การพัฒนาอาหารที่เสริมด้วยฟาร์นีซอลต่อการวางไข่และการเติบโตของกุ้งก้ามกราม *Macrobrachium rosenbergii*

นางสาวนิปกร แจวศรีทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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DEVELOPMENT OF FARNESOL SUPPLEMENTED DIETS ON SPAWNING AND GROWTH OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii*

Miss Nippagorn Jeawsrithong

A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	DEVELOPMENT OF FARNESOL SUPPLEMENTED DIETS ON SPAWNING AND GROWTH OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii*
By	Miss Nippagorn Jeawsrithong
Field of Study	Biotechnology
Thesis Advisor	Associate Professor Somkiat Piyatiratitivorakul, Ph.D.

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นิปกร แจวศรีทอง: การพัฒนาอาหารที่เสริมด้วยฟาร์นีซอลต่อการวางไข่และการเติบโตของกุ้งก้ามกราม *Macrobrachium rosenbergii* (DEVELOPMENT OF FARNESOL SUPPLEMENTED DIETS ON SPAWNING AND GROWTH OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. สมเกียรติ ปิยะธีรธิติวรกุล, 92 หน้า.

กุ้งก้ามกรามเป็นสัตว์น้ำที่มีความสำคัญทางเศรษฐกิจของประเทศ กุ้งก้ามกรามเพศเมียเกิดภาวะเจริญพันธุ์ก่อนวัย ทำให้กุ้งเพศเมียมีขนาดตัวที่เล็กกว่าตัวผู้เพราะต้องนำสารอาหารที่ได้ไปสร้างไข่และดูแลไข่ งานวิจัยนี้มีจุดมุ่งหมายเพื่อสร้างสูตรอาหารเสริมฟาร์นีซอล เพื่อยับยั้งการสืบพันธุ์ในกุ้งก้ามกรามเพศเมีย ทำการห่อหุ้มสารฟาร์นีซอลด้วยแอลจิเนตในอัตราส่วน 1:0.67 และไคโตซาน นำสารฟาร์นีซอลที่ทำการหุ้มแล้วมาทำแห้งแบบแช่เยือกแข็ง พบว่าประสิทธิภาพในการหุ้มฟาร์นีซอลด้วยแอลจิเนตและไคโตซานก่อนและหลังทำแห้งเท่ากับ 82.6±1.21 และ 83.41±0.23 เปอร์เซ็นต์ และทำการสกัดฟาร์นีซอลจากบีดแอลจิเนตและไคโตซาน พบปริมาณฟาร์นีซอลในบีดเท่ากับ 63.8±0.66 เปอร์เซ็นต์นำสารที่ผ่านกระบวนการทำแห้งมาผสมลงในอาหารทดลองที่ความเข้มข้นของสารฟาร์นีซอลเท่ากับ 0, 0.5, 1, 2 กรัม/ 100 กรัมของอาหารทดลอง จากนั้นทำการเลี้ยงกุ้งก้ามกรามเพศเมีย180 ตัว ความยาวประมาณ10 เซนติเมตรในถังพลาสติกความจุ 375 ลิตร ถังละ 15 ตัว ให้อาหารวันละ 3 มื้อ เป็นเวลา 8 สัปดาห์ ทำการทดลอง 3 ซ้ำต่อชุดการทดลอง พบว่าที่ความเข้มข้นของฟาร์นีซอล 2 กรัม/ 100 กรัมของอาหารทดลอง มีน้ำหนักไข่กุ้งน้อยที่สุดมีค่าเฉลี่ยเท่ากับ 1.07 ± 0.19 กรัม และกุ้งก้ามกรามมีการลอกคราบได้เร็วกว่าทุกสูตรอาหาร(ที่ 15.92 วัน)

สาขาวิชา .....เทคโนโลยีชีวภาพ.....ลายมือชื่อนิสิต.....

ปีการศึกษา.....2554.....ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก.....

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NIPPAGORN JEAWSRITHONG: DEVELOPMENT OF FARNESOL SUPPLEMENTED DIETS ON SPAWNING AND GROWTH OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii*. ADVISOR: ASSOC. PROF. SOMKIAT PIYATIRATITIVORAKUL, Ph.D., 92 pp.

The giant freshwater prawn *Macrobrachium rosenbergii* is an economically important cultured species in Thailand. Early maturation of female prawn caused restrict on growth. In this study, female prawn was used to study effect of farnesol on spawning and growth. Farnesol used in a representation of the MF was initially encapsulated with farnesol/ alginate by 1:0.67 ratios. The overall encapsulation efficiency at before and after freeze-dried processes was 82.6±1.21 % and 83.41±0.23 %, respectively. After freeze drying farnesol capsules were extracted and analyzed. The recovered farnesol was 63.8±0.66 %. One hundred and eighty matured female giant freshwater prawn with average body length 10 cm were obtained from a local farm. Fifteen prawn were reared in close re-circulated water system amount 375 L per culture tank and fed 3 times/day at amount of 5% total body weight for 8 weeks. The tested diets were 0, 0.5, 1 and 2 g farnesol per 100 gram diet. The result showed that farnesol 2 g/ 100 gram diet decreased egg weight and percent number of female spawns and shortened the molting duration. Significantly reduced the egg weight from 2.01 ± 0.32 g to 1.07 ± 0.19 g and shortened the molt duration at 15.92 day.

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2011 Advisor's Signature .....

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LIST OF ABBREVIATIONS

A_{310}	absorbance at 310 nanometer
β	beta
ºC	degree Celsius
$CaCl_2 \cdot 2H_2O$	calcium chloride
cm	centrimeter
20E	20-hydroxyecdysone
ESA	eye stalk ablation
FA	farnesoic acid
g	gram
g/L	gram per liter
g/mL	gram per milliliter
hr	hour
JH	juvenile hormone
MF	methyl farnesoate
m	meter
cm	centimeter
µM	micromolar
µm	micrometer
mM	millimolar
mL	milliliter
mg	milligram
µg	microgram
µg/g	microgram per gram

µg/mL	microgram per milliliter
µg/L	microgram per liter
µL	microliter
mm	millimeter
mg/ml	milligram per milliliter
min	minute
M	molar
nM	nanomolar
ng	nanogram
nm	nanometer
o/w	oil in water
/	per
%	percent
% EE_{BD}	percent of encapsulation efficiency before drying
% EE_{AD}	percent of encapsulation efficiency after drying
vol%	percent of volume
pM	picomolar
pg/hr	picogram per hour
rpm	revolutions per minute
v/v	volume by volume
w/o	water in oil
wt	weight
w/w	weight by weight
w/v	weight by volume

CHAPTER I

INTRODUCTION

1.1 Background of the study

The giant freshwater prawn, *Macrobrachium rosenbergii*, is an economically important cultured species in Thailand. Entirely, the output of *M. rosenbergii* from aquaculture expanded during the decade 1993-2002 from 17,000 tonnes to 195,000 tonnes with an average annual production of 31 % per year. While the farm production is rapidly expanding in Asia, the prawn product in India and Thailand expanded by more than 50 % per year between 1999 and 2002 (Wickins and Lee, 2002). There is also potential for expansion in Bangladesh, Indonesia, Viet Nam and Brazil. In 2009, the frozen prawn was the most exported in 1562.2 tons at a value of 353.9 million baht (MB) and the fresh prawn volume was 57.6 tons at a valve of 13 MB as shown in Table 1.1. To content the growing demand prawn, production by aquaculture must be increased by utilizing advanced technology. However, if an expansion of 10 percent per year occurs, global farmed production of *M. rosenbergii* will have significantly exceeded 400,000 tonnes by 2010. Thus, the way in which the prawn are managed (for example, the frequency of selecting large prawn, mostly males or increased female weight) influence total productivity. Early maturation of female prawn that cause limiting of growth since the prawn had spawned, the overall nutrient lead to produce eggs and reduce weight gain of the prawn. Therefore, the female prawn grow slower than male in the same stock that result in female prawn were depreciated in the market and distinct lower price from other marine shrimp. It may be better, if we can grow the same size of prawn in a single stock.

Methyl farnesoate (MF) is an unepoxidated sesquiterpene structurally related to a juvenile hormone (JH III) of insects, synthesized by a crustacean mandibular organ (MO). The JH III plays important roles in the regulation of insect development

and reproduction (Borst *et al*., 1987) which depressed the rate of juvenile metamorphosis and inhibiting reproduction. As the similar functions of MF in crustaceans was mediated the growth and development of the reproductive system, morphotypic differentiation, mating behavior, and crustacean vitellogenesis (Laufer et al., 1993). Maturation of female prawn is controlled by several neuropeptides (Okumura, 2004).

Table 1.1 Volume and value on the frozen and fresh prawn exports and gross domestic products in 2009-2010.

Year trimester	Frozen prawn		Fresh prawn	
	Tons	Million Baht (MB)	Tons	Million Baht (MB)
		2009		
1	674.2	151.7	26.5	5.3
2	350.5	76.4	15.8	3.6
3	537.5	125.8	15.3	4.1
Total	1562.2	353.9	57.6	13
		2010		
1	629	133.4	0.1	0.02
2	596.2	110.7		
3	282.5	46.8		
Total	1507.7	290.9	0.1	0
% GDP of 2009	47.4	-62.8		
% GDP of 2010	-52.6	-57.7		

Source: Cultured Aquatic Species Information Programs. Fisheries and Aquaculture Department in 2009-2010.

According to farnesoic acid O-methyltransferase (FAMeT) is an enzyme which stimulates the final step in MF biosynthetic pathway. Enzyme FAMeT, present in the insect copora allata, complete the methylation of farnesoic acid (FA) to titers MF using the cofactor S-adenosyl-L methionine (Gunawardene *et al*.,2002). Thus in both insects and crustaceans have an enzyme O-methyltransferase that plays a role in the regulation of MF synthesis, get mediates an effect on vitellogenesis and metamorphosis of these animals.

Encapsulation is of technology of reserving solid, liquid or gaseous materials in small blocked up capsules for release at controlled rates using desired release triggers. Microcapsule consists of a core surrounded by a wall or barrier. The core is the component insisting on protection that featured of one or more ingredients. The wall may be single or multi-layered (Pothakamury and Barbosa, 1995). This technology is used in drugs, foods and beverages to control the release of active ingredients, keep ingredients from the environment, lower flavor loss during the product shelf-life, disperse the flavor perception and enhance the ingredient bioavailability and efficacy (Shefer and Shefer, 2003; Berry, 2004).

In this study, *M. rosenbergii* was used as a model species to study the role of MF in inhibiting of reproduction, increase of molting cycle and weight. Farnesol used in immediate precursor of the methyl farnesoate was initially encapsulation with chitosan/calcium alginate capsules.

1.2 Objectives of the study are:

1. To study encapsulation efficiency of farnesol with alginate-chitosan .
2. To study effects of farnesol in dietary supplement on female *M. rosenbergii*., as
   2.1 The effect of farnesol on spawning, egg production.
   2.2 The effect of farnesol on molting cycle, and effect on growth rate and survival rate.

1.3 Hypothesis

Methyl farnesoate (MF) or juvenile hormone analogue had importance function of development and reproductive role in crustacean. Especially, the JH or JH analogue serving a major role in female reproduction that expected inhibited female spawn and increased growth rate on female *M. rosenbergii*.

1.4 Scope of the study

The scope of this research covers the effects of farnesol which divided into 4 concentrations (0, 0.5, 1, 2 g farnesol/ 100g diets) and combined in dietary supplement after that fed on female prawn for 8 weeks. For studies the encapsulation efficiency of farnesol with alginate-chitosan beads before and after dried. Further prawn was detected in survival rate, growth rate, molting cycle and egg weight in 4 diets. Analysis of water quality in every 2 weeks and examined the percentage of lipid, protein, ash and humid.

1.5 The expected results of the study

The following results should be obtained from the present studies:

1.5.1 The encapsulation efficiency of farnesol for suitable biopolymer which control the farnesol to approach target and protect farnesol from the environment.

1.5.2 Application of farnesol added diets on spawning of female freshwater prawn in order to increase growth rate.

CHAPTER II

LITERATURE REVIEWS

The giant freshwater prawn *Macrobrachium rosenbergii* also known as the giant river prawn, Malaysian prawn, freshwater scampi (especially in India). The prawns live native to the Indo-Pacific region, northern Australia and Southeast Asia that are in tropical freshwater environments influenced by adjacent brackishwater areas. Global production of giant freshwater prawn, *Macrobrachium rosenbergii* has been increased from 17,129 to 180,221 tonnes between 1993 and 2003 (FAO, 2005). The giant freshwater prawn is one of the most commercially important species in South East Asia. Farming of *M. rosenbergii* has received a considerable economic and social importance in the region, including of a significant source of income and employment.

