estuary (Portugal), were examined on the monthly basis by lipid extraction, thin layer chromatography (TLC) and capillary GC. *N. diversicolor* is by far the dominant species among polychaeta and the main food item in the natural diet of several flatfishes. The fatty acid composition was rather unsaturated with a 1 : 2 mean ratio of *n*-3 : *n*-6. The major fatty acids were C16:0, C18:l *n*-9, C18:2 *n*-6 and C20:5 *n*-3; there were smaller amounts of C18:0, Cl8:l *n*-ll, C18:1 *n*-7, C18:3 *n*-3, C20:1, C20:2 *n*-6, C20:4 *n*-6, C22:2, C22:5 *n*-3 and many other fatty acids were detected at trace levels [11].

The lipid composition was determined for 5 species of polychaete annelids collected by the Deep Submergence Vehicle ALVIN from high temperature chimneys of the East Pacific Rise. Lipid content was low in all samples (1.6–35.9 mg/g wet mass) and was dominated by polar lipid (78–90% of total lipid) with 8–19% sterol and very low storage lipid (triacylglycerol and wax ester). Total polyunsaturated fatty acids were moderately high (22–31% of total fatty acids) with extremely low or no docosahexaenoic acid (DHA, C22:6 *n*-3). Eicosapentaenoic acid (EPA, C20:5 *n*-3) levels were 5–6% in *Alvinella pompejana* and *A. caudata* and 10.3–13.7% in an errantiate polychaete (likely Hesionidae) and *Hesiolyra bergii*. There were greater PUFA and a greater EPA / ARA ratio in the anterior versus the posterior half of *A. pompejana*, which may correlate to the strong temperature gradient reported in its tube. The principal monounsaturated fatty acids (MUFA) included C18:1 *n*-7 (14–19%), C16:1 *n*-7 (2.6–10%) and C20:1 *n*-11 (3–7% of total fatty acids). The major sterol in the polychaetes is cholesterol (89–98% of total sterol) with less cholesterol in the gut contents of *A. pompejana* [54].

Wild *Litopenaeus annamei* females in different stages of sexual maturation were sampled, including spent females and their nauplii, for determination of the lipid content, lipid class composition, fatty acid composition, vitamin C content and vitamin E content. Free fatty acid, triacylglycerol, phosphatidylcholine (PC) and

sterol esters were the dominant lipid class in the midgut gland. Triacylglycerol and phospholipids, mainly PC and phosphatidylethanolamine (PE), were the dominant ovarian lipid class. Neutral lipids prevailed over polar lipids in midgut gland lipids, while ovarian lipids displayed an inverse relationship. An increase in ovarian total lipids was observed from stage 0 (immature) to stage 1 (early maturing). In both midgut gland and ovaries, C16:0, C18:0, C16:1 *n*-7, C18:1 *n*-9, C20:4 *n*-6 (ARA), C20:5 *n*-3 (EPA) and C22:6 *n*-3 (DHA) were the principal FA. All tissues and nauplii displayed *n*-3 > *n*-6 and EPA > DHA relationships, and contained high proportions of *n*-3 highly unsaturated FA (*n*-3 HUFA) [55].

In general, the predominant fatty acids in mature ovaries in most Penaeid shrimp species are C16:0, C16:1 *n*-7, C18:0, C18:1 *n*-9, C20:4 *n*-6, C20:5 *n*-3 and C22:6 *n*-3. The ovarian lipids contain higher proportions of *n*-3 HUFA, particularly C20:5 *n*-3 and C22:6 *n*-3, than those of the hepatopancreas, for which it is believed that they play a crucial role in shrimp reproduction. The importance of *n*-3 HUFA has also been deduced from their presence in natural food organisms that are successful maturation diets, e.g. squid and bloodworm [56, 3]. Additionally, several studies have evaluated the effect of dietary *n*-3 HUFA levels on broodstock performance. Therefore, Xu et al. [57] postulate that C20:5 *n*-3 plays a specific role in the ovarian development process of *Penaeus chinensis* (Chinese white shrimp), whereas C22:6 *n*-3 may play some other role in early embryogenesis.

Recent broodstock nutrition studies have contributed further to a better knowledge of the requirements of lipids, vitamins and carotenoids for the maturation process in Penaeid shrimp. Biochemical studies have detected high levels of triacylglycerol, phospholipids and cholesterol in the ovaries of mature shrimp, eggs and nauplii. Furthermore, *n*-3 HUFA were shown to be the predominant fatty acids in the tissues of shrimp broodstock and its offspring, and their importance was confirmed through diet studies. While the fatty acid profile of natural food organisms is classically used to predict their suitability for shrimp broodstock nutrition, some studies demonstrated that other nutrients and hormonally active dietary substances do also play a crucial role in the stimulation of ovarian maturation. Biochemical and experimental work provided increasing evidence of the important role of natural anti-

oxidants for optimal shrimp maturation, reproduction and larval quality, namely carotenoids and vitamins E and C [1].

Phospholipids are predominant in shrimp ovaries, mainly phosphatidylcholine and phosphatidylethanolamine [58, 59, 60, 61]. Shrimp broodstock seem to have a dietary requirement for phospholipids. Bray et al. [62] improved nauplii production, hatching and spermatogenesis in *Penaeus stylirostris* broodstock by supplementing the diet with 1.5% soybean lecithin. Alava et al. [63] showed that ovarian maturation in *Marsupenaeus japonicus* was retarded when their diet did not contain phospholipids. The dietary phospholipid levels affected phospholipid concentration in *Penaeus vannamei* eggs in a study performed by Cahu et al. [64], who sustain that a broodstock diet should contain more than 2% phospholipids in order to assure that 50% of the total egg lipids is represented by phospholipids, and for maintaining high spawn frequency and fecundity.

Cholesterol is also an important lipid class in mature shrimp ovaries [56, 59]. Cholesterol must be provided through the diet for growth of shrimp juveniles [65], and is assumed to be an essential dietary lipid for shrimp maturation and reproduction [66]. The success of several fresh food items is attributed to their cholesterol content (e.g. squid, clam). Cholesterol is indeed known to fulfill several endocrinological functions, and its mobilization during maturation was reviewed by Harrison [67].

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

EXPERIMENTAL

3.1 Biological Materials

Farm-raised sandworm (*P. nuntia*, *P. aibuhitensis and P. quatrefagesi*) in different age of 2, 4, 6 and 8 months old was collected from the commercial sandworm farms in Chumporn, Chonburi, Rayong and Samutsakorn province, Thailand. Farmed sandworm was fed with a commercial shrimp diet that contained 38% protein and 5% lipid. Upon arrival, farmed sandworm was put on ice and frozen at -70 ^{o}C. Live wild-caught sandworm (*P. nuntia and P. quatrefagesi*) which collected in winter and summer season was collected from shore line of Chumporn, Chonburi and Rayong province, Thailand. Wild sandworm was put on ice and frozen at -70 ^{o}C until use. Both groups of sandworm were heated in hot air oven at 100 ± 5 ^{o}C until dried, cooled down in desiccators and weighed.

3.2 Chemicals and Reagents

Petroleum ether	Merck KGaA, Germany
Sodium hydroxide (NaOH)	Merck KGaA, Germany
Boron trifluoride-methanol (BF_3-MeOH)	Merck KGaA, Germany
Heptane	Merck KGaA, Germany
Sodium chloride (NaCl)	Merck KGaA, Germany
Standard Fatty Acid Methyl Ester	Sigma Chemicals Co., USA
Dichloromethane (CH_2Cl_2)	Fisher Scientific, UK

Methanol (MeOH)	Merck KGaA, Germany
Standard Cholesterol	Sigma Chemicals Co., USA
Standard Progesterone	Sigma Chemicals Co., USA
Standard Stigmasterol	Fluka Chemika, Switzerland
Acetone	Fisher Scientific, UK
N,O-Bis-(trimethylsilyl)trifluoro acetamide (BSTFA)	Fluka, Switzerland
Hexane	Fisher Scientific, UK
FL-PR Florisil	Phenomenex, USA
Isopropanol	Fisher Scientific, UK
Formic acid	Merck KGaA, Germany
Ammonia	Merck KGaA, Germany

3.3 Apparatus and Instruments

Freezer -70 ^{o}C	Sanyo Ultra Low, Japan
Gas chromatography	Agilent Technology 6890 N, USA
Hot air oven	Contherm, New Zealand
High performance liquid chromatography	Agilent Technology 1100, USA

Ion trap mass spectrometer	Bruker Daltonics, Germany
Micropipette	Pipetteman, Gilson, France
Refrigerator	Sanden Intercool, Thailand
Refrigerated centrifuge	Himac CR20B2, HITACHI, Japan
Vortex mixer	Vortex-genie 2, Sciencetific Industries
Water bath	Aquatherm, USA

3.4 Procedures

3.4.1 Fatty Acid

3.4.1.1 Extraction of Fat by Soxhlet Extraction [68]

Two grams of dried sandworms were placed in an extraction thimble and placed all in the middle part of soxhlet apparatus. The known accurate weight round bottom flask was filled with 200 mL of petroleum ether then placed as lower part. The condenser, upper part was connected to cooling water then three parts of apparatus were assembled. The round bottom flask was placed in water bath at 60 ^{o}C. The condensing vapor was filled the middle part and carried the dissolved fat into the flask by a siphoning. Sandworms were extracted for 10 hours, the apparatus was disconnected and evaporated solvent out from the flask, then put into hot air oven until constant weight. The % fat content was calculated as equation (1).

$$\% \text{ Fat} = \frac{[\text{g of flask and fat} - \text{g of flask}] \times 100}{\text{g of dry sandworms}} \quad \text{.....(1)}$$

3.4.1.2 Fatty Acid Methyl Ester [69]

Fat extract was derivatized to fatty acid methyl esters (FAMEs). A portion of extract (0.04 gram) was placed into a round bottom flask and added 4 mL of 0.5 N

NaOH in methanol and heated at 76 - 80 ^{o}C for 15 minutes. Then, 4 mL of boron trifluoride – methanol complex (20% solution in methanol, Assay BF_3) was added and heated for 3 minutes, followed by 1 mL of heptane and heated for 1 minute. After cooling, FAMEs were extracted by addition saturated NaCl into the flask and collected the upper phase (FAMEs in heptane) 200 μL to a screw cap tube and then 50 μL of the internal standard (IS)(heptadecanoic acid methyl ester; C17:0 methyl ester) was added for quantitative analysis.

3.4.1.3 GC Conditions

For identification, the FAMEs were analyzed by Gas chromatography (GC). FAMEs were determined on an Agilent Technologies 6890 N gas chromatography equipped with INNOWAX capillary column and a flame ionization detector (FID) system. The injector and detector temperatures were set at 150 and 250 ^{o}C, respectively. Helium was used as carrier gas. The flow rate of the carrier gas was 2.3 mL/min. The column was a 30 m x 0.3 mm id., 0.25 μm film thickness. GC chromatographic temperature program was set as follows: initial temperature was 150 ^{o}C, increase to 180 ^{o}C at 10 ^{o}C/min, increase to 200 ^{o}C at 5 ^{o}C/min, increase to 205 ^{o}C at 0.5 ^{o}C/min, with hold for 2 min, increase to 250 ^{o}C at 5 ^{o}C/min and then hold for 5 min (total runtime 33 min). Individual FAMEs were identified by comparison with known standards. The fatty acid compositions were calculated to mg per g of dry weight of sandworm as equation (2, 3, 4 and 5).

$$C_2 = \frac{C_1 \text{ x peak area of individual FAME}}{\text{peak area of IS}} \quad \text{.....(2)}$$

$$W_1 = \frac{C_2 \text{ x } 250\ \mu L}{1000\ \mu L} \quad \text{.....(3)}$$

$$W_2 = \frac{W_1 \text{ x } 1000\ \mu L}{200\ \mu L} \quad \text{.....(4)}$$

$$W_4 = \frac{W_2 \text{ x } W_3}{0.04\ g} \quad \text{.....(5)}$$

Where C_1 = mg/mL of internal standard (IS)

C_2 = mg/mL of FAME

W_1 = mg of FAME in 200 µL heptane

W_2 = mg of fatty acid in 0.04 g of fat

W_3 = g of fat per 1 g of dry sandworms

W_4 = mg of fatty acid per 1 g of dry sandworms

3.4.2 Phospholipids

3.4.2.1 Extraction of Phospholipids

One gram of dried sandworms was placed in a centrifuge tube. One mL of dichloromethane was added and vortex for 1 min. And 2 mL of methanol was added and vortex for 1 min (Bligh & Dyer method with modification (1959) [70]; 1 part of sample to 3 parts of 1:2 dichloromethane/methanol). Then 1 mL of dichloromethane was added and vortex for 1 min, following which 1 mL of distilled water was added and centrifuged 3,000 rpm for 10 min. After centrifuge, the system was separated into two layers and the lower phase (dichloromethane) was collected. Dichloromethane phase containing lipids were evaporated under a flow of nitrogen.