In Thailand, *M. rosenbergii* had been intensively cultured and expanded more than 50 % a year between 1999 and 2002 and trend is expected to continue. In total, the production from aquaculture expanded during the decade 2000 – 2010 from 13,311 tonnes to 25,606 tonnes. Also the total income, number of farm and the employer had increased as shown in Table 2.1.

Table 2.1 Production, value and number of farm in Thailand during 2000-2010.

Year	Production (tonnes)	To Change (%)	Value (MB)	To Change (%)	Area unit (rai)	To Change (%)	Number of farm	To Change (%)
2000	13,311	-	1,588	-	21,071	-	1,719	-
2002	15,393	15.6	1,775	11.8	41,823	98.5	3,126	81.7
2003	28,151	82.9	2,945	65.9	42,412	1.4	3,371	7.8
2004	32,583	15.7	3,805	29.2	93,395	120.2	5,742	70.3
2005	28,740	-11.8	3,660	-3.8	85,566	-8.4	6,492	13.1
2006	25,353	-11.8	3,302	-9.8	79,403	-7.2	6,353	-2.1
2007	32,148	26.8	3,989	20.8	96,628	21.7	6,915	8.8
2008	33,189	3.2	4,178	4.7	97,991	1.4	7,143	3.3
2009	32,175	-3.1	4,505	7.8	95,000	-3.1	6,700	-6.2
2010	25,606	-20.4	3,636	-19.3	93,500	-1.6	6,750	0.7

Source: Research and fisheries statistic analysis of Department of fisherie

2.1 Biology of *Macrobrachium rosenbergii*

2.1.1 Taxonomy of *M. rosenbergii*

The giant freshwater prawn is classified in the animal kingdom. The scientific name of this species is *Macrobrachium rosenbergii* and the common name is giant freshwater prawn or giant river prawn. The taxonomy of freshwater prawn is shown below.

Phylum Arthropoda

Class Crustacea

Order Decapoda

Suborder Natatia

Family Palaemonidae

Subfamily Palaemoninae

Genus *Macrobranchium*

Species *M. rosenbergii* (de Man, 1879)

2.1.2 Life cycle and Distribution

Macrobrachium rosenbergii is widely distributed in the tropical and subtropical countries of the Indo-Pacific Region. Almost of the countries especially in South and Southeast Asia, the quantities inhabiting rivers, streams, lakes, swamps and even reservoirs which have connection with the sea. For the prawn lives in tropical freshwater environments that are influenced by near brackish water areas and often found in extremely turbid condition. Gravid females migrate downstream into estuaries where the eggs hatch as free-swimming larvae. From egg hatching until metamorphosis into postlarvae (PL) after metamorphosis, PL is approach a benthic

lifestyle and beginning to migrate upstream towards freshwater as shown in (Figure 2.1)

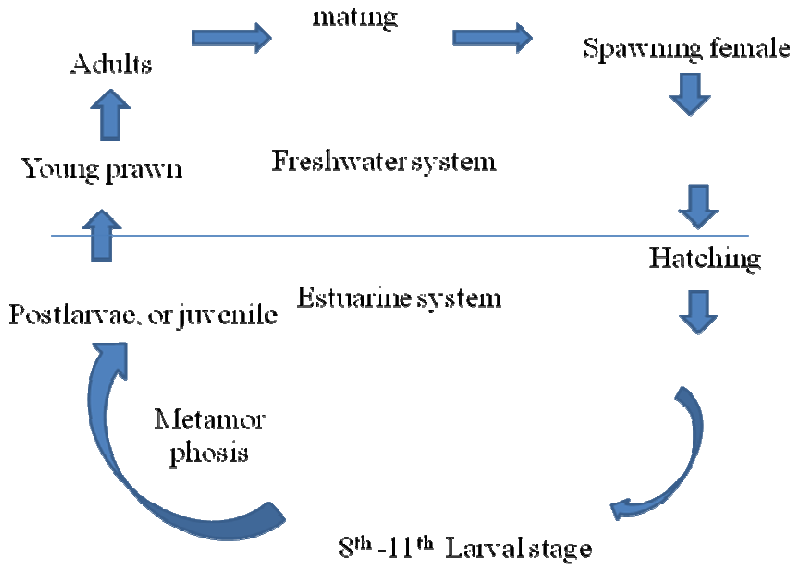


Figure 2.1 Life cycle of *M.rosenbergii* (สมเกียรติ ปียะธีรธิติวรกุล, 2521).

2.1.3 Morphology

The giant freshwater prawn is the largest species of the genus. The male can reach a total length (from tip of rostrum to tip of telson) of 32 cm, the female of 25 cm. The body is usually of a greenish to brownish grey, sometimes more bluish color. The antennae and chelipeds are often blue. The body of this species consists of two parts the cephalothorax commonly named the head and the abdomen as the tail. The carapace is smooth and hard, and it ends anterodorsally in the rostrum. The rostrum of this species is reaching beyond the antennal scale as showed in below Figure 2.2.

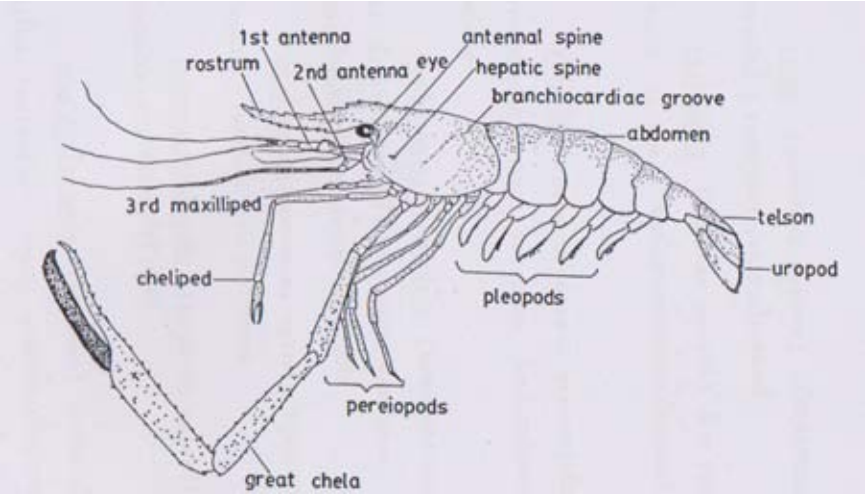


Figure 2.2 Morphology of *M.rosenbergii* (สมเกียรติ ปิยะธีรธิติวรกุล, 2521)

2.1.4 Characteristics of adults female

A female is comparatively smaller than a male with shorter and slender second thoracic legs, a smaller head and a spacious brood chamber. When the female is sexually ripe, the fully developed ovaries can be seen through the carapace. The swimmerets become slightly distended and arched outward to form a large “brood chamber” to adjust the abundant number of eggs to be laid. The basal segments of the pleopods, particularly those of the first three pairs are prolonged and are provided with branched setae on their inner margins which are developed during spawning to package the eggs which are attached in bundles to the setae through an adhesive substance as the eggs are extruded (Ismael and New 2000).

2.2 Female reproductive system

The internal reproductive structures of *M. rosenbergii* are located in the cephalothorax. In female, the paired ovaries are placed dorsally to the sromach and hepatopancreas. They are increase to a pair of oviducts which extend to the gonopores on the basal segment of the third pereiopods. Female prawn is express a brood chamber, is not appeared in penaied shrimp, as prawns never carry their eggs. Female freshwater prawn show reproductive setae on the pleopods and thorax that are mostly

pleopods guide the eggs during sprawning and the ovigerous setae that appear following a pre spawning moult and are used to secure the eggs to the pleopods for brooding (Nagamine and Knight 1980). Moreover, the female prawn can lay eggs between 80,000 to 100,000 eggs in each spawning when fully mature. However, their first broods are not over than 5,000-20,000 (New and singholka 1985). The fecundity was strongly correlated with total weight, length and carapace length.

2.3 Molting in crustacean

Molting or ecdysis is a process for growth, reproduction and metamorphosis that found both arthropod and crustacean. Crustaceans keep their old exoskeleton, resulting in somatic tissue growth. Somatic growth occurs during the intermolt period. In this phase, external dimensions occurring increase due to with the exoskeleton (Mauchline., 1977; Hartnoll., 1982). Female prawn growth is retarded after reach maturity, due to the fact that part of their energy is converted to egg production and incubation (Hartnoll, 1982). The growth rate of immature females is high, but after sexual maturation this rate reduces. Size variation decreases with time, different of female size distribution being replaced by maturation (Kurup *et al*., 1992). An adult *M. rosenbergii*, base on the hardness of the exoskeleton and internal setal development, has been identified by Peebles (1977). Molting process is according to Skinner (1985) and summarized below:

Premolt (Stage D): Calcium and other materials are reabsorbed from the old exoskeleton activating degradation, and a new exoskeleton is formed. Separation of the epidermis from the old exoskeleton is clear. Enlargement of the epidermal cells also appears.

Molt (Stage E): The animal emerges from the old exoskeleton with a newly synthesized and pliable one. Molting is accompanied by rapid uptake of water, resulting in an enhancement of animal size.

Postmolt (Stage A and B): Deposition of calcium and other materials resulting in the hardness of the exoskeleton.

Intermolt (Stage C): During the period between molts, the water absorbed during molting is gradually replaced by tissue growth, and organic and mineral reserves are stored.

Molting in crustacean is change in the biochemistry and physiology and have been reviewed by Chang (1995). In the premolt stage, ionic changes appear in different body compartments. Calcium, which is transferred from the old exoskeleton to the extracellular fluid, is excreted or carried to the hepatopancreas for storage. As a result, an increase in the concentration of calcium in the hepatopancreas and haemolymph is observed (Fieber and lutz, 1982). In the early postmolt stage, exoskeleton calcium increases while calcium levels in the extracellular fluid and hepatopancreas are decreased. In additional, the net water transport was change in midgut during the molt cycle that the water flux rates were elevated in animals approaching ecdysis and much reduced in the postmolt stage. The midgut may be the major site of water absorption at ecdysis (Fieber and lutz.1984).

2.4 Hormonal control between molting and reproduction in crustacean

Molting is one of the most important physiological process for crustacean growth since increase in body size occurs in a series of step associated with the casting of the old exoskeleton. Similarly, vitellogenin plays an important role in ovarian development because it is considered to be incorporated into the developing oocytes as a major component of yolk protein. The nature of the antagonism between reproduction and molt varies in the crustacean. It has been believed that both molting and egg development in decapods crustacean are under inhibitory hormonal control by neurosecretions released from the sinus gland (Gabe, 1966; Adiyodi and Adiyodi, 1970; Sochasky, 1973). The observation of apparently synchronous molting and reproduction in adult crabs (Panouse, 1947) led initially to the view that the processes were “synergistic” that a single hormone might control both.

2.4.1 Endocrine regulation

Essentially, the hormonal controlling mechanisms enabling the temporal separation of these two processes involve principally the inhibitory neuropeptides: vitellogenesis-inhibiting hormone (VIH) and molt-inhibiting hormone (MIH) that originating from the X-organ/sinus gland complex in the optic ganglia. It is well-known that eyestalk ablation induces ovarian development and oviposition. This is because the source (X-organ-sinus gland complex) of the VIH is removed by the ablation. VIH has been purified as a peptide in the *American lobster*, *Homarus americanus* and in the isopod, *Armadillidium vulgare* (Okumura, 2004). Thus, the hormonal coordination of both molting and vitellogenesis becomes vital to accomplishing continued body growth and increased fecundity. The endocrine factors that control vitellogenesis can be considered under two categories: gonad-inhibiting and gonad-stimulating hormones (Subramonium 2000).

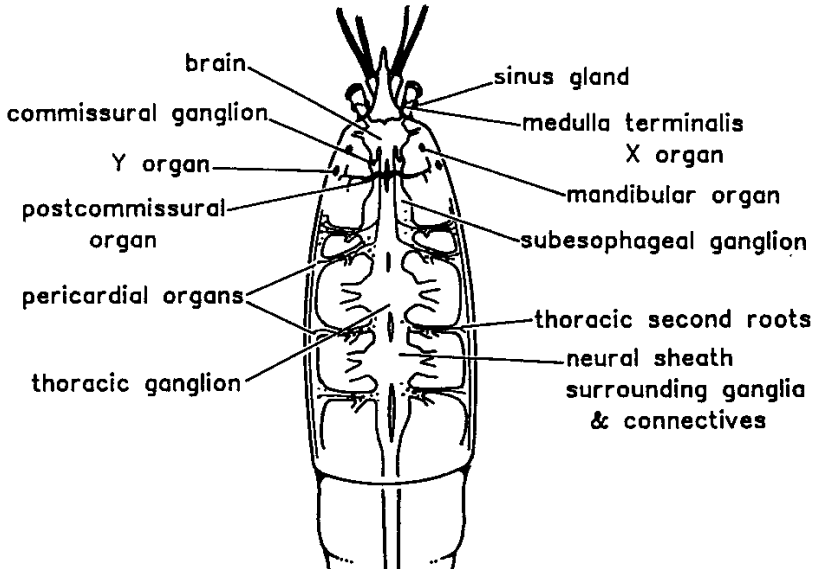


Figure 2.3 Major endocrine and neuroendocrine structures of generalized female crustacean. Included are the organs important for female reproduction, the eyestalk sinus gland x-organ, the mandibular organ, Y-organ, and thoracic ganglion (Laufer *et al*., 1991).