Prior to analysis, the extracted samples were reconstituted with 100 µL of dichloromethane/methanol (2:1, v/v) and further diluted with hexane/isopropanol (4:1, v/v), of which 5 µL was injected into HPLC-MS system.

3.4.2.2 HPLC Conditions

An HP 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used. The lipids were separated on a diol column, Nucleosil 100-OH (Macherey-Nagel, Germany), (250 mm x 3.0 mm id. x 5 µm particles size). The volume of sample injection was 5 µL. The linear solvent gradient was used according to the method described by Wang, C. et al. (2004) [71] with modification. Hexane/isopropanol (4:1, v/v) was used as mobile phase A, and mobile phase B was isopropanol/water/formic acid/ammonia (89.3:10:0.2:0.5, v/v/v/v). Separation was

obtained by using a gradient elution starting at 30% B, increasing to 60% in 22 min, then maintained for another 2 min. After that, mobile phase B was increased to 80% over the following 11 min and maintained at this level for additional 28 min. Finally, the solvent B was quickly decreased to 30% in 2 min and the column was re-equilibrated for about 10 min before next injection. The flow rate was 0.50 mL/min and the column temperature was 35 ^{o}C.

3.4.2.3 ESI-MS Conditions

The HPLC system was coupled on-line to Esquire HCT Ion trap mass spectrometer (Bruker Daltonics, GmbH, Germany) with an Electrospray Ionization source. The analytes eluted from HPLC column were directly entered the MS through a steel ESI needle set at 4.5 kV in the negative ion mode. The dry nitrogen gas flow rate was approximately 8.0 L/min at 300 ^{o}C. All ion source and ion optic parameters were optimized with respect to the negative ion of the phospholipids standard. The MS data were collected under full scan mode (500-1,000 m/z at a rate of five spectra for each time point). PE, Plasmalogen phosphatidylethanolamine, PI and PS were all analyzed in negative mode as $[M-H]^-$. PC, SM and Lyso phosphatidylcholine are zwitterionic molecules, they can be analyzed in both negative and positive mode. Thus, in this experiment, these phospholipids molecular species were well detected as the formate adduct, $[M+45]^-$.

3.4.2.4. Data Analysis

All chromatograms and spectra of phospholipids were analyzed by using Data analysis ^{TM} software version 3.2 (Bruker Daltonik GmbH, Germany). In this study, absolute phospholipids concentrations can not be shown due to the differences in the ionization efficiency and instrument response of the different head groups and also of the unsaturated degree of acyl chains in phospholipids structure [72].

3.4.3 Steroid

3.4.3.1 Extraction of Lipid by Soxhlet Extraction

Five grams of dried sandworms were placed in an extraction thimble and placed all in the middle part of soxhlet apparatus. The known accurate weight round bottom flask was filled with 200 mL of a mixture of dichloromethane/methanol (2:1, v/v) then placed as lower part. The condenser, upper part was connected to cooling water then three parts of apparatus were assembled. The round bottom flask was placed in water bath. The condensing vapor was filled the middle part and carried lipids into the flask by a siphoning. Sandworms were extracted at approximately 5-6 cycles per hour for 8 hour, the apparatus was disconnected and evaporated solvent out from the flask until constant weight.

3.4.3.2 Silyl Derivatization and Cleanup [73]

Lipid extract was derivatized with silyl derivatization. A portion of extract (0.1 gram) was transferred to 15 mL glass vial. Two mL of acetone was added and then silyl derivatization reaction was performed with 250 μL of BSTFA at room temperature. The derivatizing reagent and acetone were evaporated under gentle flow of dry nitrogen and 2.5 mL of hexane was added to the vial. The concentrated extract was subjected to a column florisil for cleanup and eluted with 12 mL of hexane. The eluent was concentrated to 1 mL with gentle flow of dry nitrogen.

3.4.3.3 GC Conditions

Steroids in the lipid extract were analyzed by Gas chromatographic (GC). The steroids were determined on an Agilent Technologies 6890 N gas chromatography equipped with HP-5 column and a flame ionization detector (FID) system. The injector and detector temperatures were set at 290 and 250 ^{o}C, respectively. Helium was used as carrier gas. The flow rate of the carrier gas was 2.3 mL/min. The HP-5 capillary column was 30 m x 0.25 mm id., 0.25 μm film thickness. GC chromatographic temperature program was set as follows: initial temperature was 80 ^{o}C hold for 1 min, increase to 200 ^{o}C at 30 ^{o}C/min, increase to 205 ^{o}C at 0.5

^{o}C/min, increase to 260 ^{o}C at 30 ^{o}C/min, increase 270 ^{o}C at 4 ^{o}C/min, increase to 320 ^{o}C at 20 ^{o}C/min and then hold for 10 min (total runtime 31.83 min). Individual steroids were identified by comparison with known standards.

3.5 Statistic Analysis

Results were expressed as % of fat and mg per g of dry weight ± SD of sandworms for fatty acid composition and mg per 100 g wet weight of sandworms for cholesterol. Analysis of variance (one-way ANOVA) in SPSS for window was used to determine statistical differences between different ages of farm-raised and different seasons of wild-caught sandworms. The Duncan test was performed for multiple comparisons. All references to significant differences are at the 5% level or lower ($p \leq 0.05$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

RESULTS AND DISCUSSION

The percentage of fat, fatty acid profiles and lipid compositions of farm-raised sandworm and wild-caught sandworm (*Perinereis* sp.) were determined in this study. Farm-raised sandworm (*P. nuntia*, *P. aibuhitensis and P. quatrefagesi*) in different age was collected from the commercial sandworm farms in Chumporn, Chonburi, Rayong and Samutsakorn province, Thailand (Table 4.1). Live wild-caught sandworm (*P. nuntia and P. quatrefagesi*) which caught in winter and summer season was collected from shore line of Chumporn, Chonburi and Rayong province, Thailand (Table 4.2). The fatty acid profiles, phospholipids and steroids of sandworm (*Perinereis* sp.) were analyzed using methods as described in the chapter III. The results have been shown and discussed in each part of this chapter. Approximate size of *P. nuntia* sandworm at aged 2, 4, 6 and 8 months has shown in Figure 4.1.

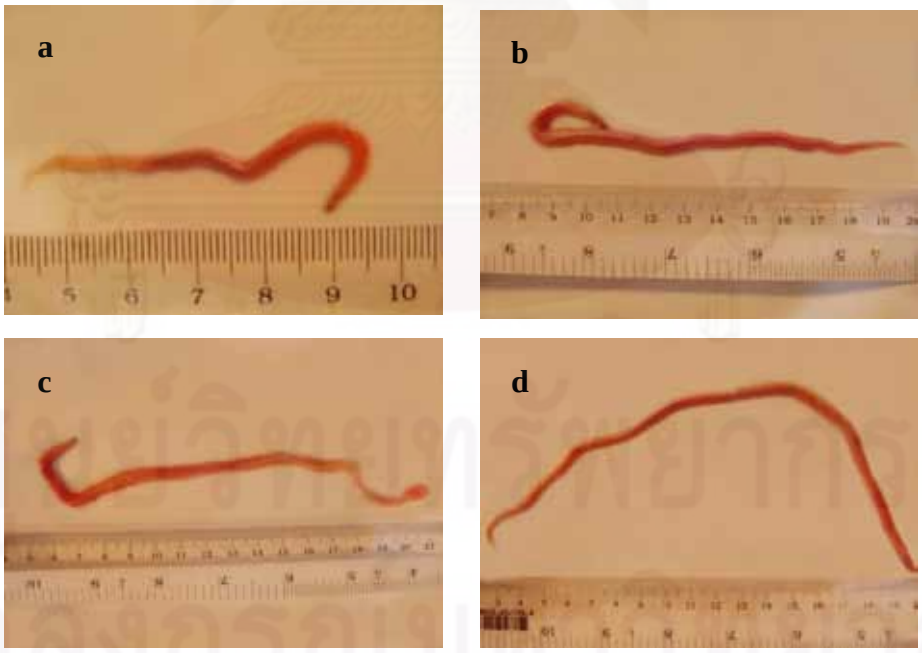

Figure 4.1 The *P. nuntia* farm-raised sandworm from Chumporm province, Thailand (a) aged 2 months, (b) aged 4 months, (c) aged 6 months and (d) aged 8 months

Table 4.1 Lists of commercial sandworm farms

Age	Commercial sandworm farms		
	P. nuntia	***P. quatrefagesi***	***P. aibuhitensis***
2m	Thai-Union Farm Chonburi province, Thailand December, 2007		
	Pankavee Farm Chumporn province, Thailand March, 2009		
4m	Ton-Aquatic Farm Samutsakorn province, Thailand July,2007		
	Pankavee Farm Chumporn province, Thailand July, 2008		
	Suchart Farm Chonburi province, Thailand September, 2008		
	Pankavee Farm Chumporn province, Thailand March, 2009		
6m	CP Farm Rayong province, Thailand January, 2008	Pankavee Farm Chumporn province, Thailand November, 2007	Pankavee Farm Chumporn province, Thailand December, 2007
	Pankavee Farm Chumporn province, Thailand April, 2008	Pankavee Farm Chumporn province, Thailand December, 2007	Pankavee Farm Chumporn province, Thailand April, 2008
	Pankavee Farm Chumporn province, Thailand July, 2008		
8m	Thai-Unian Farm Chonburi province, Thailand May, 2008		
	Pankavee Farm Chumporn province, Thailand July, 2008		

Table 4.2 Lists of shore line which caught sandworm for this resarch

Season	**Shore line**		
	P. nuntia	***P. quatrefagesi***	***P. aibuhitensis***
Summer	Chonburi province, Thailand June, 2008		
	Rayong province, Thailand June, 2008		
Winter	Chonburi province, Thailand December, 2007	Chumporn province, Thailand November, 2007	
	Rayong province, Thailand January, 2008		

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.1 Fatty Acid

4.1.1 Extraction of Fat by Soxhlet Extraction

A fat of farm-raised and wild-caught sandworms (*P. nuntia*, *P. aibuhitensis* and *P. quatrefagesi*) were extracted from dried sandworms by soxhlet extraction with petroleum ether (descripted in 3.4.1.1). The percentage of fat content in different age 2, 4, 6 and 8 months of farm-raised sandworm is summarized in Table 4.3 and that in summer and winter of wild-caught sandworm is summarized in Table 4.4.

Table 4.3 Comparison of percentage of fat in different age 2, 4, 6 and 8 months of farm-raised sandworms (*Perinereis* sp.)

Farm-raised (age, months)	**% of Fat**		
	P. nuntia	***P. aibuhitensis***	***P. quatrefagesi***
2	17.27 ± 0.98^{b}	-	-
4	17.35 ± 2.08^{b}	-	-
6	17.42 ± 1.75^{b}	18.60 ± 1.37^{b}	15.82 ± 0.72^{a}
8	20.96 ± 0.73^{c}	-	-
average	18.27 ± 2.14	-	-

- means not determined.

Data represent mean values. Within a column and a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.4 Comparison of percentage of fat in summer and winter of wild-caught sandworms (*Perinereis* sp.)

Wild-caught (season)	**% of Fat**		
	P. nuntia	***P. aibuhitensis***	***P. quatrefagesi***
winter	13.81 ± 1.89^{a}	-	16.98 ± 0.35^{b}
summer	12.97 ± 3.87^{a}	-	-
average	13.43 ± 2.83	-	-

- means not determined.

Data represent mean values. Within a column and a row, values with different superscript letters are significantly different ($P \leq 0.05$).

The fat content from fat extraction of farm-raised sandworm *P. nuntia* (average 18.27±2.14%) was higher than that of the wild-caught sandworm (average 13.43±2.83%). The percentage of fat content of farm-raised *P. nuntia* aged 8 months (20.96±0.73%), which were in the beginning stage of reproductive cell synthesis was higher than that of aged 2, 4 and 6 months (about 17.35±0.08%). The percentage of fat content of farm-raised *P. aibuhitensis* aged 6 months (18.60±1.37%) was slightly higher than that of *P. nuntia* and *P. quatrefagesi*. While that of wild-caught *P. quatrefagesi* (16.98±0.35%) was higher than that of *P. nuntia*. The difference of fat contents of farm-raised and wild-caught sandworms was resulted from diet and environment as other marine organisms [74].