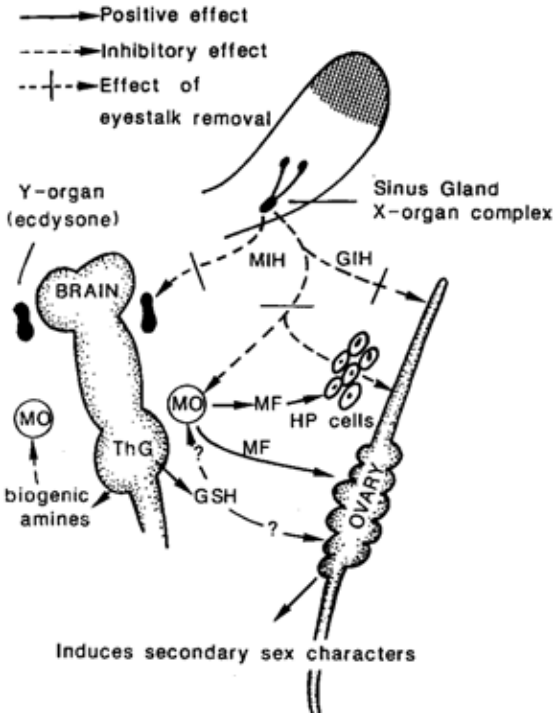


Figure 2.4 Major endocrine glands and their target tissue involve in crustacean female reproduction (Laufer *et al*., 1991).

2.4.2 Eyestalk neuropeptides from X organ-sinus gland

The neuroendocrine x organ-sinus gland complex in the crustacean eyestalk (Figure 2.4) has long been known to affect both molting and gonadal development. The eyestalk neurosecretory system of crustacean contains several peptide factors affecting various important physiological processes. These neuropeptides are synthesized in specific neurosecretory cells of the medullar terminalis X-organ (XO), stored, and released from the sinus gland (SG) in the eyestalk ganglia (XOSG complex). In addition, neuroendocrine complex produces several factors affecting important processes including sugar, calcium, and oxygen metabolism, color changes, and retinal pigment migration (Kleinholz, 1976). Quackenbush and Herrnkind 1983 reported that included of neuropeptide i.e. hyperglycemic hormone (CHH), putative molt-inhibiting hormone (MIH) and vitellogenesis or (gonad)-inhibiting hormone (VIH), (GIH) and these hormone synthesized by x-organ and mandibular organ inhibiting hormone (MOIH). Moreover, the neuropeptide transported via an axonal

tract to the principal neurohemal organ, the sinus gland (SG), where they are stored and released.

2.4.3 Homonal from Mandibular organ (MO)

The crustacean mandibular organ (MO) was first identified by Le Roux (1968) and has since been identified in a large number of crustaceans. Mandibular organs are composed of two types of cells, as opposed to the single type seen in Y-organs (Burghause, 1975). The cells of mandibular organs are connection with the epidermis is less obvious than in Y-organs. The mandibular organs are situated in a mandibulo-maxillar metameric position of the mandibles at the base of the inner adductor muscle tendon as shown in Figure 2.6 (Nagaraju., 2007). The mandibular organ secretes the sesquiterpenes methyl farnesoate (MF) and farnesoic acid (Laufer *et al*., 1987; Cusson *et al*., 1991). These are related to the 'juvenile hormones' produced by the corpora allata (CA) (Cephalic endocrine glands) of insects (Laufer *et al*., 1986, 1987; Tobe *et al*.,1989). Initial studies by several authors suggested that the MO might be involved in regulating reproduction (Le Roux, 1968; Hinsch, 1980), and molting (Aoto *et al.*, 1974; Byard *et al.*, 1975; Yudin *et al.*, 1980). However, MOIH are responsible for the negative regulation of MF synthesis and show inhibit MO activity that is presumed the function as MO inhibitor.

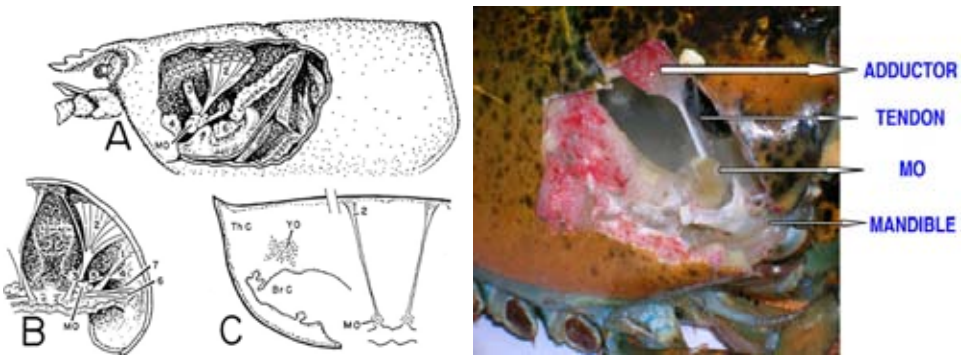


Figure 2.5 Location of mandibular organs (MO) and Y-organs (YO) in the crab *Carcinus* sp. and the lobster *Homarus* sp. Cephalothorax of adult *Homarus* viewed from lateral (A) and posterior (B) aspects, showing position of MO. Organs are also

shown in a diagrammatic transverse section of adult *Carcinus* (C) (Homola and Chang., 1997b) and mandibular organ location in *Homarus americanus* (Nagaraju., 2007).

2.4.3.1 Methyl farnesoate

MF is a compound similar to juvenile hormone (JH) in insects, which plays an important role in morphogenesis, metamorphosis, and reproduction (Laufer *et al*., 1997, 1998; Abdu *et al*., 1998; Sagi *et al*., 1994). Morphogenesis and reproduction in crustaceans may be regulated by a sesquiterpenoid hormone (MF), as in insects. A homologous organ of insect Corpora Allata (CA) is the MO in crustaceans. Both MOs and CA are ectodermally derived organs and produce similar biosynthetic products. MF(methyl-(2E,6E,10E)-3,7,11-trimethyldodecatri-2,6,10-eneoate) is a sesquiterpene that is structurally similar to insect JH III (methyl 9-(3,3-dimethyloxiranyl)-3,7-dimethyl-2,6-nonadienoate) hormone, differing only in the absence of an epoxide moiety at the terminal end as shown in Figure 2.7 (Nagaraju., 2007).

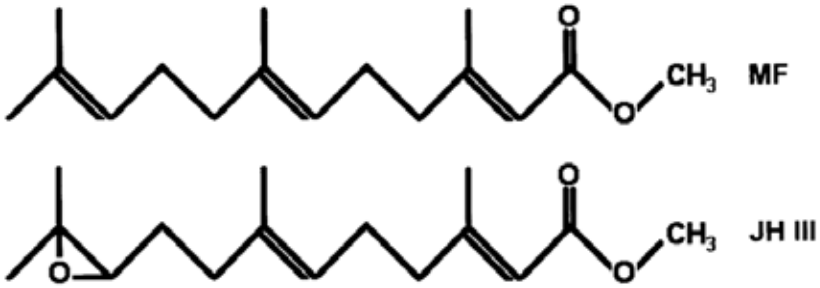
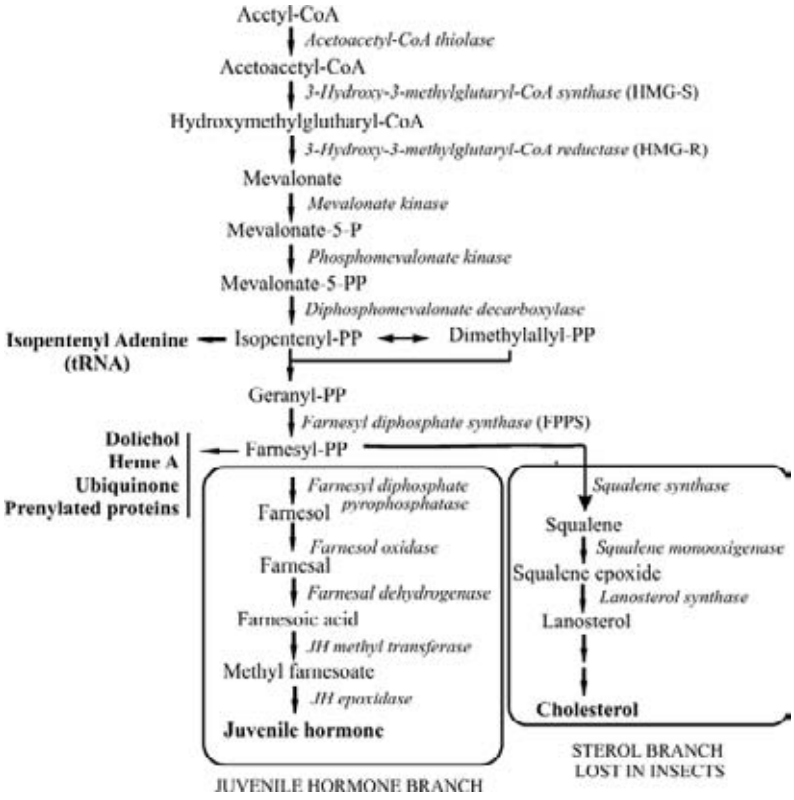


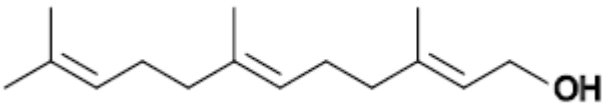
Figure 2.6 Chemical structures of methyl farnesoate (MF) and juvenile hormone III (JH III) (Nagaraju, 2007).

Methyl farnesoate or farnesoic acid (FA) is synthesized de novo by a ductless gland and MF is secreted into the hemolymph at nanomolar amounts. However, by analogy to the established functions of JH III in insects, MF has been suggested to play an important role in the regulation of growth and reproduction in crustaceans. Methyl farnesoate secretion appears to be correlated with the size of the animals. In the spider crab *L. emarginata*, larger animals (with larger MOs) had higher rates of MF secretion than smaller ones (Borst *et al*., 1987). Moreover, Sex specific differences in MF titer were confirmed in several species of crustaceans. In male lobster *H. americanus*, the MOs increase spectacularly in size after sexual maturity

(Waddy *et al*., 1995) and males have higher levels of MF (larger MO) in the hemolymph than females (Borst *et al*., 1987; Laufer *et al*., 1987b). Similar results were also found in crayfish *P. clarkii* (Landau *et al*., 1989), freshwater prawn *Macrobrachium rosenbergii* (Sagi *et al*., 1991) and crab *O. senex senex* (Nagaraju *et al*., 2004, 2006) indicated that the organs may function differently in the two sexes. Eyestalk ablation in several species had showed resulted in an elevation of MF levels. For instant, eyestalk ablation of male lobster *H. americanus* elevated MF titers in haemolymph that showed enzymes *FAMet* increase in activity after ablation (Li *et al*., 2010).

Farnesol is a precursor of MF, found structural an acyclic sesquiterpene, as a colorless liquid and miscible with oils. It has a molecular weight of 222.37 g/mol. In commercial available pure farnesol is 96 percent and has a maximum absorbance at 192-196 nm. In insect, juvenile hormone III is biosynthesized from farnesyl pyrophosphate (FPP) which is a key intermediate of the mevalonate pathway and also oxidation of farnesol to farnesal is an important step in insect juvenile hormone (JH) biosynthesis. Farnesol is a precursor of MF and elucidation of the role of circulating sesquiterpenes like MF in crustacean (Sperry and Sen, 2000).





Farnesol

Figure 2.7 Mevalonate pathway and chemical structures of farnesol(Sperry and Sen, 2000).

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The first study of farnesol metabolism, as it relates to JH biosynthesis in insects, was performed over 15 years ago (Baker *et al*., 1983). In tobacco hornworm *Manduca sexta*, indicate that farnesol oxidase of the corpus allatum plays a role in controlling JH homolog production in *M. Sexta*, and another oxidative enzyme, which is present at early stages of *M. Sexta* development, is involved in JH homolog construction (Sen *et al*., 2003). Moreover, female *M. sexta*, farnesol was sequentially converted to farnesal and farnesoic acid, and that acid formation was significantly enhanced by the addition of 2 mM NAD.