4.1.2 Identification of Fatty Acid Methyl Ester by Gas Chromatography

The FAMEs were separated and quantified using Gas chromatographic (GC). Chromatogram of farm-raised sandworm (*P. nuntia*) aged 6 months from Chumporn province, Thailand is shown in Figure 4.2 and that of wild-caught sandworm (*P. nuntia*) was caught in winter season from Chonburi province, Thailand is shown in Figure 4.3. The various fatty acids of sandworms were identified by comparing retention time of each peaks with reference standards and internal standard (heptadecanoic aicd methyl ester; C17:0 methyl ester) was added for quantitative analyze. The total and individual fatty acid profiles of farm-raised and wild-caught sandworms of *P. nuntia* are presented in Table 4.5 and 4.6, respectively. While those of *P. aibuhitensis* aged 6 months of farm-raised and *P. quatrefagesi* aged 6 months and wild-caught are shown in Table 4.7. The fatty acid analyses were grouped as saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). For *P. nuntia*, the major SFA of both farm-raised and wild-caught sandworms was C16:0 (palmitic acid), but the major MUFA and PUFA of farm-raised differed from those of wild-caught sandworm. C18:1 *n-9* (oleic acid) and C18:2 *n-6* (linoleic acid) were respectively the major of MUFA and PUFA of farm-raised sandworm, as C18:1 *n-7* (vaccenic acid) was a major MUFA of wild-caught sandworm. Comparable data were reported for *Nereis diversicolor,* the major SFA, MUFA and PUFA were C16:0, C18:1, C18:2 and C20:5 [11]. The results indicated that the most abundant fatty acid profiles of farm-raised sandworm were C16:0, C18:1

n-9 and C18:2 *n*-6, while the profiles of wild-caught sandworm were C16:0, C18:1 *n*-7 and C18:0. The high content of C18:1 *n*-9 and C18:2 *n*-6 of farm-raised sandworm may come from diet for farmed animal (Table B1) that have been improved with vegetable oil containing C18:1 *n*-9 and C18:2 *n*-6. The total of MUFA and PUFA of farm-raised sandworm were higher compared with those of wild-caught sandworm.

For *P. aibuhitensis* and *P. quatrefagesi*, the major SFA, MUFA and PUFA of farm-raised aged 6 months of both species and those of wild-caught was caught in winter of *P. quatrefagesi* were similar to those of *P. nuntia* (C16:0, C18:1 *n*-9 and C18:2 *n*-6, respectively). The most abundant fatty acid profiles from both species of farm-raised sandworm were C16:0, C18:1 *n*-9 and C18:2 *n*-6.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

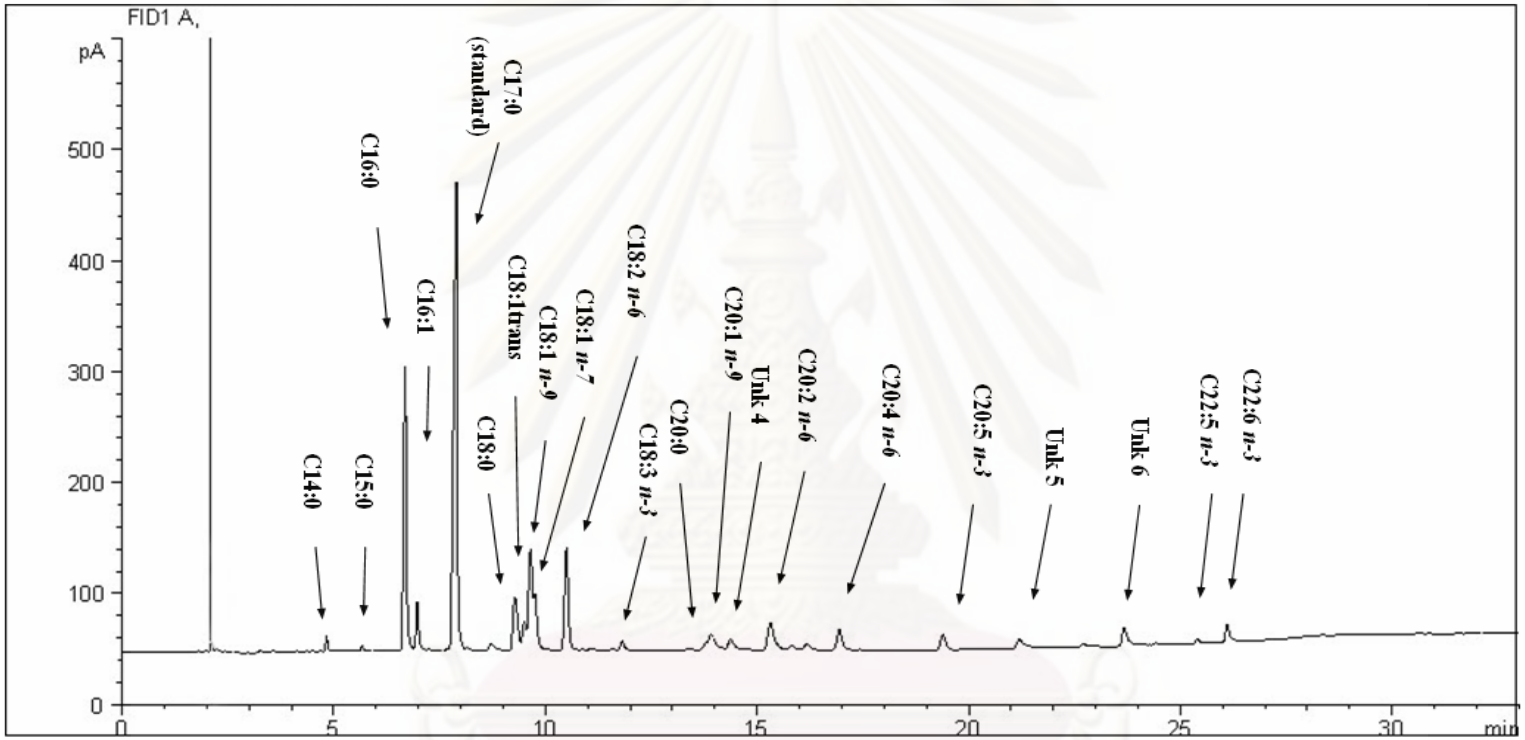

Figure 4.2 GC chromatogram of fatty acid methyl ester of farm-raised sandworm (*P. nuntia*) aged 6 months from Chumporn province, Thailand (C17:0 standard was added for quantitative analyze.)

46

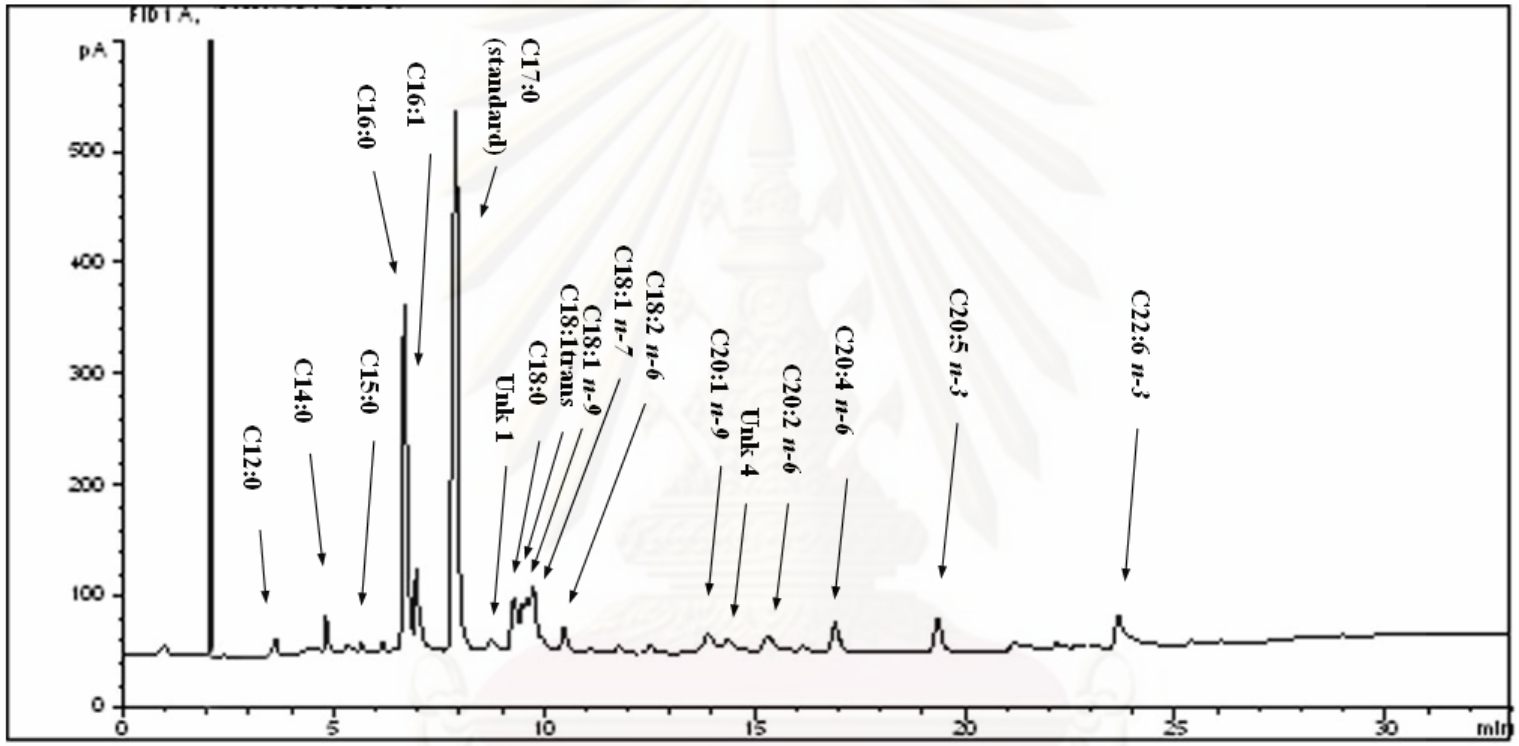

Figure 4.3 GC chromatogram of fatty acid methyl ester of wild-caught sandworm (*P. nuntia*) caught in winter season from Chonburi province, Thailand (C17:0 standard was added for quantitative analyze.)

Table 4.5 Comparison of total and individual FAME profiles (mg per g of dry weight) of farm-raised sandworm in different age 2, 4, 6 and 8 months (*P. nuntia*)

Fatty acid	Fatty acid (mg/g of dry weight)			
	2 m	**4 m**	**6 m**	**8 m**
Saturated				
12:0	0.07 ± 0.00	0.08 ± 0.08	0.05 ± 0.00	0.12 ± 0.02
14:0	0.98 ± 0.06	1.10 ± 0.13	1.12 ± 0.07	1.41 ± 0.09
15:0	0.00^{a}	0.46 ± 0.05^{b}	0.49 ± 0.05^{b}	0.58 ± 0.02^{b}
16:0	30.57 ± 1.73^{a}	35.16 ± 4.64^{a}	35.62 ± 4.04^{a}	44.12 ± 1.22^{b}
18:0	9.06 ± 0.51^{a}	10.51 ± 1.40^{ab}	10.09 ± 0.79^{ab}	11.68 ± 0.31^{b}
Total SFA	**40.69^{a}**	**47.31^{b}**	**47.38^{b}**	**57.92^{c}**
Monounsaturated				
16:1 *n*-7	5.30 ± 0.30^{a}	5.85 ± 0.74^{a}	5.28 ± 0.77^{a}	6.70 ± 0.59^{b}
18:1 *trans*	3.15 ± 0.18^{a}	4.77 ± 0.55^{b}	4.72 ± 1.10^{b}	5.40 ± 0.43^{c}
18:1 *n*-9	24.12 ± 1.37^{b}	13.09 ± 1.51^{a}	14.26 ± 1.89^{a}	22.50 ± 2.42^{b}
18:1 *n*-7	0.00^{a}	8.25 ± 1.14^{b}	7.78 ± 0.91^{b}	10.10 ± 0.83^{b}
20:1 *n*-9	0.00^{a}	5.68 ± 0.74^{b}	5.25 ± 0.59^{b}	5.45 ± 0.50^{b}
Total MUFA	**32.57^{a}**	**37.65^{a}**	**37.29^{a}**	**50.14^{b}**
Polyunsaturated				
18:2 *n*-6	17.28 ± 0.98^{b}	14.76 ± 1.79^{a}	14.23 ± 0.88^{a}	19.44 ± 2.15^{b}
20:2 *n*-6	3.45 ± 0.20^{a}	7.35 ± 0.86^{b}	7.31 ± 0.93^{b}	9.21 ± 0.80^{b}
20:4 *n*-6	4.12 ± 0.23^{a}	4.58 ± 1.10^{a}	4.54 ± 0.53^{a}	6.30 ± 0.30^{b}
18:3 *n*-3	1.31 ± 0.07	1.27 ± 0.16	1.30 ± 0.08	1.76 ± 0.11
20:5 *n*-3	3.24 ± 0.18^{a}	4.05 ± 0.53^{ab}	3.56 ± 0.42^{a}	4.85 ± 0.56^{b}
22:5 *n*-3	0.00^{a}	0.86 ± 0.11^{b}	0.85 ± 0.07^{b}	0.00^{a}
22:6 *n*-3	1.99 ± 0.11^{a}	2.88 ± 0.37^{ab}	2.77 ± 0.25^{ab}	3.89 ± 0.12^{b}
Total PUFA	**31.40^{a}**	**35.76^{a}**	**34.55^{a}**	**45.46^{b}**

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.6 Comparison of total and individual fatty acid profiles (mg per g of dry weight) of wild-caught sandworms in summer and winter (*P. nuntia*)

Fatty acid	**Fatty acid (mg/g of dry weight)**	
	summer	**winter**
Saturated		
12:0	0.00^{a}	0.58 ± 0.57^{b}
14:0	1.41 ± 0.51	1.62 ± 0.19
15:0	0.70 ± 0.18	0.54 ± 0.06
16:0	37.75 ± 7.65^{b}	27.96 ± 3.15^{a}
18:0	7.31 ± 1.96	6.72 ± 1.07
Total SFA	**47.18^{b}**	**37.41^{a}**
Monounsaturated		
16:1 *n*-7	5.85 ± 1.33	5.58 ± 0.86
18:1 *trans*	4.14 ± 0.92	3.20 ± 0.61
18:1 *n*-9	6.51 ± 2.68	5.55 ± 1.61
18:1 *n*-7	8.86 ± 2.35	7.57 ± 0.79
20:1 *n*-9	0.00^{a}	2.83 ± 1.55^{b}
Total MUFA	**25.36**	**24.72**
Polyunsaturated		
18:2 *n*-6	4.10 ± 1.55	3.78 ± 1.52
20:2 *n*-6	0.00^{a}	2.67 ± 1.46^{b}
20:4 *n*-6	4.79 ± 0.16^{b}	3.92 ± 0.24^{a}
18:3 *n*-3	0.00	0.00
20:5 *n*-3	2.77 ± 0.16^{a}	4.30 ± 0.28^{b}
22:5 *n*-3	0.00	0.00
22:6 *n*-3	0.00^{a}	1.76 ± 0.19^{b}
Total PUFA	**11.66^{a}**	**16.42^{b}**

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.7 Comparison of total and individual fatty acid profiles (mg per g of dry weight) of farm-raised (aged 6 months) in different species (*P. aibuhitensis* and *P. quatrefagesi*) and wild-caught (which caught in winter) of *P. quatrefagesi*

Fatty acid	**Fatty acid (mg/g of dry weight)**		
	age 6 m		**winter**
	P. quatrefagesi	***P. aibuhitensis***	***P. quatrefagesi***
Saturated			
12:0	0.00^{a}	0.10 ± 0.00^{b}	0.00^{a}
14:0	1.12 ± 0.25	1.20 ± 0.08	1.01 ± 0.02
15:0	0.43 ± 0.11	0.52 ± 0.07	0.56 ± 0.01
16:0	32.89 ± 0.73^{a}	40.95 ± 2.67^{ab}	44.49 ± 0.90^{b}
18:0	8.23 ± 0.18^{a}	11.91 ± 0.77^{b}	10.29 ± 0.21^{b}
Total SFA	**42.67^{a}**	**54.67^{b}**	**56.35^{b}**
Monounsaturated			
16:1 *n*-7	5.21 ± 0.17	5.63 ± 0.99	5.35 ± 0.11
18:1 *trans*	3.06 ± 0.18^{a}	4.69 ± 0.68^{b}	5.90 ± 0.12^{c}
18:1 *n*-9	20.64 ± 1.42^{b}	26.48 ± 1.82^{b}	12.25 ± 0.25^{a}
18:1 *n*-7	0.00^{a}	0.00^{a}	10.17 ± 0.21^{b}
20:1 *n*-9	2.82 ± 0.17^{a}	5.06 ± 1.18^{b}	5.92 ± 0.12^{b}
Total MUFA	**31.74^{a}**	**41.87^{b}**	**39.59^{b}**
Polyunsaturated			
18:2 *n*-6	13.57 ± 1.40^{a}	16.85 ± 1.43^{b}	11.44 ± 0.23^{a}
20:2 *n*-6	6.39 ± 0.35^{a}	8.12 ± 1.30^{ab}	9.39 ± 0.19^{b}
20:4 *n*-6	4.03 ± 0.22^{a}	6.35 ± 1.27^{b}	6.39 ± 0.13^{b}
18:3 *n*-3	1.29 ± 0.21^{b}	1.47 ± 0.16^{b}	0.00^{a}
20:5 *n*-3	3.95 ± 0.12^{a}	4.88 ± 0.59^{b}	4.22 ± 0.09^{ab}
22:5 *n*-3	0.00	0.00	0.00
22:6 *n*-3	3.77 ± 0.14^{b}	3.45 ± 0.39^{b}	2.12 ± 0.04^{a}
Total PUFA	**33.00^{a}**	**41.13^{b}**	**33.57^{a}**

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Fatty acids are an important factor to consider when providing a maturation diet for shrimp, for example fatty acid profile in life feed especially in polychaete such as bloodworms and sandworms have been used successfully as a feed source to induce maturation in peneaid shrimp [1, 3]. The fatty acid class and ratio of fatty acid compositions in different age 2, 4, 6 and 8 months of farm-raised and wild-caught sandworms (*P. nuntia*) which caught in summer and winter are listed in Table 4.8 and 4.9, respectively. The results indicated that the total fatty acid of farm-raised sandworm aged 8 months was significantly higher than that in difference age. While the total fatty acid of farm-raised was higher than that of wild-caught sandworm. The ratio of SFA : MUFA : PUFA of all of farm-raised were about 1.3 : 1.0 : 1.0 but the ratio of those of wild-caught sandworm was caught summer and winter season were 4.0 : 2.2 : 1.0 and 2.3 : 1.5 : 1.0, respectively. The ratio of ARA : EPA : DHA of farm-raised was about 1.6 : 1.3 : 1.0 to stand in age 4, 6 and 8 months, while ratio of those of wild-caught was caught summer was 1.7 : 1.0 : 0.0 and that in winter was 0.9 : 1.0 : 0.4. The ratios of those of wild-caught were different because of less amount of DHA. In the results, the ratio of ARA : EPA of wild-caught sandworm which caught in summer (1.73) was higher than that of sandworm which caught in winter (0.91). Same as in marine fish, JIRCAS Annual report 2005 [75] reported the ratio of ARA : EPA of tropical marine fish species was higher than that in cold water fish species. While ratio of *n*-3 : *n*-6 fatty acid in difference age of farm-raised sandworm were not different (1.0 : 3.0, to stand in age 4, 6 and 8 months) but of wild-caught, the *n*-3 : *n*-6 fatty acid in the summer (1.0 : 3.2) was less than those caught during winter season (1.0 : 1.7). The ratio of LC : VLC fatty acid in difference age of farm-raised were not significantly different in age 4, 6 and 8 months (about 2.9 : 1.0) as of wild-caught were significantly different. From these results, the fatty acid profiles and ratio of fatty acid composition of farm-raised sandworm were not different (to stand) in aged 4, 6 and 8 months, while those of wild-caught sandworm which caught in summer and winter were different. The different of those may arise from diets, environmental temperature and habitat of worms. Cowey and Sargent (1972) [76] reported the fatty acid composition of marine animals is influenced by several factors including modes of feeding, gametogenesis and probably also environmental temperature.

Table 4.8 Fatty acid class composition (mg per g of dry weight) and ratio of fatty acid compositions in different age 2, 4, 6 and 8 months of farm-raised sandworm (*P. nuntia*)

Fatty acids	**Farm-raised**			
	2 m	**4 m**	**6 m**	**8 m**
Total FA	106.81[a]	130.68[b]	128.76[b]	158.32[c]
Total SFA	40.69[a]	47.31[b]	47.38[b]	57.92[c]
Total MUFA	32.57[a]	37.65[a]	37.29[a]	50.14[b]
Total PUFA	31.40[a]	35.76[a]	34.55[a]	45.46[b]
SFA:MUFA:PUFA	1.3 :1.0 :1.0	1.3 :1.1 :1.0	1.4 :1.1 :1.0	1.3 :1.1 :1.0
ARA	4.12 ± 0.23[a]	4.58 ± 1.10[a]	4.54 ± 0.53[a]	6.30 ± 0.30[b]
EPA	3.24 ± 0.18[a]	4.05 ± 0.53[ab]	3.56 ± 0.42[a]	4.85 ± 0.56[b]
DHA	1.99 ± 0.11[a]	2.88 ± 0.37[ab]	2.77 ± 0.25[ab]	3.89 ± 0.12[b]
ARA : EPA : DHA	2.1 :1.6 :1.0	1.6 :1.4 :1.0	1.6 :1.3 :1.0	1.6 :1.2 :1.0
*n*3	6.54[a]	9.07[b]	8.48[b]	10.51[b]
*n*6	24.85[a]	26.69[a]	26.08[a]	34.95[b]
n-3 : *n*-6	1.0 : 3.8	1.0 : 2.9	1.0 : 3.1	1.0 : 3.3
LC-FA	90.80[a]	93.67[a]	93.87[a]	111.08[b]
VLC-FA	14.96[a]	33.28[b]	32.65[b]	37.81[b]
LC : VLC	6.1 :1.0	2.8 :1.0	2.9 :1.0	2.9 :1.0

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 4.9 Fatty acid class composition (mg per g of dry weight) and ratio of fatty acid compositions in summer and winter of wild-caught sandworm (*P. nuntia*)

Fatty acids	Wild-caught	
	summer	winter
Total FA	107.18[b]	80.10[a]
Total SFA	47.18[b]	37.41[a]
Total MUFA	25.36	24.72
Total PUFA	11.66	16.42
SFA:MUFA:PUFA	4.0 : 2.2 :1.0	2.3 :1.5 :1.0
ARA	4.79 ± 0.16[b]	3.92 ± 0.24[a]
EPA	2.77 ± 0.16[a]	4.30 ± 0.28[b]
DHA	0.00[a]	1.76 ± 0.19[b]
ARA : EPA : DHA	1.7 :1.0 :0.0	0.9 :1.0 :0.4
n-3	2.77[a]	6.06[b]
n-6	8.89[a]	10.37[b]
n-3 : *n*-6	1.0 : 3.2	1.0 : 1.7
LC-FA	74.53[b]	60.35[a]
VLC-FA	9.60[a]	16.26[b]
LC : VLC	7.8 :1.0	3.7 :1.0

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

The fatty acid class and ratio of fatty acid compositions of farm-raised (aged 6 months) of *P. quatrefagesi*, *P. aibuhitensis* and *P. nuntia* are summarized in Table 4.10. In addition, those of farm-raised (aged 6 months) and wild-caught (which caught in winter) of *P. quatrefagesi* are summarized in Table 4.11. The results indicated that the total fatty acid in aged 6 months of *P. aibuhitensis* was significantly higher than that of *P. nuntia* and *P. quatrefagesi*. While total fatty acid of wild-caught of *P. quatrefagesi* which caught in winter was significantly higher than that of farm-raised. The ratio of SFA : MUFA : PUFA and *n-3* : *n-6* fatty acid of both farm-raised *P. quatrefagesi* and *P. aibuhitensis* were similar to those of *P. nuntia* were about 1.3 : 1.0 : 1.0 and about 1.0 : 3.0, respectively. The ratio of ARA : EPA : DHA of farm-raise of *P. quatrefagesi* and *P. aibuhitensis* were 1.1 : 1.0 : 1.0 and 1.8 : 1.4 : 1.0,

respectively. The ratio of LC : VLC fatty acid of both species of farm-raised were not significantly different (about 3.5 : 1.0) and significantly higher than that of *P. nuntia* (2.9 : 1.0).