2.4.4 Effect of Methyl farnesoate and the immediate precursor of MF on ovarian development and/or molting of crustacean

2.4.4.1 Farnesoic acid (FA)

Farnesoic acid, the immediate precursor of MF may also be involved in gonad maturation, either directly or indirectly. It is also unclear how the MO regulates gonad maturation. To address these issues, MF or FA biosynthesis by the MO during the female reproductive cycle has been studied (Tiu *et al*., 2012).

Tiu *et al* (2012) showed possible functional correlation between farnesoic acid and methyl farnesoate production during lobster reproduction. The rates of biosynthesis of FA and MF were involved with maximal production occur during secondary vitellogenesis. The rates of biosynthesis of FA were low during the immature stages but began to increase as females matured which increased sharply and reached a maximum at 2756.2 ± 381.1 pmol/h then declined towards the end of cycle. Rate of MF biosynthesis was low at the beginning of the reproductive cycle (stages 1 and 2), and increased during maturation. However, maximal rates of MF biosynthesis at 1425.9 ±203.9 pmol/h. A potential juvenoid receptor, retinoid X receptor (RXR), *HaRXR*, was characterized using PCR cloning techniques.

Expression of *HaRXR* was also related to reproductive stage, and maximal level of expression was observed at stage 4, in which secondary vitellogenesis is occurring.

Tiu *et al* (2010) studied the effect of farnesoic acid (FA) and 20-hydroxyecdysone (20E) on production of vitellogenin by hepatopancreas gene (*HaVg1*). *HaVg1* was expressed by FA or 20E in a dose-dependent manner. A 2-fold and 2.2-fold increase in *HaVg1* gene expression which 4.2 µM FA and 0.7 µM 20E levels were observed. When increasing of FA concentration to 0.4 µM, resulted in a 2.5-fold increase in *HaVg1* gene expression. The increase in *HaVg1* gene expression reached a peak of approximately 4-fold compared to the control at 4.2 µM FA concentration. FA concentration at 42.3 µM–4.2 mM, showed in a decrease *HaVg1* expression level.

2.4.4.2 Farnesoic acid O-methyltransferase (FAMeT) in crustacean

Farnesoic acid O-methyltransferase (FAMeT) is the enzyme that conversed FA to MF in the final step of MF biosynthetic pathway. FAMeT catalyzes the methylation that transfer of a methyl group from S-adenosylmethionine to farnesoic acid for produce MF, similarly in the *O*-methyl transferase in the insect corpus allatum (Holford *et al*., 2004). FAMeT has detected in the MO of crustaceans (Claerhout *et al*., 1996; Wainwright *et al*., 1998). In both insects and crustaceans, O-methyltransferase plays a role in the regulation of MF synthesis and thereby mediated an effect on vitellogenesis and metamorphosis of these animals through MF.

Hui et al (2008), the *Litopenaeus vannamei* have two forms of *FAMeT*s (i.e. LvFAMeT-S and LvFAMeT-L), that analyzed *FAMeT* expression and its functions. LvFAMeT expression may be related to its role in growth and regulation of molting with RNA interference technique. Double stranded RNA for *LvFAMeT* knocked down *FAMeT* expression. *LvFAMeT* was knockdown, affected the regulation of two molt-relating genes, *cathepsin-L and the hemocyanin* gene, resulting in failure to molt and mortality. The role of *FAMeT* in regulation of MF production and *FAMeT* wide distribution in shrimp tissue suggested that the gene role might not be limited to only reproduction and molting-related function, and that their effect might be extensive.

2.4.4.3 Methyl farnesoate (MF)

Reproductive roles of MF in crustaceans are not universal, several evidence that MF has a stimulatory effect upon reproduction. While contrasting of MF inhibited ovarian developments was revealed in female, male and some crustacean species. In adult male crayfish *P. clarkii* investigated the hormonal control of these transitions in two ways. Eyestalk ablation accelerates molting and increases MF levels in the blood. These results were compared with two groups of Form I (intact animal) and II (eyestalk ablation) in each treatment (eyestalk intact, eyestalk ablated and eyestalk intact with MF). MF premolt in blood levels suggested that Form IIs were produced the presence 1.3 ng/ml of MF, while Form I result from MF levels less than 0.5 ng/ml is described by (Laufer *et al*., 2005).

In 2003, Nagaraju *et al* examined the effect of MF injection on gonad of female *Macrobranchium malcomsonii*. The result showed that increased the ovarian index at 0.54 ± 0.03 from the control at 0.34±0.04. Moreover, the oocyte diameter showed higher with MF injection at 10.2 ±2.69 µm than the control group at 5.6±1.86 µm. Maturation of the ovary also includes an increase in size, as the oocyte also increase in diameter due to yolk deposition. Administration of MF resulted in a significant ($p < 0.01$) increase in testicular index, whereas no significant change was observed in the testicular index of control prawns. These results provide evidence to support the hypothesis that MF has a role in stimulating gonad growth in crustaceans.

Paran and Tsukimura (2010) was studied the effect of MF administration on vitellogenesis of penaeoidean shrimp, *Sicyonia ingentis*, 0.1 and 1.0 µg concentration MF were injection. Haemolymph Vitellogenin (Vg) levels declined over the experimental period in all the treatments, the Vg levels decreased significantly only in the short-term treatment with 1.0 µg MF. The present MF data showed did not increase the Vg level in any of the treatments (short term and long term/0.1 and1.0 mg MF). According with, in the crayfish, *Cherax quadricarinatus*, Medesani *et al* (2011) examined the effect of methyl farnesoate on the vitellogenin content of ovary and hepatopancreas. The MF exposed in vitro to 0.15, 1.5, or 15 µM of MF. A significant accumulation of vitellogenin was showed in ovaries treat with 15 µM of MF during the early pre-reproductive period. On the other hand, no stimulating effect of MF on the vitellogenin content of the hepatopancreas was seen in any period. These results

support the hypothesis of the endogenous vitellogenin synthesis stimulated by MF during the early ovarian maturation but this hormone was not able to stimulate vitellogenin synthesis in the ovary during the reproductive period.

Additionally, MF plays a role in crustacean development that described by Borst *et al*., 1987 who investigated MF with *Libinia emarginata* and *Homarus americanus*. The treatment of lobster larvae with MF caused retardation when compared with untreated larvae from 22.0±1.3 to 20.8±1.5 days, respectively. Further, Abdu *et al* (1998a) administered concentration of MF (0.21, 0.35, 0.59 µg/ml) via an artemia vector in *M. rosenbergii* larvae. Higher MF levels caused earlier retardation of late larval growth, and the highest dose retarded larval development. The control group and the larvae fed on Artemia that were enriched with the lowest concentration of MF exhibited the fastest grown, development was significantly ($P< 0.001$) retarded when compared with control group. After day 10, developmental progress was again markedly slower, and on day 12 the average stage of this treatment group was significantly behind that of the control group ($P < 0.001$). According with, Abdu *et al* (1998b) administered of MF through the artemia vector on *M. rosenbergii* larvae. The result showed that MF freshly administered daily to larvae caused retardation. The control group grew fastest after 8 days the average carapace length was over 1 mm, and after 14 days the average carapace length was 1.2 mm but in larvae fid on artemia enriched with higher doses of MF (0.11 and 0.20 µg MF/ml) grew significantly slower than the control group, starting from 4 day ($P < 0.001$).

In several studies indicated three major physiological effects of MF as a regulation of reproduction, molting, and juvenile development. The reproductive roles of MF in crustaceans are not universal. Several evidences express that MF may have a stimulatory effect upon reproduction in both female and male crustaceans but in several contrasting result. For example, in 2008, Marsden *et al* was indicated an inhibitory role for MF in the late stage of ovary development on the shrimp *Penaeus monodon*. MF was orally administered to ablated *P. monodon* at a concentration of 5.5 µg MF per gram of diet. Three diets were evaluated in a natural diet, a formulated maturation diet (named BIARC) and the BIARC+MF diet. A range of reproductive performance criteria was measured. The addition of MF to the artificial diet

significantly reduced the number of spawns per prawn from an average of 3±0.4 (BIARC) to 1.8±0.3 (BIARC+MF) and also significantly ($P<0.01$) reduced average fecundity of the first three spawns from 4.1 thousand to 3.2 thousand eggs per gram of prawn. Following with, Tsukimura *et al* (2006) examined whether MF which administered to juveniles by ingestion via biological vector (*Artemia nauplii*), MF-coated food pellets, and MF liposome food pellets in the tadpole shrimp, *Triops longicaudatus*. Pellets were made containing three MF concentrations by weight composed of 0.1 µg, 1 µg, and 10 µg per gram of diets. At day 5, MF treatment significantly decreased the number of oocytes from a mean of 33.2 oocytes in controls to 16.5 oocytes in the MF-treated. Similarly, at day 10, the MF artemia vector decreased ovarian weights in the treatment group (mean = 0.6 mg) as compared with controls group (mean =1.1 mg). However, MF 10 µg/g titer decreased both ovarian weights and lengths as compared to controls.

MF is a unepoxidated form of the insect JH III that has been found inmore than 35 crustacean species. The physiological role of this compound (MF) has been subject to contest (Nagaraju, 2007). In the Arthropoda, hormonal controls of reproductive processes have been established. In the Class Insecta (subphylum Uniramia), Juvenile Hormone III has a dual role: (1) in juveniles, depressing the rate of juvenile metamorphosis and inhibiting reproduction; and (2) in adults, acting as a necessary component of oocyte production. Many juvenile hormone analogs have been shown to have effects on metamorphosis and reproduction in the Crustacean. It is a possibility that MF mimics the function of a similar chemical that is a native regulator of development. However, of the known juvenile hormone terpenoids, only farnesoic acid (FA) and MF, two successive precursors to JH III, have been detected in significant levels in the Crustacean. Additionally, previous studies have shown that the final enzyme (*FAMeT*) in the MF synthetic pathway is highly regulated, suggesting that MF is the physiologically active terpenoid (Tsukimura, 2006).

2.5 Encapsulation

Encapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material, and the coating material is called shell, wall material, carrier or encapsulant (Madene *et al*., 2006). The development of microencapsulation products started in 1950s in the research into pressure-sensitive coatings for the manufacture of carbonless copying paper (Green and Scheicher, 1955). Encapsulation technology is now well developed and accepted within the pharmaceutical, chemical, cosmetic, foods and printing industries (Augustin *et al*., 2001; Heinzen, 2002). In food products, fats and oils, aroma compounds and oleoresins, vitamins, minerals, colorants, and enzymes have been encapsulated (Dziezak, 1988; Jackson and Lee, 1991; Shahidi and Han, 1993). An encapsulation matrix should have good solubility in water, emulsifying properties, drying properties and low viscosity at high solids concentration (Kim et al., 1996; Reineccius, 1988). In 1999, Ribeiro *et al*, was studied on chitosan coated with alginate in lipophilic drugs which Dye release into simulated gastric at pH 1.2 and intestinal at pH 7.5 media (USP XXIII) was determined. Drugs release within the first hour under gastric conditions was lower than 10% of the total release. In simulated intestinal fluid, dye release was rapid to levels approaching 85% of total release within 10–15 min. Less than drugs release observed with uncoated microspheres, reaching a maximum of 35% after 2 h. The level of release is depended on the molecular weight of chitosan. While dye release was lower in comparison to uncoated microspheres, it reached 90% of the total release after 10 min and the membrane thickness be expected to have an influence on the release profile of encapsulated dye. For example, 75 kDa chitosan, a more rapid release of dye was obtained under intestinal conditions for membrane coating times of 10 min compared to 30 min. Dye release approached percentage of 35 and 18, respectively. Chitosan membranes are microporous with the pore size increasing with pH levels above 6 due to electrostatic repulsion For chitosan molecular weights less than 300 kDa, a longer coating time results in thicker membranes reducing the release rate. In intestinal fluid, alginate matrix erosion leads to a total dye release within 0.5 h whereas in coated microspheres, dye release was limited by membrane diffusion. An optimum dosage

form must deliver drug immediately and in a controlled fashion over 8–10 h to respond to the short half-life of the lipophilic drug's availability (Lee and Min, 1996).

2.5.1 Biopolymer for encapsulation

2.5.1.1 Alginates

Alginate is a family of linear anionic polysaccharides, which can be considered as copolymers of (1-4) linked β-L-guluronic acid (G) and β-D-mannuronic acid (M) residues. The linear chains are composed of homopolymeric regions of G- and M-blocks interspaced with regions of mixed sequences, MG-blocks as shown in Figure 2.8 (Zuidam and Shimoni, 2010). The structure shows a part of a sodium alginate chain. Depending on the seaweed extract, the isolation process, or the biotechnological procedure, the proportion and sequential arrangement of the two structural units vary widely. M and G blocks of various lengths can be present in the polymer chain. The selectivity for cation binding and gel forming properties strongly depend on the composition and sequence. Divalent cations preferably bind to the G-blocks. The ability to form ionotropic gels is based on this selective binding of cations. Therefore, solution viscosity was strongly that depend on the ionic strength (Patil *et al*., 2010).