For *P. quatrefagesi*, the ratio of SFA : MUFA : PUFA, ARA : EPA : DHA , *n*-3 : *n*-6 and LC : VLC of wild-caught (1.7 : 1.2 : 1.0, 3.0 : 2.0 : 1.0, 1.0 : 4.3 and 2.8 : 1.0, respectively) were different from farm-raised sandworm and *P. nuntia* wild-caught sandworm which caught winter.

Table 4.10 Fatty acid class composition (mg per g of dry weight) and ratio of fatty acid compositions in different species (*P. quatrefagesi*, *P. aibuhitensis* and *P. nuntia*) of farm-raised sandworms (aged 6 months)

Fatty acids	**Farm-raised sandworm age 6 months**		
	P. quatrefagesi	***P. aibuhitensis***	***P. nuntia***
Total FA	110.08[a]	144.91[c]	128.76[b]
Total SFA	42.67[a]	54.67[b]	47.38[a]
Total MUFA	31.74[a]	41.87[b]	37.29[ab]
Total PUFA	33.00[a]	41.13[b]	34.55[a]
SFA:MUFA:PUFA	1.3 :1.0 :1.0	1.3 :1.0 :1.0	1.4 :1.1 :1.0
ARA	4.03 ± 0.22[a]	6.35 ± 1.27[b]	4.54 ± 0.53[a]
EPA	3.95 ± 0.12[a]	4.88± 0.59[b]	3.56 ± 0.42[a]
DHA	3.77 ± 0.14[b]	3.45 ± 0.39[b]	2.77 ± 0.25[a]
ARA : EPA : DHA	1.1 :1.0 :1.0	1.8 :1.4 :1.0	1.6 :1.3 :1.0
n-3	9.01[b]	9.80[b]	8.48[a]
n-6	23.99[a]	31.33[b]	26.08[ab]
n-3 : *n*-6	1.0 : 2.7	1.0 : 3.2	1.0 : 3.1
LC-FA	84.89[a]	107.99[b]	93.87[a]
VLC-FA	23.63[a]	32.20[b]	32.65[b]
LC : VLC	3.6 :1.0	3.4 :1.0	2.9 :1.0

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.11 Fatty acid class composition (mg per g of dry weight) and ratio of fatty acid compositions in farm-raised and wild-caught sandworms (*P. quatrefagesi*)

Fatty acids	**Farm-raised age 6 months**	**Wild-caught winter**
Total FA	110.08[a]	140.43[b]
Total SFA	42.67[a]	56.35[b]
Total MUFA	31.74[a]	39.59[b]
Total PUFA	33.00	33.57
SFA:MUFA:PUFA	1.3 :1.0 :1.0	1.7 :1.2 :1.0
ARA	4.03 ± 0.22[a]	6.39 ± 0.13[b]
EPA	3.95 ± 0.12	4.22 ± 0.09
DHA	3.77 ± 0.14[b]	2.12 ± 0.04[a]
ARA : EPA : DHA	1.1 :1.0 :1.0	3.0 :2.0 :1.0
n-3	9.01[b]	6.34[a]
n-6	23.99	27.23
n-3 : *n*-6	1.0 : 2.7	1.0 : 4.3
LC-FA	84.89[a]	102.44[b]
VLC-FA	23.63[a]	36.41[b]
LC : VLC	3.6 :1.0	2.8 :1.0

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

ศูนย์วิทยทรัพยากร
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4.2 Phospholipids

The HPLC chromatogram of phospholipids from *P. nuntia* farm-raised sandworm aged 6 months, which was extracted by modified method of Bligh and Dyer (1959) [70], was shown in Figure 4.4. The major component of the lipid of sandworms was phospholipids. Lee et al. (2005) [77] was suggest that in polychaete *Neanthes arenaceodentata*, the lipid component included phospholipids (92 % of lipid), triacylglycerol (3 % of lipid) and cholesterol (3 % of lipid). HPLC was used for identified characteristic of phospholipids, phosphatidylcholine (PC) was the major phospholipid classes of all groups of sandworms. Same as in *Perinereis cultrifera* oocyte, most of the associated phospholipids of that were PC (77 % of phospholipid), X (non identified phospholipids, 13.3 % of phospholipids) and LPC (lysophosphatidylcholine, 4.2 % of phospholipids) [78]. The kinds of fatty acid within PC was identified by ESI-MS. The MS spectrum of PC from *P. nuntia* farm-raised sandworm aged 6 months is shown in Figure 4.5. The percentage of each species of PC in different age 2, 4, 6 and 8 months of farm-raised sandworm (*P. nuntia*) is summarized in Table 4.12 and that in summer and winter of the wild-caught is summarized in Table 4.13. While those in aged 6 months of farm-raised *P. aibuhitensis* and *P. quatrefagesi* and wild-caught *P. quatrefagesi* was caught in winter are also shown in Table 4.14. Most, second and third PC species of each farm-raised and wild-caught sandworms were concluded in Table 4.15. The results indicated that the most abundant of PC species in farm-raised sandworm of all species were PC 16:0/18:1, PC 16:0/18:2 and PC 18:0/18:2. Those of all of wild-caught *P. nuntia* were PC 16:0/18:1, PC 16:0/20:4 and PC 18:0/20:4, while those of *P. quatrefagesi* were PC 16:0/18:1, PC 16:0/20:4 and PC 16:0/18:2.

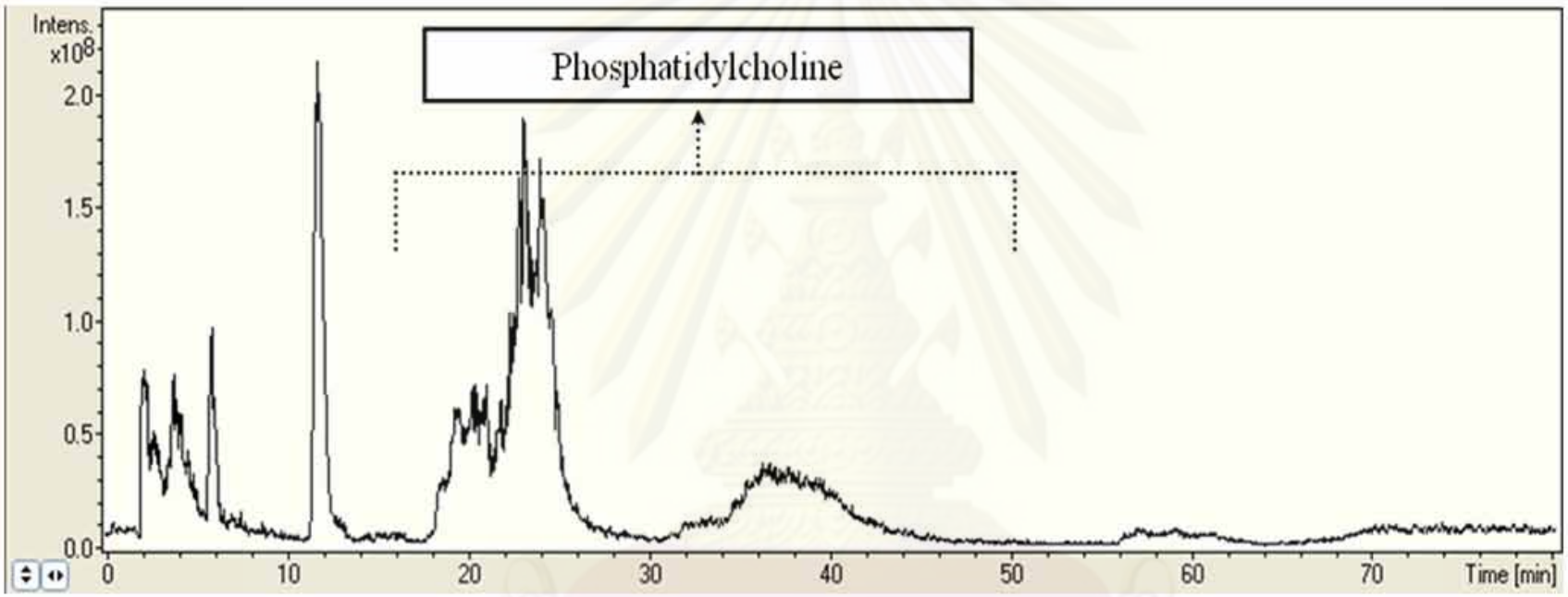

Figure 4.4 HPLC chromatogram of phospholipids extraction from *P. nuntia* farm-raised sandworm aged 6 months by modified method of Bligh and Dyer (1959)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

57

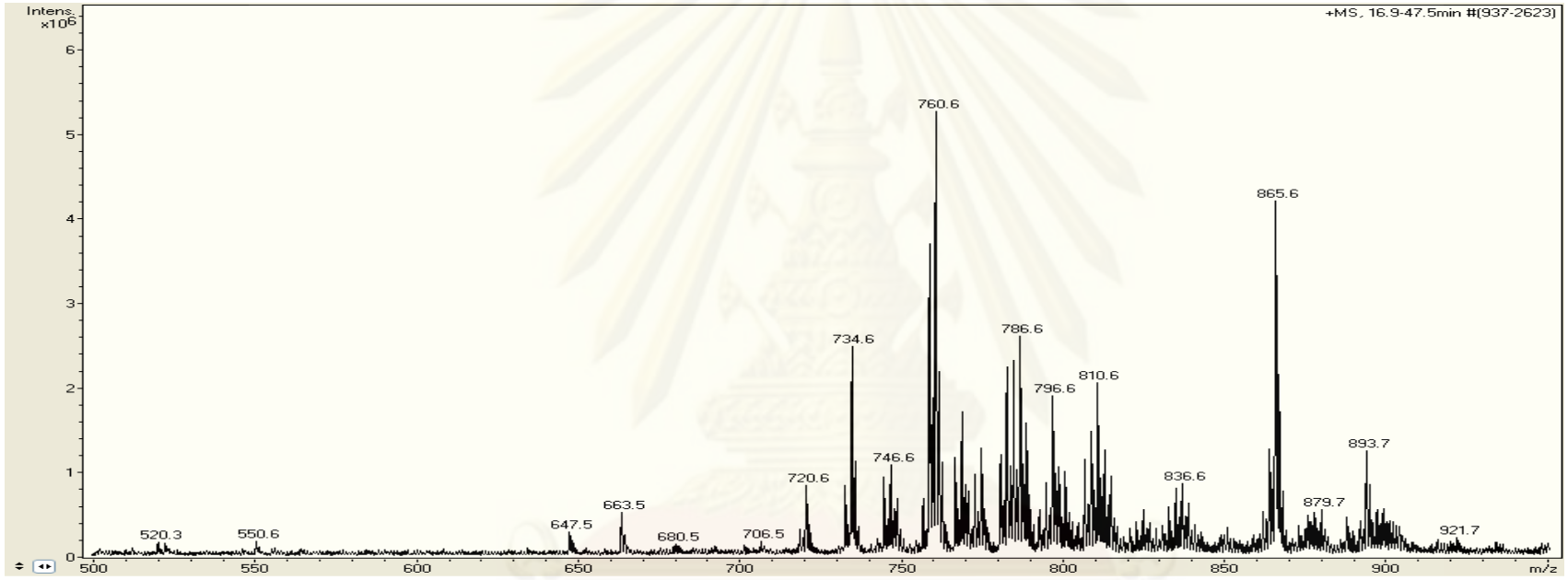

Figure 4.5 MS spectrum of phospholipids from *P. nuntia* farm-raised sandworm aged 6 months