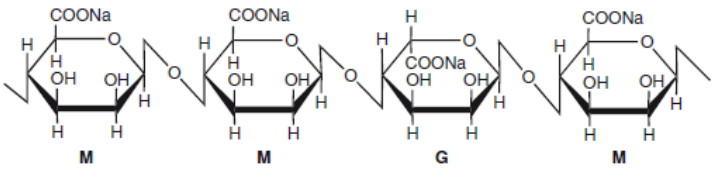

Figure 2.8 Chemical structural units of a sodium alginate polymer chain

2.5.1.2 Chitosan

Chitosan is a linear polysaccharide, which can be considered as a copolymer consisting of randomly distributed β (1-4) linked D-glucosamine and N-acetyl-D-glucosamine as shown in Figure 2.9. The composition is indicated by the degree of acetylation (DA), the fraction of acetyl-glucosamine units. The molar mass depends on the source and the isolation technology (Zuidam and Shimoni, 2010). Chitosan is

a biopolymer which could be used for the preparation of various polyelectrolyte complex products with natural polyanions such as xanthan, alginate, and carrangeenan. The strong electrostatic interaction of amine groups of chitosan with the carboxyl groups of alginate lead to the formation of chitosan-alginate complex. The chitosan-alginate gel beads with a chitosan core and a chitosan-alginate skin are prepared by dropping a solution of alginate into chitosan solution. Due to the protonation of amino group on chitosan and the ionization of carboxylic acid group on alginate, the stability of chitosan influenced by the environmental parameters such as pH and ionic strength (Patil *et al*., 2010). Mechanical Strength of chitosan found the tension strength that prepared from chitosan 90 % DD increase molecular weight of chitosan and had a higher mechanical strength. The effect of low molecular weight polymer did not have sufficient cross-link network to form films. Moreover, chitosan polymer had a swelling property which all polymers dissolved in pH 1.2 HCl-KCl buffer and normal saline. Molecule of chitosan is formed in acetate salts during film formation in acetic acid solution (Nunthanid *et al*., 2001).

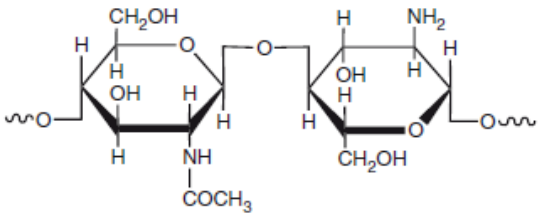

Figure 2.9 Chemical structure of chitosan, deacetylated (right) and acetylated (left) chain units.

2.5.2 Encapsulation process

2.5.2.1 Emulsification

Emulsions are kinetically rather than thermodynamically stable two-phase systems and ultimately, both the oil and water phase will separate. Proper formulation design of both phases and the interface, including choice of ingredients like emulsifiers, might prevent that (McClements 2005; Appelqvist *et al*., 2007). Emulsions are commonly made under high shear with, e.g., homogenizer, colloid mill, high shear mixer, or stirred vessel preferably equipped with baffles (Figure 2.10).

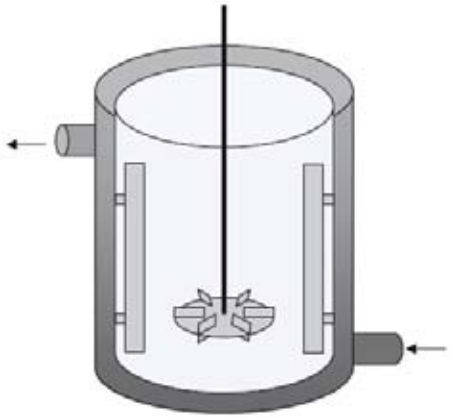

Figure 2.10 Set-up of a stirred, double-wall vessel with 3–4 baffels and Rushton-impeller, which might be used for the preparation of emulsions or complex coacervates. This set-up can be used at both a lab scale and a factory scale.

Water soluble food active agent might be encapsulated in water-in-oil (w/o) emulsions or double emulsions of the type w/o/w (Appelqvist *et al*., 2007). Furthermore, oil-in-water (o/w) emulsions may affect taste (e.g., salt) by changing the aqueous phase volume and thus the concentration of taste molecules in water, and by suppressing contacts of salt with taste receptors. Lipophilic active agent (e.g., aroma, cartonenoids such as lycopene and beta-carotene, plant sterols, vitamin E, dietary fats) might be protected and delivered to consumers via o/w emulsions. Oil-in-water emulsions might be dried by, e.g., spray-drying or freeze-drying to provide a powder. Such dry emulsions might be encapsulates or an instant formulation of beverages or

other food products. Emulsion droplets might also be prepared during the processing of encapsulates (such as extrudates or co-extrusion, or act as templates for further processing such as complex coacervates, microspheres or emulsions with multi-layers (Zuidam and Shimoni, 2010). A layer around "primary" emulsions with ionic emulsifier(s) can be formed by adsorbing oppositely charged polyelectrolytes to form "secondary" emulsions with a two-layer interface. This procedure can be repeated to form emulsion droplets with three or more layers at their interface (Guzey and McClements, 2006). Removal of excess free polyelectrolytes by, e.g., centrifugation or filtration between the steps might be necessary.

2.5.2.2 Coacervation

Coacervates are made via a liquid–liquid phase separation mechanism of an aqueous solution into a polymer-rich phase and a polymer-poor phase. According to the number of polymer type(s) present, the process can be identified as (simple) coacervation when only one type of polymer is involved or complex coacervation when two or more types of polymers of opposite ionic charges are present. Complex coacervates are commonly made from an o/w emulsion with gelatin and gum arabic at a 1:1 w/w ratio and at a 2–4% w/w of each polymer dissolved in the water phase via adjusting the pH from neutral to about 4 under turbulent conditions in a stirred vessel Zuidam and Shimoni (2010) (Figure 2.11). A mixture with two phases was formed, one of which was the heavier phase containing the encapsulated material (Madene *et al*, 2006). This creates three immiscible phases (oil, polymer-rich, and polymer-poor phase), and the polymerrich phase droplets will deposit on the emulsion surfaces because of interfacial sorption. Alternatively, complex coacervation can be induced by dilution instead of pH adjustment; oil is emulsified in 8–11% (w/w) gelatin solution, followed by addition of gum arabic and dilution water (Thies, 2007). Simple coacervation has been used less to encapsulate active agent. Examples are the encapsulation of o/w emulsions in gelatin where solubility is reduced by temperature or sodium sulfate, in 0.2 wt. % chitosan by increasing the pH with 0.1–1.5 wt. % sodium hydroxide (Hsieh *et al*., 2006).

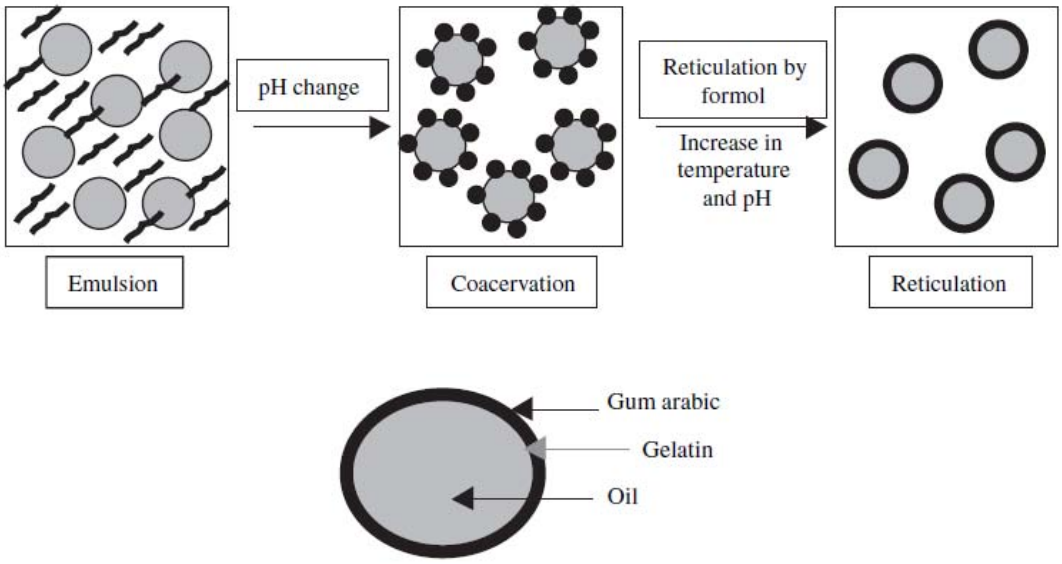

Figure 2.11 Principle of the complex coacervation method (Madene *et al*., 2006).

2.5.2.3 Freeze drying

The freeze-drying technique, which is lyophilization, is one of the most useful processes for drying thermosensitive substances that are unstable in aqueous solutions. In this process, upon water crystallization, the non-frozen solution is viscous and the diffusion of flavours is retarded. Upon starting freeze drying, the surface of the solution becomes an amorphous solid in which selective diffusion is possible. Freeze drying is the process that gives the most desirable properties to spray-dried powder (Madene *et al*., 2006). Active agent and carrier material dissolved in water can be freeze-dried to produce a porous, nonshrunken structure. Firstly, the sample is frozen at temperatures between -90 and -40 ºC and then dried by direct sublimation under low pressure and reduced temperature (between -90 and -20 ºC). After drying, the brittle cake obtained can be broken into smaller pieces by, e.g., grinding, if necessary. The major disadvantages of freeze-drying are the high energy use, the long processing time, and the open porous structure obtained, which is in general not a very good barrier between the active and its surroundings. Compared to spray-drying, freeze-drying is up to 30–50 times more expensive (Gharsallaoui *et al*., 2007).

2.5.3 Complex of encapsulation

Microspheres are microbeads composed of a biopolymer gel network entrapping an active. The microspheres are commonly prepared in the presence of the active, but post loading of blank microspheres containing oil droplets with, e.g. aroma is also an option. Calcium-alginate gel is the best known gelling system used for the preparation of gel beads to encapsulate a wide variety of active agent, such as oil droplets containing aroma, cells, probiotics, yeast, or enzymes to name a few. These active agent are relatively large in size, as smaller ones will diffuse easily through the porous biopolymer network. Gelation of alginate in the presence of divalent cations can be easily controlled and does not require heating like other gelling biopolymers like agarose, agar, or carrageenan (Madene *et al*., 2006).

2.5.3.1 Ionotropic gelation technique

Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogels. Since, the use of alginates, gellan gum, chitosan, and carboxymethyl cellulose for the encapsulation of drug and even cells, ionotropic gelation technique has been widely used for this purpose. The natural polyelectrolytes inspite, having a property of coating on the drug core and acts as release rate retardants contains certain anions on their chemical structure. These anions forms meshwork structure by combining with the polyvalent cations and induce gelation by binding mainly to the anion blocks. The hydrogel beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuses into the drug-loaded polymeric drops, forming a three dimensional lattice of ionically crossed linked moiety. Biomolecules can also be loaded into these hydrogel beads under mild conditions to retain their three dimensional structure (Patil *et al*., 2010).

2.5.3.2 Polyelectrolyte complexation technique

The quality of hydrogel beads prepared by ionotropic gelation method can also be further improved by polyelectrolyte complexation technique. The mechanical strength and permeability barrier of hydrogels can be improved by the addition of oppositely charged another polyelectrolyte to the ionotropically gelated hydrogel

beads. For instance, addition of polycations allows a membrane of polyelectrolyte complex to form on the surface of alginate bead. Large numbers of natural and chemically modified polyelectrolytes have been investigated and a schematic diagram of the preparation of hydrogel beads through ionotropic gelation and polyelectrolyte complexation (Patil *et al*., 2010).