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

58

Table 4.12 The percentage of each species of PC in different age 2, 4, 6 and 8 months of farm-raised sandworm (*P. nuntia*)

m/z	PC species	Combine species	% of PC			
			2 m	4 m	6 m	8 m
706	14:0/16:0	12:0/18:0	1.83 ± 0.00^{c}	1.24 ± 0.00^{b}	0.61 ± 0.15^{a}	0.68 ± 0.22^{a}
730	14:0/18:2	-	1.96 ± 0.00^{b}	1.51 ± 0.00^{b}	0.48 ± 0.18^{a}	0.58 ± 0.11^{a}
732	16:0/16:1	14:0/18:1	2.52 ± 0.10	2.70 ± 0.00	2.42 ± 0.02	2.39 ± 0.27
734	16:0/16:0	14:0/18:0	5.40 ± 1.97^{a}	7.57 ± 1.20^{b}	7.47 ± 2.39^{b}	6.03 ± 2.49^{ab}
754	14:0/20:4	16:1/18:3	2.21 ± 0.20^{c}	1.74 ± 0.10^{b}	0.75 ± 0.25^{a}	0.80 ± 0.11^{a}
756	16:1/18:2	16:0/18:3	2.19 ± 0.29^{b}	1.44 ± 0.17^{a}	2.03 ± 0.04^{b}	1.97 ± 0.47^{ab}
758	16:0/18:2	14:0/20:2	6.45 ± 0.82^{a}	11.34 ± 2.35^{b}	10.03 ± 1.39^{b}	10.69 ± 2.15^{b}
760	16:0/18:1	14:0/20:1	10.76 ± 2.38^{a}	15.63 ± 2.41^{b}	14.12 ± 2.29^{b}	13.49 ± 3.91^{b}
762	16:0/18:0	-	2.66 ± 0.37^{a}	4.28 ± 2.10^{b}	2.33 ± 1.49^{a}	2.14 ± 1.09^{a}
778	14:0/22:6	16:1/20:5	2.06 ± 0.30^{c}	1.25 ± 0.02^{b}	0.38 ± 0.03^{a}	0.61 ± 0.12^{a}
780	16:1/20:4	16:0/20:5 14:0/22:5	3.79 ± 0.13^{a}	4.53 ± 0.15^{ab}	5.07 ± 2.41^{b}	4.54 ± 0.68^{ab}
782	16:0/20:4	-	6.10 ± 1.11^{a}	8.70 ± 1.03^{b}	7.05 ± 0.63^{b}	7.26 ± 0.82^{b}
784	18:1/18:2	18:0/18:3 16:1/20:2	5.22 ± 0.15^{a}	5.38 ± 0.94^{a}	6.30 ± 0.04^{b}	6.38 ± 0.54^{b}
786	18:0/18:2	16:0/20:2 18:1/18:1	6.65 ± 1.19^{a}	10.19 ± 1.53^{b}	8.16 ± 1.25^{ab}	7.33 ± 1.01^{ab}
788	18:0/18:1	16:0/20:1	4.28 ± 0.92^{a}	6.04 ± 0.37^{b}	4.91 ± 2.15^{ab}	4.54 ± 1.83^{ab}
790	18:0/18:0	-	1.84 ± 0.57^{b}	1.89 ± 1.25^{b}	0.78 ± 0.32^{a}	1.02 ± 0.22^{a}
806	16:0/22:6	16:1/22:5	3.74 ± 0.86^{ab}	3.41 ± 1.23^{a}	3.86 ± 0.26^{ab}	4.40 ± 0.94^{b}
808	18:1/20:4	18:0/20:5 16:0/22:5	4.75 ± 0.51	4.92 ± 2.01	5.46 ± 1.72	4.69 ± 0.43
810	18:0/20:4	-	5.96 ± 1.64^{a}	8.50 ± 3.41^{b}	6.76 ± 1.37^{a}	6.06 ± 0.59^{a}
812	18:1/20:2	-	3.27 ± 0.59	3.57 ± 0.21	2.19 ± 1.96	3.35 ± 0.25
814	18:0/20:2	18:1/20:1	3.56 ± 0.78^{b}	2.81 ± 1.17^{a}	3.88 ± 2.51^{b}	2.57 ± 0.48^{a}
816	18:0/20:1	18:1/20:0	2.05 ± 0.30^{c}	1.62 ± 0.08^{b}	0.76 ± 0.07^{a}	0.79 ± 0.12^{a}
818	18:0/20:0	-	2.04 ± 0.25^{b}	2.08 ± 1.11^{b}	0.76 ± 0.23^{a}	0.77 ± 0.16^{a}
832	18:1/22:6	-	2.82 ± 0.14	2.00 ± 1.13	2.08 ± 0.24	2.25 ± 0.04
834	18:0/22:6	18:1/22:5	2.97 ± 0.08	2.33 ± 1.06	2.36 ± 0.08	2.77 ± 0.78
836	18:0/22:5	18:1/22:4	3.08 ± 0.45	2.67 ± 1.23	2.31 ± 0.14	2.74 ± 0.39
838	18:0/22:4	-	2.47 ± 0.21^{b}	2.44 ± 0.52^{b}	1.73 ± 0.15^{a}	1.83 ± 0.14^{a}
840	18:1/22:2	-	2.05 ± 0.05^{b}	1.94 ± 0.39^{b}	1.08 ± 0.23^{a}	0.98 ± 0.26^{a}

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.13 The percentage of each species of PC in summer and winter of wild-caught sandworm (*P. nuntia*)

m/z	PC species	Combine species	% of PC	
			summer	winter
706	14:0/16:0	12:0/18:0	0.85 ± 0.00[a]	2.05 ± 0.00[b]
730	14:0/18:2	-	0.50 ± 0.00[a]	2.43 ± 0.00[b]
732	16:0/16:1	14:0/18:1	2.36 ± 0.46[a]	3.57 ± 0.72[b]
734	16:0/16:0	14:0/18:0	4.10 ± 2.24	3.92 ± 2.80
754	14:0/20:4	16:1/18:3	1.97 ± 0.17	2.49 ± 1.12
756	16:1/18:2	16:0/18:3	1.58 ± 1.08	2.07 ± 1.28
758	16:0/18:2	14:0/20:2	7.32 ± 1.64[b]	4.98 ± 1.29[a]
760	16:0/18:1	14:0/20:1	10.20 ± 2.27[b]	6.83 ± 2.65[a]
762	16:0/18:0	-	1.19 ± 0.87[a]	2.16 ± 1.74[b]
778	14:0/22:6	16:1/20:5	0.60 ± 0.03[a]	2.43 ± 0.52[b]
780	16:1/20:4	16:0/20:5 14:0/22:5	5.23 ± 1.36[b]	3.92 ± 1.38[a]
782	16:0/20:4	-	11.38 ± 3.62[b]	5.22 ± 2.82[a]
784	18:1/18:2	18:0/18:3 16:1/20:2	3.62 ± 1.63	3.81 ± 0.95
786	18:0/18:2	16:0/20:2 18:1/18:1	4.78 ± 1.86	4.51 ± 1.03
788	18:0/18:1	16:0/20:1	3.01 ± 0.28	3.68 ± 2.01
790	18:0/18:0	-	0.72 ± 0.52[a]	2.91 ± 1.26[b]
806	16:0/22:6	16:1/22:5	3.32 ± 0.72	2.91 ± 1.69
808	18:1/20:4	18:0/20:5 16:0/22:5	6.79 ± 0.09[b]	3.79 ± 0.64[a]
810	18:0/20:4	-	11.93 ± 1.73[b]	5.66 ± 1.94[a]
812	18:1/20:2	-	1.94 ± 0.77	2.54 ± 0.87
814	18:0/20:2	18:1/20:1	1.73 ± 0.98[a]	3.29 ± 1.84[b]
816	18:0/20:1	18:1/20:0	0.85 ± 0.30[a]	2.26 ± 1.08[b]
818	18:0/20:0	-	1.09 ± 0.11[a]	2.49 ± 1.09[b]
832	18:1/22:6	-	1.58 ± 0.23	2.13 ± 1.86
834	18:0/22:6	18:1/22:5	2.32 ± 0.45	2.46 ± 1.79
836	18:0/22:5	18:1/22:4	3.48 ± 0.28[b]	2.34 ± 1.55[a]
838	18:0/22:4	-	2.98 ± 0.87	2.20 ± 0.38
840	18:1/22:2	-	0.67 ± 0.09[a]	1.86 ± 0.21[b]

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.14 The percentage of each species of PC in aged 6 months of farm-raised *P. aibuhitensis* and *P. quatrefagesi* and wild-caught *P. quatrefagesi* which caught in winter

m/z	PC species	Combine species	% of PC		
			age 6 m		winter
			P. quatrefagesi	*P. aibuhitensis*	*P. quatrefagesi*
706	14:0/16:0	12:0/18:0	0.50 ± 0.03	0.93 ± 0.08	0.45 ± 0.05
730	14:0/18:2	-	0.56 ± 0.10	0.64 ± 0.31	0.36 ± 0.09
732	16:0/16:1	14:0/18:1	2.74 ± 0.35	2.76 ± 0.20	2.47 ± 0.63
734	16:0/16:0	14:0/18:0	4.95 ± 2.62	6.01 ± 1.00	4.60 ± 2.22
754	14:0/20:4	16:1/18:3	0.84 ± 0.15	0.94 ± 0.23	0.87 ± 0.64
756	16:1/18:2	16:0/18:3	2.13 ± 1.16^{b}	1.72 ± 0.16^{a}	1.70 ± 0.45^{a}
758	16:0/18:2	14:0/20:2	13.75 ± 1.37^{b}	10.20 ± 2.23^{a}	10.51 ± 1.73^{ab}
760	16:0/18:1	14:0/20:1	11.51 ± 3.28^{a}	13.22 ± 2.43^{b}	11.86 ± 2.77^{a}
762	16:0/18:0	-	1.23 ± 0.75^{b}	1.74 ± 0.18^{b}	0.99 ± 0.02^{a}
778	14:0/22:6	16:1/20:5	0.50 ± 0.03	0.68 ± 0.23	0.42 ± 0.17
780	16:1/20:4	16:0/20:5 14:0/22:5	3.23 ± 1.21^{a}	3.59 ± 0.23^{ab}	4.19 ± 2.30^{b}
782	16:0/20:4	-	7.41 ± 2.89^{ab}	6.31 ± 0.44^{a}	8.06 ± 2.11^{b}
784	18:1/18:2	18:0/18:3 16:1/20:2	7.26 ± 1.58^{b}	5.87 ± 0.81^{a}	5.98 ± 0.33^{a}
786	18:0/18:2	16:0/20:2 18:1/18:1	7.64 ± 1.25	7.80 ± 0.06	7.01 ± 1.68
788	18:0/18:1	16:0/20:1	3.81 ± 0.44^{a}	4.28 ± 0.19^{b}	3.56 ± 1.99^{a}
790	18:0/18:0	-	0.73 ± 0.04	1.07 ± 0.14	0.65 ± 0.05
806	16:0/22:6	16:1/22:5	3.24 ± 0.35	3.23 ± 0.00	3.64 ± 0.69
808	18:1/20:4	18:0/20:5 16:0/22:5	4.10 ± 0.11^{ab}	3.96 ± 0.08^{a}	5.45 ± 0.24^{b}
810	18:0/20:4	-	6.08 ± 1.72^{a}	6.01 ± 0.46^{a}	7.96 ± 1.54^{b}
812	18:1/20:2	-	3.28 ± 0.35	2.94 ± 0.25	3.30 ± 0.76
814	18:0/20:2	18:1/20:1	2.51 ± 0.43	2.61 ± 0.03	2.16 ± 1.06
816	18:0/20:1	18:1/20:0	0.77 ± 0.20^{ab}	1.18 ± 0.23^{b}	0.54 ± 0.11^{a}
818	18:0/20:0	-	0.78 ± 0.01	0.94 ± 0.22	0.93 ± 0.25
832	18:1/22:6	-	1.87 ± 0.24	1.90 ± 0.09	2.10 ± 0.83
834	18:0/22:6	18:1/22:5	1.73 ± 0.82^{a}	2.22 ± 0.03^{b}	2.48 ± 1.03^{b}
836	18:0/22:5	18:1/22:4	2.27 ± 0.55^{a}	2.31 ± 0.10^{a}	3.19 ± 1.83^{b}
838	18:0/22:4	-	1.53 ± 0.06	1.62 ± 0.43	1.81 ± 0.36
840	18:1/22:2	-	1.13 ± 0.08	1.15 ± 0.03	1.36 ± 0.09

Data represent mean values. Within a row, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.15 Comparative of the first, second and third abundant of PC species in each farm-raised and wild-caught sandworm

Sandworm		PC species		
		First	**Second**	**Third**
P. nuntia	2 m	16:0/18:1	18:0/18:2	16:0/18:2
	4 m	16:0/18:1	16:0/18:2	18:0/18:2
	6 m	16:0/18:1	16:0/18:2	18:0/18:2
	8 m	16:0/18:1	16:0/18:2	18:0/18:2
P. nuntia	summer	18:0/20:4	16:0/20:4	16:0/18:1
	winter	16:0/18:1	18:0/20:4	16:0/20:4
P. quatrefagesi	6 m	16:0/18:2	16:0/18:1	18:0/18:2
	winter	16:0/18:1	16:0/18:2	16:0/20:4
P. aibuhitensis	6 m	16:0/18:1	16:0/18:2	18:0/18:2