CHAPTER III

MATERIALS AND METHODS

3.1 Equipments and chemical use

- Beaker, model: 50, 250, 600 and 1000 ml, Pyrex®, Germany
- Automatic micropipette, model: P200 and P1000 µl, Gilson, France
- Pipette tips (catalog# 10040), model: 200 and1000 µl, Sorenson, Bioscience, Inc., USA
- Spectrophotometer, model : Lambda 25, perkin elmer instruments, USA
- Spectrophotometer, model :Milton Roy, USA
- Flask, model: 50, 500 and 1000 ml, Schott Duran, Germany
- Hot plate stirrer, Schott, Germany
- Mixture, model: Ultra-Turrax 25 ,Janke and Kunkellka- Labortechnik, Germany
- Blender, model: Mulinex, USA
- Cuvette, model: 1.5 ml, Sterna scientific Ltd.
- Freeze-drier, Labconco freeze dry system, USA
- Pellet machine, model: California pelleting mill,CO., USA
- Test tube, model: 3 ml, Pyrex, Mexico
- Vortex mixer, model: MS I Minishaker, IKA-works, Inc., USA
- VBC test kit
- Centrifuge, Falcon 6/300, MSE Laboratory Centrifuges., UK
- Lipid extract thimble: soxtherm., Gerhardt, Germany
- Dry oven: Fisher scientific, USA
- Filter paper, Whatman No 1, whatman international Ltd., England
- Farnesol (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol, $C_{15}H_{26}O$, M.W.= 222.36634 (g/mol), Sigma-Aldrich, USA
- Chitosan, %DD 91.4 %, Union chemical 1986 Co., LTD, Thailand
- Sodium alginate powder, Union chemical 1986 Co., LTD, Thailand

- Acetic acid, CH_3COOH, 60.05, Merck., Germany
- Calcium cholride, $CaCl_2 \cdot 2H_2O$, 147.02, Ajax finechem Ltd., Australia
- Petroleum ether, Mallinckrodt chemical., USA
- Hydrochloric acid, HCl, M.W. = 36.46, J.T baker., USA
- Sulfuric acid, H_2SO_4, M.W.= 98.08., QREC., New Zealand
- Coppersulfate, $CuSO_4 \cdot 5H_2O$, M.W.= 249.70, Merck., Germany
- Sodium hydroxide, NaOH, M.W.= 40.00, Merck., Germany
- Boric acid**,** H_3BO_3, M.W.= 61.83, Ajax finechem Ltd., Australia
- Acetronitrile, M.W. = CH_3CN, M.W.= 41.05, Lab scan analytical science, Thailand

3.2 Measuring concentrations of farnesol by spectrophotometry

Farnesol in oil form as a precursor of methyl farnesoate and purchased from Sigma-Aldrich, USA. (*trans,trans*-Farnesol, density 0.887 g/ml at 25 °C). Farnesol was detected at maximum wave length (nm) by spectrophotometer as shown in Table 1A at Appendix A. The maximum wave length are used to make the standard curve, plotting farnesol concentration on the X axis, and assay the maximum absorbance (A_{max}) measurement on the Y axis. Farnesol was diluted by acetronitrile solution that the original stock expressed a ratio in 600 farnesol: 3,600 acenitrile µl or 140.42 mg/ml and had seen formulated in Appendix A. After that, the stock solution was prepared in 8 tubes from different volume of the farnesol. Tube 1 had a farnesol stock 100 µl. Therefore, the tube 1 had a farnesol in 14.042 mg and the quantities of farnesol mg were found in all 8 tubes is shown in Table 2A of Appendix A. Standard curve made from difference ratios of the quantities of farnesol (mg) in acetonitrile solution that were diluted from farnesol stock and measured an absorbance Milton Roy spectrophotometer at A_{310} nm.

3.3 Encapsulation process

3.3.1 Preparation of the encapsulate solution

Sodium alginate powder was dissolved in distilled water and intensively mixed to 0.67, 2 and 3.33 % (w/v) of alginate solution. Solution was stood for at least 1 hour without aeration. A calcium chloride 0.4 M dehydrate solution was dissolved in distilled water. Chitosan solution 1% (w/v) was prepared in acetic acid solution.

3.3.2 Preparation of emulsion

Following by Ribeiro *et al*. 1999, 200 ml of alginate solution was dispersed in a beaker without baffles by an impeller at a speed of 800 rpm was used to mix the solution for 3 min with high speed homogenizer Ultra-Turrax T25 for encapsulation process. The farnesol (oil form) was droplets to the alginate solution during mixing with high speed homogenizer for 20 min at 2400 rpm to desired concentration as shown in Table 3.1.

Table 3.1 The ratio of farnesol and sodium alginate.

Sample	Initial of farnesol (g)	Alginate solution (%w/v)
1	0.5	0.67
2	1	0.67
3	2	0.67
4	1	2
5	3	3.33
6	5	2

3.3.3 Encapsulation of farnesol

After 5 min emulsification, 150 ml of the alginate-oil emulsion was assembled by 0.4 M calcium chloride gelling solution and agitated by Ultra-Turrax T25 to produce the microencapsulation capsule at 9500 rpm. The mixing rate was varied from 800 to 9500 rpm after stirrer of gelling solution. Moreover, homogenized emulsion was coated with 1% (v/v) chitosan solution and gradually mixed by magnetic stirrer at 40 min. The microencapsulation was varied comparison in

different ratio of farnesol (g) per sodium alginate (% w/v) is shown in Table 3.1 and the process can be explained in Figure 3.1.

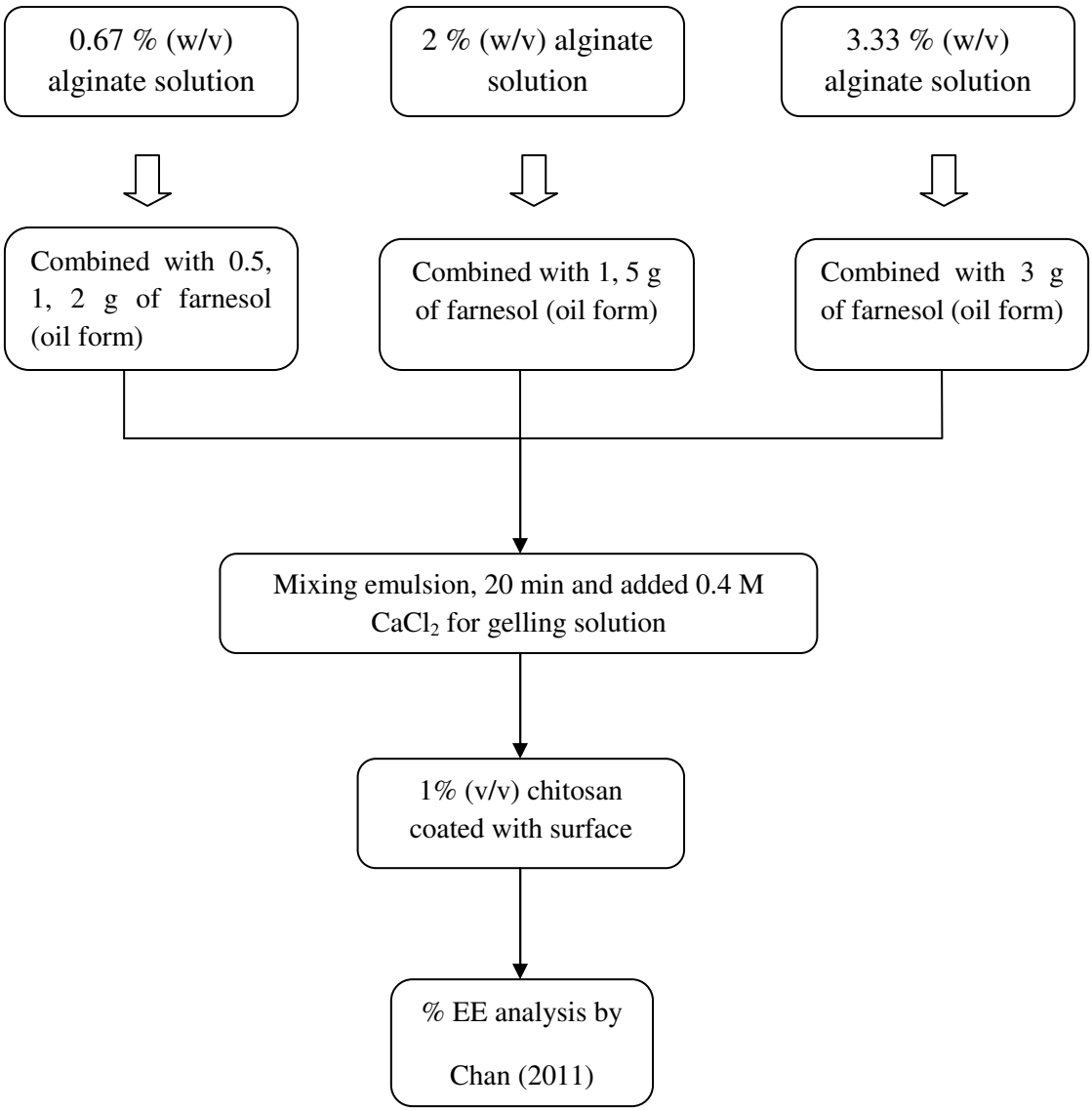

Figure 3.1 Encapsulation process of farnesol in oil form.

3.3.4 Determination of encapsulation efficiency before drying (% EE_{BD})

The encapsulation process was followed by Chan (2011). The microencapsulation beads were separated from the gelling solution using a sieve. Encapsulated beads were washed with acetonitrile solution to remove dissolved surface oil. Free oil removed on the surface of gelling solution as well as the surface on the wet beads. Non-encapsulated farnesol (oil) before drying was measuring the quantities farnesol on surface with serial dilution (0, 2, 4, 8, 16, 32 fold) and determined by spectrophotometer at A_{310} nm. The difference ratio between initial amount of farnesol (W_1) and the non-encapsulated farnesol before drying (W_2) was the amount of farnesol encapsulated (W_3), as shown in Equation 1. The encapsulation efficiency before drying (% EE_{BD}) was showed as the percentage of oil encapsulated with aspect to the initial of oil used, as shown in Equation 2.

$$W_3 = W_1 - W_2 \qquad \text{.....} (1)$$

$$EE_{BD}\,(\%) = \frac{W_3}{W_1} \times 100\% \qquad \text{.....} (2)$$

A standard curve was determined using absorbance of farnesol mixed in acetonitrile (v/v). A blank sample of acetonitrile was used. Absorbance of the acetonitrile was measured with spectrophotometer at 310 nm in acryl cuvettes (Sterna scientific Ltd., dimensions 10×10×48 mm). This wavelength was found to be maximum absorbance of farnesol.

3.3.5 Freeze-drying

The microencapsulation beads were frozen in foil paper at -50 ºC on 24 hours. After freeze-drying the emulsions were stored in vacuum-sealed desiccators at room temperature. For this experiment, the dried bead was powdered by mulinex blender.

3.3.6 Determination of encapsulation efficiency after drying (%EE $_{AD}$)

After dried process, the total weight of dried particles were measured and 1 g were dissolved in acetonitrile 20 ml and agitated with magnetic stirrer at 5 min. Encapsulated farnesol was extracted with acetonitrile by heating the samples in beaker at 50ºC for 15–20 min. Beside, the difference between the amount of oil encapsulated before drying (W_3) and the non-encapsulated oil after drying (W_4) gives the amount of oil encapsulated after drying (W_5), as shown in Equation 3. The encapsulation efficiency after drying (%EE $_{AD}$) was revealed of oil encapsulated (W_5) with aspect to the amount of oil encapsulated before drying (W_3), as shown in Equation 4.

$$W_5 = W_3 - W_{4s} \quad \text{..... (3)}$$

.

$$EE_{AD}\,(\%) = \frac{W_5}{W_3} \times 100\% \quad \text{..... (4)}$$

3.3.7 Determination of the extractable oil (% extractable oil)

The extractable oil was exhibited after grinding of freeze-dried particles by a pestle and mortar. One gram of powder was dissolved in 20 ml acetonitrile, beaker and mixed with magnetic stirrer at 50ºc, 15 min, and centrifuged at 3000 rpm, 20 min. The extractable oil (%) was determined at 310 nm by spectrophotometer. The amount of extractable oil was calculated between the weights of beads before and after extraction. It was obvious calculated as the percentage of the extractable oil (W_6) with respect to the amount of oil encapsulated before drying (W_3), as shown in Equation 5. A flow chart of encapsulation efficiency and extractable oil determination is shown in Figure 3.2.

$$\text{Extractable oil (\%)} = \frac{W6}{W3} \times 100\% \quad \text{..... (5)}$$

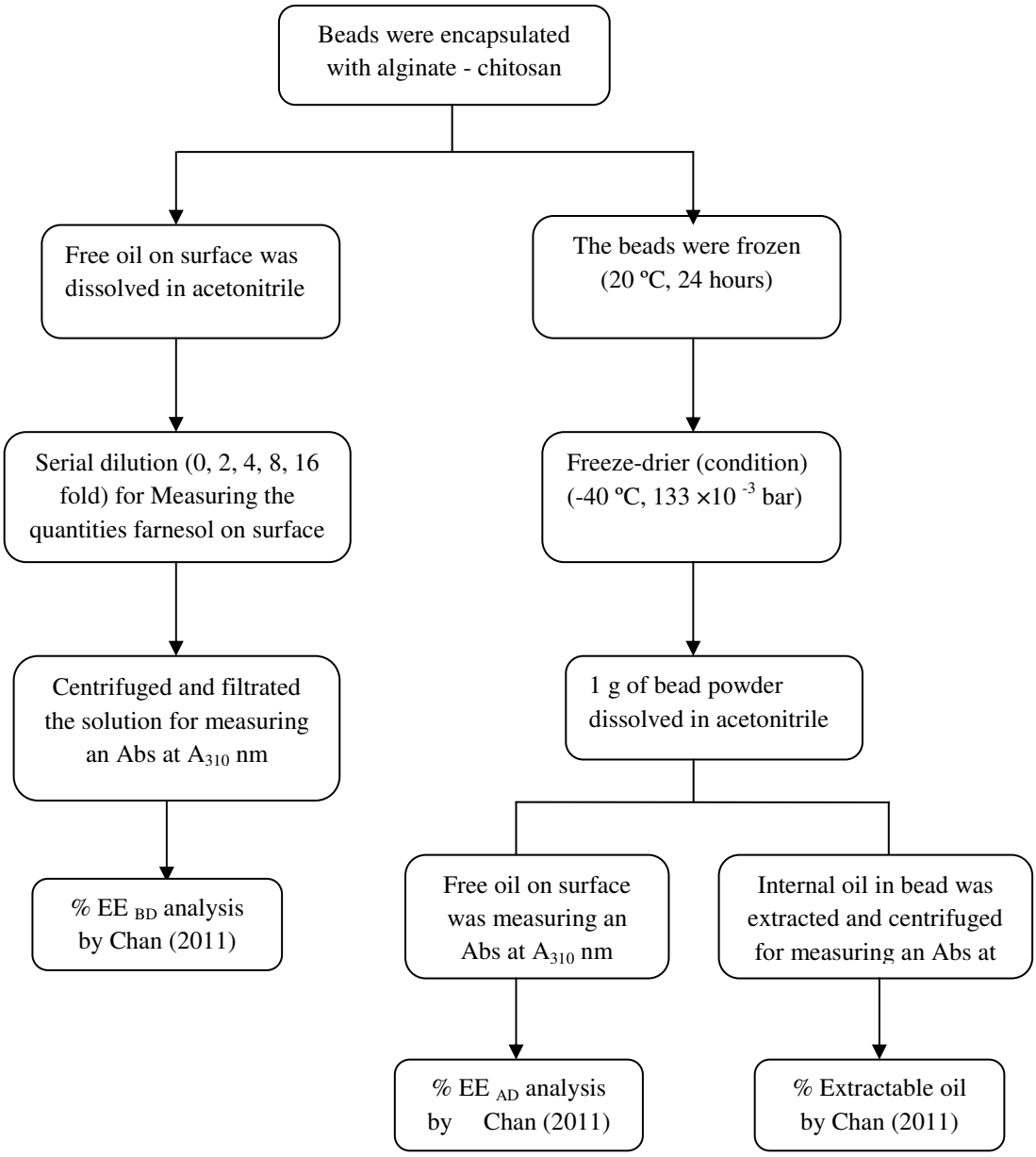

Figure 3.2 Encapsulation efficiency and percent of extractable oil

3.4 Formulation of prawn diet with farnesol

After encapsulation process, the encapsulated farnesol was divided to 0.5, 1, 2 g farnesol per 100 gram of diet and balanced ratio with alginate-chitosan particle. The diet ingredients are shown in Table 3.2 and pelleted by California pelleting machine at a size of 0.2mm × 5mm. The pellet was steamed 90 ºC, 5 min and oven dried at 90 ºC, 2 hr. The pellet was assorted from the excessive using a sieve and kept in dry bag at 4 ºC 10 g of each diet was sampled and proximate analysis was determined using AOAC (1980) method are described in Appendix C.

Table 3.2 The ingredients ratio of prawn diets.

Ingredients (grams)	**Treatments**			
	1	2	3	4
Fish meal	38	38	38	38
Shrimp meal	4	4	4	4
Wheat flour	25	25	25	25
Soy bean meal	15	15	15	15
Wheat gluten	5	5	5	5
Fish oil	3	3	3	3
corn oil	3	3	3	3
Vitamin mix	2	2	2	2
Minerals	2	2	2	2
Lecithin	1	1	1	1
Alginate-chitosan	2	1.5	1	0
Farnesol	0	0.5	1	2

* Vitamins (% Kg^{-1} diet): vitamin A 107 IU, vitamin D 106 IU, vitamin E 0.01%, vitamin K 0.001%, vitamin B_1 0.0005%, vitamin B_6 0.01%, Methionin 0.016%.

**Minerals (% Kg^{-1} diet): dicalcium phosphate 14.7%, phosphorus 14.7%, manganese oxide 1.0%, copper sulphate 0.36%, iron sulphate 0.20%, potassium iodide 0.10%, cobalt sulphate 0.10%, selenium oxide 0.006%.

3.5 Giant freshwater prawn preparation

One hundred and eighty matured female giant freshwater prawn with average body length at 10 cm were obtained from local farm in Ratchaburi province, Thailand. Immidiatly after prawn were arrived at Ang Sila Station, Department of marine science, prawn were acclimated in blue fiber tank with aeration. Prawn were fed before starting the experiment in a control diet 3 times per day at 8.00 am, 12.00 am and 6.00 pm at amount of 5% total body weight.

3.6 Farnesol dietary test on female prawns

After acclimating 2 weeks, the prawn were sampled and transferred the experiment unit (0.5 × 1.5 × 0.5 m) and fifteen prawn per replicate were used. The experiment was run in 3 replicates. Prawn were fed 3 times at 8.00 am, 12.00 am and 6.00 pm with a feed as randomly to the designed treatment. Every morning, unfed feed and feces were siphoned out to clean the experimental units. The experiment was run for 2 months. All prawn were number tagged individually on carapace for identification. During rearing period, prawn size was determined at initial and monthly. Molting and egg spawning time, number and weight of eggs per spawn were determined. Biological parameter measurement for this study is described in Table 3.3.

Table 3.3 Female prawn parameters study

Parameter	Detail of study
Survival rate (%)	= 100 × (final prawn number / initial prawn number)
Weight gain(%)	= 100 × (final prawn weight – initial weight/initial weight)
Percent of females spawned (%)	= 100 × (number of spawns / total female)
Egg production weight (g)	= Weight of egg (gram)
Molting periods (day)	= Time at prawns molt – Time at start as day
Length gain (%)	= 100 × (Final prawn length – initial length /initial length)
Specific growth rate (%)	= 100 × [ln final prawn weight – ln initial weight / culture periods (day)]

3.7 Culture system

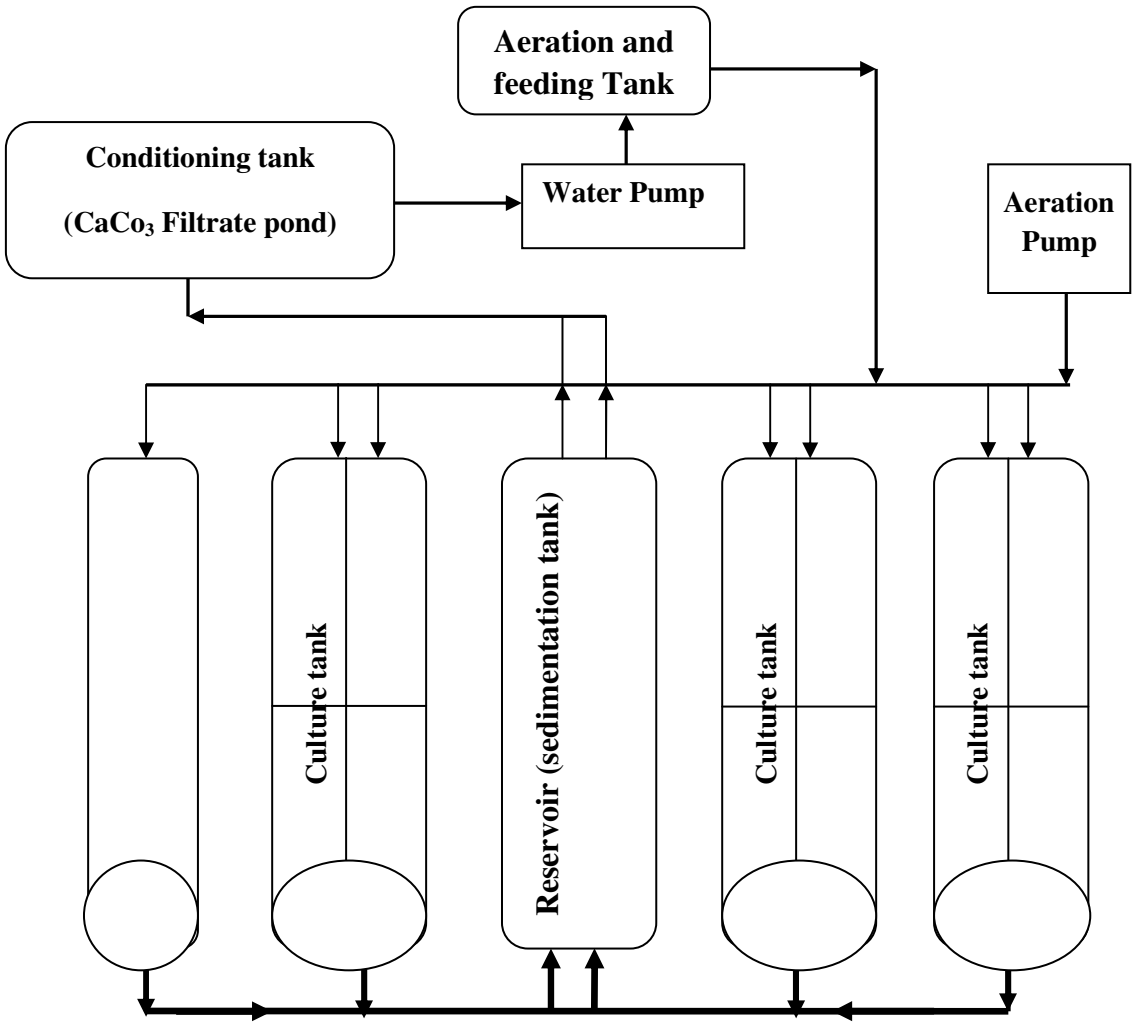

Figure 3.3 Water re-circulating system

Culture system a closed re-circulating system (Figure 3.3) was used in this study. The system was consisted of 2×6 rearing units, sedimentation units, a condition unit and a feeding unit. The detail of the system was described (Piyatiratitivorakul *et al*., 2001). Water quality (Table 3.4) was determined every two weeks.

Table 3.4 Water quality parameter

Parameters	Equipment/determination technique
pH	pH meter model
Dissolve oxygen	YSI model
Temperature	YSI model
Alkalinity	VBC test kit
Ammomium	VBC test kit
Nitrite	VBC test kit

3.8 Statistical analysis

The data were analyzed using by one-way ANOVA. Difference between treatments means were analyzed by Duncan's Multiple Range Test. The level of significance for result was 95% confidential limit.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Standard curve from the quantities of farnesol

The quantities of farnesol in oil form (mg) was measured an absorbance at A_{310} nm by spectrophotometer. Original stock of farnesol was taken a serial dilution by acetronitrile solution, following with a ratio farnesol 600: 3,600 acenitrile µl. The result is shown in Table 4.1 and Figure 4.1. The quantitative relationship between farnesol (mg) (x) and absorbance value (y) is $y = 0.0045x + 0.0016$; $R^2 = 0.9944$

Table 4.1 Standard curve from the quantities of farnesol

Tube number	Farnesol Stock (µl)	Quantities of farnesol (mg)	Absorbance at A_{310} (nm)
Blank	0	0	0
1	100	14.042	0.063
2	200	28.084	0.107
3	300	42.126	0.186
4	400	56.17	0.28
5	500	70	0.324
6	600	84.25	0.403
7	800	112	0.514
8	1000	140.42	0.617

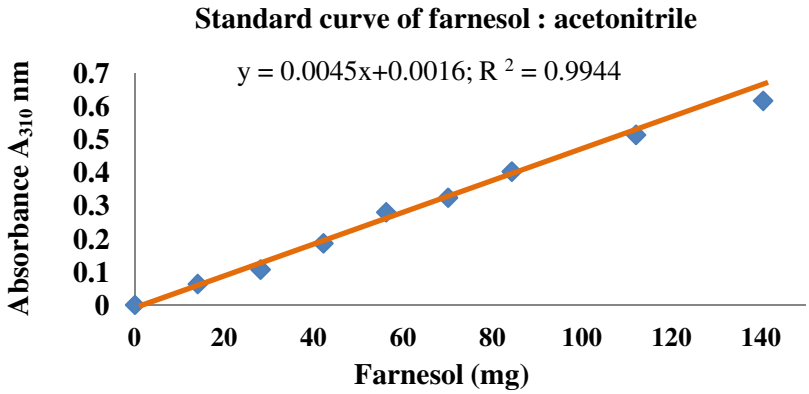

Figure 4.1 Standard curve of farnesol in acetonitrile solution

4.2 Encapsulation Efficiency

4.2.1 Encapsulation Efficiency before drying (% EE_{BD}) and after drying (% EE_{AD})

Encapsulated farnesol in oil form before and after freeze dried shown in Figure 4.2 indicated that encapsulated efficiency relation between quantities of farnesol and alginate concentration were used. The EE_{BD} increased from 77.6±2.07 to 98.03±0.2% when raised proportion of farnesol and alginate solution as shown in Table 4.2. Encapsulation before dried was found a maximum efficiency 98.03±0.2% when used 3.33 % (w/v) of alginate solution, following with 2 % (w/v) of alginate solution showed 97.6±0.2 and 93±0.19%. Efficiency of encapsulated before drying can be achieved over 90% when used alginate higher concentrations in 3.33 and 2 % (w/v). Lowering of alginate concentration 0.67 % (w/v) and farnesol loading up to 0.5,1,2 g, the efficiency of encapsulated was decreased.