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.3 Steroid

The steroid compositions of farm-raised and wild-caught sandworms were analysed by GC (as descripted in 3.4.3). In the present study, cholesterol was the most components in steroid extracted. Same as in deep-sea polychaetes, Phleger et al. [79] present the total sterol from deep-sea polychaetes and found the most component was cholesterol (89-98 % of total sterol). Cholesterol is an important membrane component in earthworms [80]. The amount of cholesterol in different age of farm-raised sandworm and that in winter and summer of wild-caught sandworm were presented in Table 4.16 and 4.17, respectively. For *P. nuntia*, the amount of cholesterol in aged 4, 6 and 8 months was about 60.21±0.73 mg cholesterol per 100 g ww sandworms, but significantly higher than that in 2 months (33.20±0.18 mg cholesterol per 100 g ww sandworms). While cholesterol of wild-caught sandworm was slightly different in summer and winter (about 81.43±4.16 mg cholesterol to 100 g ww sandworms) and significantly higher than that of farm-raised sandworm. While that of wild-caught *P. quatrefagesi* which caught in winter (102.75±3.18 mg cholesterol per 100 g ww sandworms) was also significantly higher than that of farm-raised sandworm aged 6 months (78.58±1.75 mg cholesterol per 100 g ww sandworms). The results indicated that the cholesterol of wild-caught sandworm was higher than that of farm-raised because the diet of wild-caught sandworm was remains of marine. The cholesterol of farm-raised *P. aibuhitensis* aged 6 months (86.57±4.16 mg cholesterol per 100 g ww sandworms) was slightly higher than that of *P. quatrefagesi* and *P. nuntia*. While, cholesterol of wild-caught *P. quatrefagesi* which caught in winter was significantly higher than that of *P. nuntia*. In most marine animals, cholesterol was important in oocyte and sperm development [77, 81, 82, 83] and mature animals store cholesterol in tissue for these activities [84]. Therefore, farm-raised sandworm which was older and more mature than other was found to contain higher cholesterol. The other steroid compositions were not detected in this study.

Table 4.16 Comparison of mg cholesterol per 100 g wet weight of sandworms in different age 2, 4, 6 and 8 months of farm-raised sandworm (*Perinereis* sp.)

Farm-raised (age, months)	**Cholesterol (mg / 100 g ww)**		
	P. nuntia	***P. aibuhitensis***	***P. quatrefagesi***
2	33.20 ± 0.18^{a}	-	-
4	59.48 ± 0.21^{b}	-	-
6	60.21 ± 0.29^{b}	86.57 ± 3.07	78.58 ± 1.75
8	60.94 ± 0.14^{b}	-	-
average	53.46 ± 13.52	-	-

- means not determined

Data represent mean values. Within a column, values with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.17 Comparison of mg cholesterol per 100 g wet weight of sandworms in summer and winter of wild-caught sandworm (*Perinereis* sp.)

Wild-caught (season)	**Cholesterol (mg / 100 g ww)**		
	P. nuntia	***P. aibuhitensis***	***P. quatrefagesi***
Winter	84.37 ± 0.46^{a}	-	102.75 ± 3.18
Summer	78.49 ± 1.07^{b}	-	-
average	81.43 ± 4.16	-	-

- means not determined

Data represent mean values. Within a column, values with different superscript letters are significantly different ($P \leq 0.05$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER V

CONCLUSION

The main fatty acid compositions of *Perinereis* sp. from farm-raised and wild-caught sandworms were C16:0, C18:1 *n*-9, C18:2 *n*-6, C18:1 *n*-7 and C18:0, respectively. The percentage of fat and total fatty acid of farm-raised sandworm aged 8 months was significantly higher than those in difference age, while total fatty acid of farm-raised sandworm was higher than that of wild-caught. On the basis of the results, the most of ratio of fatty acid profiles did not change during maturation (in aged 4, 6 and 8 months).

The major component of the lipid of *Perinereis* sp. was phospholipids, especially phosphatidylcholine (PC). The most abundant of phosphatidylcholine of all of farm-raised and wild-caught sandworm were PC 16:0/18:1, PC 16:0/18:2, PC 18:0/18:2, PC 16:0/20:4 and PC 18:0/20:4.

Cholesterol was the most component in steroid extracted. The results indicated that cholesterol of wild-caught sandworm was significantly higher than that of farm-raised sandworm. Other sterol components were not detected in this present study.

The fatty acid profiles and lipid compositions of sandworms *Perinereis* sp. were dependent on the estuarine environment, most probably on their different feeding modes. These results revealed that fatty acid profiles and lipid compositions of farm-raised sandworms could be used to improve the quality of sandworms for broodstock shrimp and help reduce the destruction of environment from worms collecting activities.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Suggestion for future work

The percentage of fat and total fatty acids of farm-raised sandworms was the highest at the age of 8 months, but steroid hormones could not be detected which may because of age or maturity. Consequently, the farm-raised sandworms at the age of 10 to 12 months and the reproductive male and female sandworms should be analysed.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A1 Fatty acid profiles (mg per g dry weight) of *P. nuntia* farm-raised sandworm aged 2 months

Fatty acid	Age 2 m			Average	SD
	Chonburi	Chumporn			
12:0	0.08	0.07	0.07	**0.07**	**0.00**
14:0	1.05	0.96	0.95	**0.98**	**0.06**
16:0	32.56	29.77	29.39	**30.57**	**1.73**
16:1	5.65	5.16	5.10	**5.30**	**0.30**
18:0	9.65	8.82	8.71	**9.06**	**0.51**
18:1 *trans*	3.35	3.06	3.02	**3.15**	**0.18**
18:1 *n*-9	25.69	23.48	23.19	**24.12**	**1.37**
18:2 *n*-6	18.41	16.83	16.62	**17.28**	**0.98**
18:3 *n*-3	1.40	1.28	1.26	**1.31**	**0.07**
20:2 *n*-6	3.68	3.36	3.32	**3.45**	**0.20**
20:4 *n*-6	4.39	4.01	3.96	**4.12**	**0.23**
20:5 *n*-3	3.45	3.15	3.11	**3.24**	**0.18**
unk6	2.29	2.10	2.07	**2.15**	**0.12**
22:6 *n*-3	2.12	1.94	1.92	**1.99**	**0.11**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A2 Fatty acid profiles (mg per g dry weight) of *P. nuntia* farm-raised sandworm aged 4 months

Fatty acid	Age 4 m						Average	SD
	Samutsakorn	Chonburi		Chumporn				
12:0	0.00	0.00	0.00	0.16	0.13	0.17	**0.08**	**0.08**
14:0	1.12	0.99	1.23	1.16	0.91	1.19	**1.10**	**0.13**
15:0	0.46	0.41	0.51	0.50	0.39	0.51	**0.46**	**0.05**
16:0	37.46	33.23	41.35	35.26	27.48	36.21	**35.16**	**4.64**
16:1	5.61	4.98	6.19	6.53	5.09	6.71	**5.85**	**0.74**
18:0	11.23	9.96	12.40	10.50	8.18	10.79	**10.51**	**1.40**
18:1 *trans*	4.75	4.22	5.25	5.12	3.99	5.26	**4.77**	**0.55**
18:1 *n*-9	13.03	11.56	14.38	14.11	11.00	14.50	**13.09**	**1.51**
18:1 *n*-7	7.72	6.85	8.52	9.41	7.33	9.66	**8.25**	**1.14**
unk3	0.00	0.00	0.00	1.71	1.33	1.75	**1.60**	**0.23**
18:2 *n*-6	14.33	12.71	15.81	16.29	12.69	16.73	**14.76**	**1.79**
18:3 *n*-3	1.22	1.08	1.35	1.42	1.11	1.46	**1.27**	**0.16**
20:1 *n*-9	6.04	5.36	6.67	5.71	4.45	5.87	**5.68**	**0.74**
unk4	2.52	2.23	2.78	2.51	1.96	2.58	**2.43**	**0.29**
20:2 *n*-6	7.53	6.68	8.31	7.69	5.99	7.90	**7.35**	**0.86**
20:4 *n*-6	5.49	4.87	6.05	3.94	3.07	4.05	**4.58**	**1.10**
20:5 *n*-3	4.31	3.83	4.76	4.07	3.17	4.18	**4.05**	**0.53**
unk5	0.00	0.00	0.00	2.75	2.14	2.82	**2.57**	**0.37**
unk6	4.06	3.60	4.48	2.86	2.23	2.94	**3.36**	**0.84**
22:5 *n*-3	0.91	0.81	1.01	0.87	0.68	0.89	**0.86**	**0.11**
22:6 *n*-3	2.76	2.44	3.04	3.22	2.51	3.31	**2.88**	**0.37**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A3 Fatty acid profiles (mg per g dry weight) of *P. nuntia* farm-raised sandworm aged 6 months

Fatty acid	Age 6 m					Average	SD
	Rayong			Chumporn			
12:0	0.00	0.00	0.00	0.05	0.05	**0.05**	**0.00**
14:0	1.19	1.03	1.20	1.12	1.08	**1.12**	**0.07**
15:0	0.54	0.47	0.55	0.46	0.45	**0.49**	**0.05**
16:0	40.38	34.99	40.64	33.09	32.06	**35.62**	**4.04**
16:1	6.17	5.34	6.21	4.73	4.58	**5.28**	**0.77**
unk1	1.21	1.05	1.22	0.00	0.00	**1.16**	**0.10**
18:0	11.00	9.53	11.07	9.80	9.50	**10.09**	**0.79**
18:1 *trans*	5.93	5.13	5.96	3.81	3.70	**4.72**	**1.10**
18:1 *n*-9	13.25	11.48	13.33	16.09	15.59	**14.26**	**1.89**
18:1 *n*-7	8.85	7.67	8.91	7.20	6.97	**7.78**	**0.91**
18:2 *n*-6	14.59	12.65	14.69	14.70	14.25	**14.23**	**0.88**
18:3 *n*-3	1.37	1.19	1.38	1.32	1.28	**1.30**	**0.08**
20:1 *n*-9	5.94	5.15	5.98	4.88	4.73	**5.25**	**0.59**
unk4	2.43	2.11	2.45	1.87	1.82	**2.09**	**0.30**
20:2 *n*-6	8.39	7.27	8.44	6.68	6.47	**7.31**	**0.93**
20:4 *n*-6	5.15	4.47	5.19	4.20	4.07	**4.54**	**0.53**
20:5 *n*-3	4.06	3.52	4.09	3.29	3.19	**3.56**	**0.42**
unk5	3.09	2.68	3.11	2.00	1.93	**2.46**	**0.57**
unk6	4.52	3.92	4.55	3.38	3.28	**3.83**	**0.60**
22:5 *n*-3	0.93	0.81	0.94	0.82	0.79	**0.85**	**0.07**
22:6 *n*-3	3.07	2.66	3.09	2.64	2.56	**2.77**	**0.25**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A4 Fatty acid profiles (mg per g dry weight) of *P. nuntia* farm-raised sandworm aged 8 months

Fatty acid	Age 8 m						Average	SD
	Chumporn		Chonburi			Rayong		
12:0	0.14	0.15	0.10	0.09	0.12	0.13	**0.12**	**0.02**
14:0	1.30	1.40	1.47	1.51	1.45	1.30	**1.41**	**0.09**
15:0	0.57	0.62	0.57	0.58	0.56	0.55	**0.58**	**0.02**
16:0	42.17	45.46	44.18	45.37	43.75	43.81	**44.12**	**1.22**
16:1	5.95	6.42	7.18	7.37	7.11	6.17	**6.70**	**0.59**
18:0	11.20	12.08	11.65	11.97	11.54	11.62	**11.68**	**0.31**
18:1 *trans*	5.54	5.98	5.00	5.14	4.96	5.76	**5.40**	**0.43**
18:1 *n*-9	25.81	24.57	22.81	20.12	22.01	19.68	**22.50**	**2.42**
18:1 *n*-7	9.52	9.34	10.10	9.82	10.18	11.66	**10.10**	**0.83**
18:2 *n*-6	16.75	17.06	21.36	20.93	21.49	19.05	**19.44**	**2.15**
18:3 *n*-3	1.59	1.72	1.86	1.91	1.76	1.74	**1.76**	**0.11**
20:1 *n*-9	5.68	6.12	4.97	5.10	4.97	5.85	**5.45**	**0.50**
20:2 *n*-6	9.64	10.39	8.36	8.58	8.67	9.63	**9.21**	**0.80**
20:4 *n*-6	6.28	6.77	6.03	6.20	5.98	6.53	**6.30**	**0.30**
20:5 *n*-3	5.12	5.52	4.37	4.48	4.22	5.38	**4.85**	**0.56**
unk6	5.88	6.34	3.94	4.04	3.90	4.83	**4.82**	**1.07**
22:6 *n*-3	3.79	4.09	3.82	3.92	3.78	3.94	**3.89**	**0.12**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A5 Fatty acid profiles (mg per g dry weight) of *P. nuntia* wild-caught sandworm which caught in summer