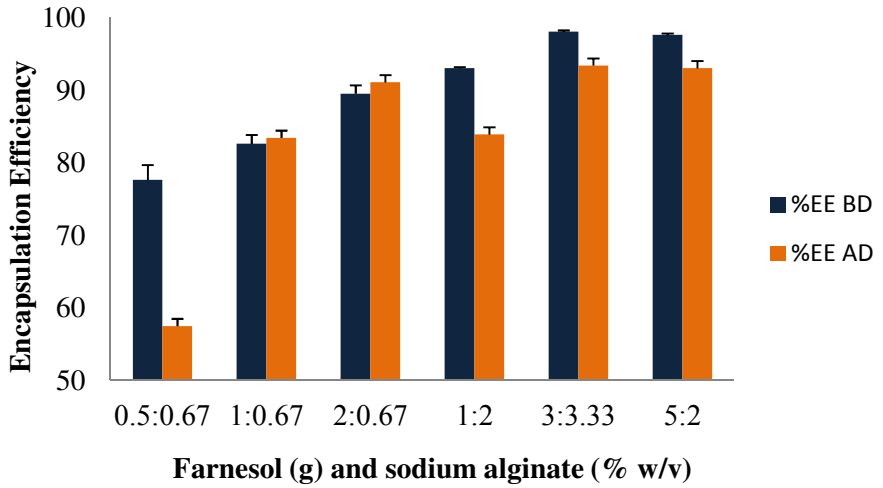

Figure 4.2 Encapsulation efficiency before (% EE_{BD}) and after drying (% EE_{AD}) of farnesol (g) and sodium alginate (% w/v) in different ratio.

The results of encapsulation efficiency after drying (% EE_{AD}) are presented in below Table 4.2. Percent of farnesol encapsulated after drying that showed relative as the %EE_{BD} in a similar trend. The EE_{AD} was found a maximum value amount 93.38% in the 3:3.33 ratio of solution and had a minimum value at 57.47±1.04% of (0.5:0.67), whereas the ratio at 1:2 had higher value both encapsulate efficiency and extractable oil. The result indicated on the influence of sodium alginate concentration and farnesol ratio was used.

In this study, the alginate-farnesol emulsion, prepare from different ratio of alginate concentration and farnesol. The encapsulation efficiency depends on the degree of cross-linking at the surface of the extruded emulsion droplet. If there was a lower ratio, once the emulsion droplet dropped into gelling bath, there was insufficient cross-linking between the alginate and calcium ions at the droplet surface (Chan, 2011). The beads or capsule produced from polyelectrolyte complexes through the mixing of oppositely charged and combinations of polyanion-polycation can be used to form capsule. In generally, sodium alginate solution containing the material to be encapsulated into a calcium chloride solution to form calcium alginate beads and the beads are immersed in solution of poly-L-lysine or chitosan. Calcium alginate beads are produced by ionotropic gelation of alginate in the presence of calcium ions that had been hydrogel formation of guluronic unit is one for the cross

linking reaction and the properties of the beads (Peniche *et al*., 2004). Influence of sodium alginate concentration depended on the mechanical stability of the capsules (Peniche *et al*., 2004). Capsules formed using 2% (w/v) alginate solution were very weak and loosen, whereas capsules prepared from 6 % alginate solution were more compact that appropriated mechanical resistance and greater encapsulated than 2% alginate solution. Higher alginate concentrations were not used because they were difficult to process due to their high viscosities. Moreover, influence of the amount of oil loading had an importance which capsule using constant of 25 g/L alginate concentration and various oil loading ranged from 10-60% when oil loading was increased 60%, the encapsulation efficiency decreased drastically. The emulsion was found to be stable for oil loading up to 40 %v/v when increased the oil loading to 60% emulsion sharply decrease and the oil was separated out from the initial emulsion. The effect of higher oil loading as expect increased the number density of oil droplet within the emulsion and raised the oil sizes (Chan., 2011). Huang *et al* (2001) reported that increased the concentration of many hydrocolloid gums would increase viscosity of the continuous phase surrounding the oil droplets inhibiting their movement and absorption of the gum at the oil-water interphase causing a reduction in interfacial tension.

Table 4.2 Encapsulation efficiency in various ratio of farnesol (g) and sodium alginate solution (% w/v).

Farnesol (g) / alginate ratio (% w/v)	**EE before drying (% EE_{BD})**	**EE after drying (%EE_{AD})**	**Extractable oil (%)**
0.5 : 0.67	77.6±2.07	57.47±1.04	97.61±3.72
1:0.67	82.6±1.21	83.41±0.23	63.8±0.66
2:0.67	89.5±1.15	91.06±2.61	60±0.86
1:2	93±0.19	83.87±0.25	97.78±0.57
3:3.33	98.03±0.2	93.38±0.5	27.52±1.67
5:2	97.6±0.2	93±3.73	24.34±1.46

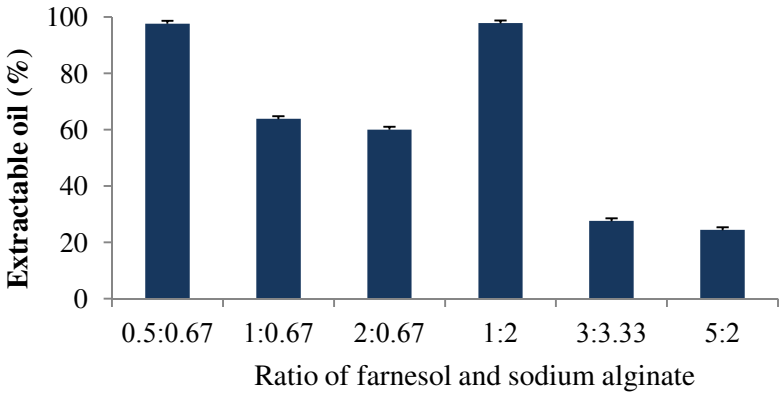

Figure 4.3 Percentage of extractable oil after freeze dried

4.2.2 Percentage of the extractable oil

In this study, oil extraction from alginate-chitosan bead after dried was extracted by mortar and dissolved with acetonitrile for 15 min. The purpose was to detect an oil distribution profile on beads surface. The results of 0.5:0.67 and 1:2 ratio expressed higher percent of extractable oil more than 97%, but in the ratio of 3:3.33 and 5:2 showed the least percentage on 27.52±1.67 and 24.34±1.46, respectively (showed in Table 4.2 and Figure 4.3). The results showed a reverse trend from encapsulated efficiency decreased sharply when used difference ratio both farnesol and alginate concentration.

Freeze-dried beads have a macroporous surface and internal structures. The characteristic was caused by sublimation of water crystals from the alginate-chitosan matrix, therefore in blank spaces and minor structural reduction (Chan, 2011). The drying process of coated beads is known by its effect on the release behavior as a result of destroying the formed alginate–chitosan films. As a result, the oil could be easily extracted from the open pores at the bead surface which gave percentage of extractable oil decrease sharply when used different alginate solution ratio. In the ratio of 5:2 showed the least of extractable oil at 24.34±1.46 % because the oil extraction can be related to the structure properties of beads which depended on alginate hydrogel network, in less alginate concentration thus increasing the porosity of the beads that cause a higher amount of oil extracted. The importance of coating the calcium alginate beads with the alginate-chitosan polyelectrolyte complex in order

to control the permeability of these capsules bead. The quality of hydrogel beads prepared by ionotropic gelation method can also improved by polyelectrolyte complexation technique. The mechanical strength and permeability block of hydrogels can be improved by an addition of oppositely charged polyelectrolyte to the ionotropically gelated hydrogel beads. For instance, addition of polycations allows a membrane of polyelectrolyte complex to form on the surface of alginate beads (Patil *et al.,* 2010). Elzatahry *et al* (2008) indicated that the molecular weights of chitosan involved the rate of diffusion of chitosan molecules into the three-dimensional network of calcium alginate and the extent of the reaction between chitosan and sodium alginate molecules. If chitosan had a higher molecular weight that lead to high diffusion resistance and causes a low diffusion rate, so in conversely when chitosan with a low molecular weight beads with a thick and strong membrane and had a strong antiswelling ability.

4.3 Effects of farnesol on female prawns (*M. rosenbergii*)

4.3.1 The effect of farnesol on growth

In order to study the effects of farnesol on *M. rosenbergii* under different concentration of farnesol in diets, freshwater prawn were divided into four treatments and three replicates: a control group was fed with basal diet, and three treatments fed with farnesol divided to 0.5, 1 and 2g/100 g of diets for 8 weeks, respectively.

The effect of the dietary with farnesol titers on growth parameter of female prawn is presented in Table 4.3 and 4.4. There was no significant difference ($p>0.05$) among the treatments on weight and length gain for 8 weeks. In accordance with, the ANOVA results revealed significantly difference ($p<0.05$) in percent of weight gain and specific growth rate that showed increasing of average. Percentage of weight gain was expressed highest in treatment 2 22.06±3.09% and reduced to 21.11±2.74, 13.36±1.62 and 18.89±1.73 % in control, treatment 3 and 4, respectively. There are correlation trends with the weight specific growth rate (%). Additional, the weight specific growth rate was derived from different proportion of initial weight, final weight and all trail days. In treatment 2 (0.5g farnesol) was expressed the highest value from other treatment that prawn were showed the most average percentage of growth rate for 0.33±0.057 and treatment 1 (control; no have farnesol) lead to

0.317±0.0515. Further, in the treatment 3 and 4 showed the lowering significant of specific growth rate in 0.208±0.0298 and 0.289±0.0419, respectively (as shown in Figure 4.4 and Table 4.5. At 8 weeks the length gain increased from initial in every treatment and had the highest length gain in treatment 2 (0.5g farnesol) to 4.73±0.95 %. However, in treatment 4 (2g farnesol) showed the least of length gain from other treatment on 2.48±1.47 % as shown in Table 4.4. Obviously, when additional of MF were higher doses, the weight gain, weight specific growth rate and also length gain had decreased.

Table 4.3 Growth by weight of *M. rosenbergii* in different treatment for 8 weeks**.**

Treatment	Initial weight (g)	Final Weight (g)	Weight gain (%)	Specific growth rate (%)
1 (control)	16.17±3.16	19.56±3.685	21.11±2.74[a]	0.317±0.05[a]
2 (Farnesol 0.5 g)	16.16±3.18	19.628±3.79	22.06±3.09[a]	0.33±0.06[a]
3 (Farnesol 1 g)	16.28±2.45	18.388±2.729	13.36±1.62[b]	0.208±0.03[b]
4 (Farnesol 2 g)	16.78±3.42	19.921±3.37	18.89±1.73[a,b]	0.289±0.04[a,b]

Note: Different superscripts within columns indicate significant ($p<0.05$) differences between diets treatment.

Table 4.4 Length of *M. rosenbergii* in different treatment for 8 weeks.

Treatment	Initial length	Final Length (cm)	Length gain (%)
1 (control)	11.584±0.78	12.042±0.6	4.135±2.327
2 (Farnesol 0.5 g)	11.43±0.72	11.96±0.634	4.72±0.948
3 (Farnesol 1 g)	11.41±0.57	11.862±0.709	4.147±2.727
4 (Farnesol 2 g)	11.42±0.76	11.92±0.847	2.48±1.47

Note: Different superscripts within columns indicate significant ($p<0.05$) differences between diets treatment.

Table 4.5 The weight of specific growth rate

Treatment	Week 4	Week 8
1	0.304±0.07	0.317±0.05[b]
2	0.282±0.15	0.330±0.06[b]
3	0.138±0.03	0.208±0.03[a]
4	0.274±0.02	0.289±0.04[a,b]

Note: Different superscripts within columns indicate significance ($p<0.05$) differences between diets treatment.