Fatty acid	Summer				Average	SD
	Chonburi		Rayong			
14:0	1.02	1.29	1.70	2.32	**1.41**	**0.51**
15:0	0.50	0.53	0.85	0.90	**0.70**	**0.18**
16:0	29.59	29.22	44.01	48.05	**37.75**	**7.65**
16:1	4.32	4.99	6.97	7.37	**5.85**	**1.33**
unk1	2.85	3.07	3.32	2.97	**3.24**	**0.42**
18:0	4.93	8.91	8.89	9.39	**7.31**	**1.96**
18:1 *trans*	3.16	3.35	4.83	5.10	**4.14**	**0.92**
18:1 *n*-9	3.71	4.33	8.82	9.31	**6.51**	**2.68**
18:1 *n*-7	6.74	6.65	10.85	11.46	**8.86**	**2.35**
unk3	2.47	3.62	6.83	4.72	**5.00**	**1.94**
18:2 *n*-6	2.43	2.79	5.69	5.51	**4.10**	**1.55**
unk4	4.26	3.89	4.29	6.18	**4.86**	**0.90**
20:4 *n*-6	4.58	4.87	4.64	4.90	**4.79**	**0.16**
20:5 *n*-3	2.64	2.80	2.84	2.51	**2.77**	**0.16**
unk5	3.89	4.38	5.11	4.89	**4.75**	**0.54**
unk6	3.79	5.28	6.37	5.35	**5.14**	**0.96**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A6 Fatty acid profiles (mg per g dry weight) of *P. nuntia* wild-caught sandworm which caught in winter

Fatty acid	Winter				Average	SD
	Rayong		Chonburi			
12:0	1.09	1.10	0.07	0.06	**0.58**	**0.57**
14:0	1.75	1.77	1.63	1.39	**1.62**	**0.19**
15:0	0.58	0.59	0.54	0.47	**0.54**	**0.06**
16:0	30.13	30.50	28.32	24.19	**27.96**	**3.15**
16:1	6.25	6.33	5.39	4.60	**5.58**	**0.86**
18:0	5.78	5.85	8.44	7.21	**6.72**	**1.07**
18:1 *trans*	3.70	3.74	2.97	2.53	**3.20**	**0.61**
18:1 *n*-9	4.08	4.13	7.74	6.62	**5.55**	**1.61**
18:1 *n*-7	8.09	8.19	7.73	6.61	**7.57**	**0.79**
18:2 *n*-6	2.40	2.42	5.71	4.88	**3.78**	**1.52**
20:1 *n*-9	2.80	2.83	0.00	0.00	**2.83**	**1.55**
unk4	0.35	0.36	0.74	0.62	**0.53**	**0.19**
20:2 *n*-6	2.64	2.67	0.00	0.00	**2.67**	**1.46**
20:4 *n*-6	3.86	3.91	4.37	3.73	**3.92**	**0.24**
20:5 *n*-3	4.35	4.40	4.67	3.99	**4.30**	**0.28**
unk6	1.32	0.88	1.35	0.43	**1.03**	**0.39**
22:6 *n*-3	1.69	2.03	1.96	1.68	**1.76**	**0.19**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A7 Fatty acid profiles (mg per g dry weight) of *P. quatrefagesi* farm-raised sandworm aged 6 months

Fatty acid	**Age 6 m**			**Average**	**SD**
	Chumporn				
14:0	1.12	1.17	1.08	**1.12**	**0.25**
15:0	0.38	0.45	0.53	**0.43**	**0.11**
16:0	33.88	33.42	31.74	**32.89**	**0.73**
16:1	5.19	5.44	5.09	**5.21**	**0.17**
18:0	8.09	8.30	8.36	**8.23**	**0.18**
18:1 *trans*	3.05	3.20	2.78	**3.06**	**0.18**
18:1 *n*-9	20.56	21.57	18.37	**20.64**	**1.42**
18:2 *n*-6	13.51	14.18	11.13	**13.57**	**1.40**
18:3 *n*-3	1.28	1.35	0.93	**1.29**	**0.21**
20:1 *n*-9	2.81	2.95	2.57	**2.82**	**0.17**
20:2 *n*-6	6.37	6.68	5.85	**6.39**	**0.35**
20:4 *n*-6	4.01	4.21	3.72	**4.03**	**0.22**
20:5 *n*-3	3.93	4.13	3.86	**3.95**	**0.12**
unk6	2.66	2.79	2.44	**2.67**	**0.16**
22:6 *n*-3	3.76	3.94	3.64	**3.77**	**0.14**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A8 Fatty acid profiles (mg per g dry weight) of *P. aibuhitensis* farm-raised sandworm aged 6 months

Fatty acid	**Age 6 m**				**Average**	**SD**
	Chumporn					
12:0	0.00	0.00	0.10	0.10	**0.10**	**0.00**
14:0	1.30	1.14	1.20	1.18	**1.20**	**0.08**
15:0	0.64	0.56	0.45	0.44	**0.52**	**0.07**
16:0	45.59	39.95	40.03	39.43	**40.95**	**2.67**
16:1	5.28	4.63	6.39	6.29	**5.63**	**0.99**
18:0	13.14	11.52	11.75	11.57	**11.91**	**0.77**
18:1 *trans*	5.78	5.07	4.09	4.03	**4.69**	**0.68**
18:1 *n*-9	28.57	25.03	26.71	26.31	**26.48**	**1.82**
18:2 *n*-6	17.64	15.46	17.48	17.22	**16.85**	**1.43**
18:3 *n*-3	1.49	1.30	1.57	1.55	**1.47**	**0.16**
20:1 *n*-9	6.73	5.89	3.98	3.92	**5.06**	**1.18**
20:2 *n*-6	10.16	8.90	6.95	6.84	**8.12**	**1.30**
20:4 *n*-6	8.21	7.20	5.19	5.11	**6.35**	**1.27**
20:5 *n*-3	5.88	5.15	4.37	4.31	**4.88**	**0.59**
unk5	2.41	2.24	2.02	1.83	**2.09**	**0.22**
unk6	6.30	5.52	4.91	4.87	**5.16**	**0.74**
22:6 *n*-3	3.48	3.05	3.70	3.64	**3.45**	**0.39**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table A9 Fatty acid profiles (mg per g dry weight) of *P. quatrefagesi* wild-caught sandworm which caught in winter

Fatty acid	**Winter**		**Average**	**SD**
	Chumporn			
14:0	0.99	1.03	**1.01**	**0.02**
15:0	0.55	0.57	**0.56**	**0.01**
16:0	43.48	45.23	**44.49**	**0.90**
16:1	5.23	5.44	**5.35**	**0.11**
unk1	2.50	2.60	**2.56**	**0.05**
18:0	10.05	10.46	**10.29**	**0.21**
18:1 *trans*	5.77	6.00	**5.90**	**0.12**
18:1 *n*-9	11.97	12.45	**12.25**	**0.25**
18:1 *n*-7	9.94	10.34	**10.17**	**0.21**
18:2 *n*-6	11.19	11.64	**11.44**	**0.23**
20:1 *n*-9	5.79	6.02	**5.92**	**0.12**
unk4	2.46	2.56	**2.52**	**0.05**
20:2 *n*-6	9.18	9.55	**9.39**	**0.19**
20:4 *n*-6	6.25	6.50	**6.39**	**0.13**
20:5 *n*-3	4.12	4.29	**4.22**	**0.09**
unk6	5.72	5.95	**5.85**	**0.12**
22:6 *n*-3	2.07	2.16	**2.12**	**0.04**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX B

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table B1 Fatty acid profiles (percentage of fatty acid) of diet for farm-raised sandworm from Pankavee Farm, Chumporn province, Thailand

Fatty acid	Average	SD
14:0	**2.23**	**0.35**
15:0	**0.48**	**0.12**
16:0	**20.58**	**4.05**
16:1	**2.83**	**0.19**
18:0	**6.12**	**1.43**
18:1 *n*-7	**2.51**	**0.07**
18:1 *n*-9	**25.07**	**5.38**
18:2 *n*-6	**24.58**	**4.28**
18:3 *n*-3	**2.67**	**0.27**
20:1	**1.19**	**0.01**
20:4 *n*-6	**0.87**	**0.02**
20:5 *n*-3	**2.45**	**0.38**
22:5 *n*-3	**0.57**	**0.05**
22:6 *n*-3	**5.26**	**1.84**
unidentified	**2.84**	**0.26**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX C

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

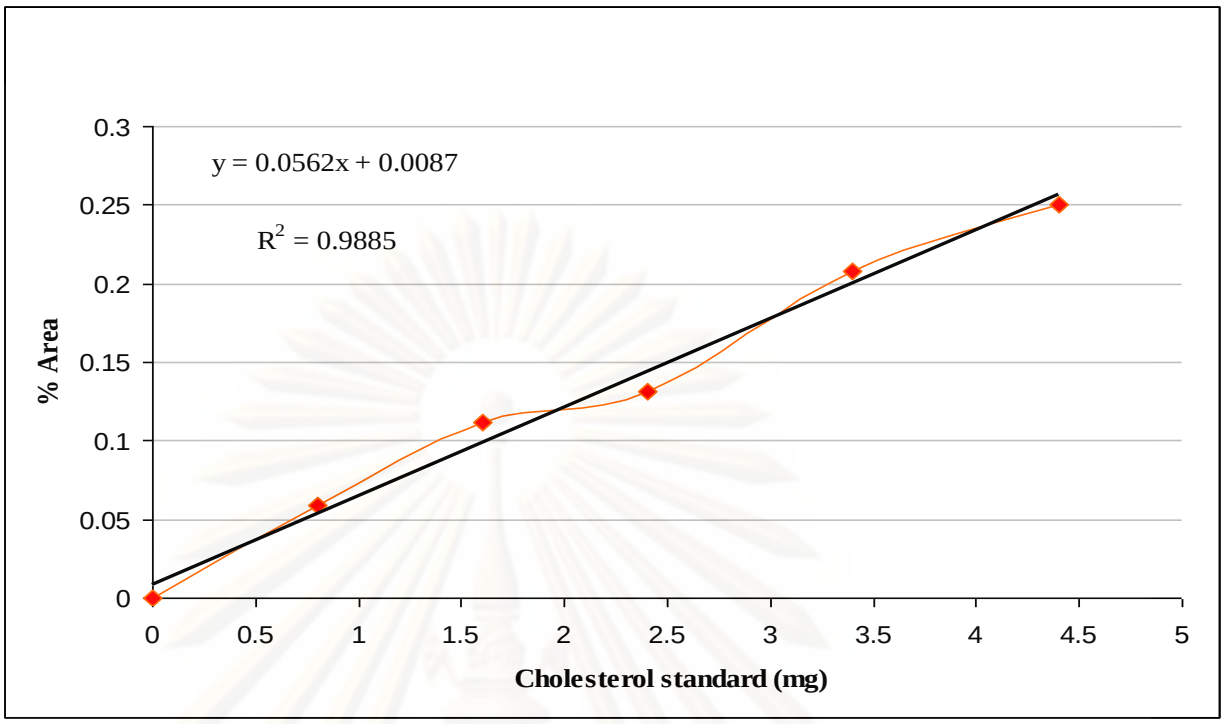

Figure C1 Standard curve of cholesterol

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

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Conference

Maneerat Limsuwatthanathamrong, Sarintip Sooksai, Suraphol Chunhabundit, Sajee Noitung, Nattaya Ngamrojanavanich, Amorn Petsom "Fatty acid profile of farm-raised and wild-caught sandworms (*Perinereis nuntia*)" *4th BUU Grad. Research Conference*, Burapha University, Chonburi, Thailand, March 13, **2009**, pp 88.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย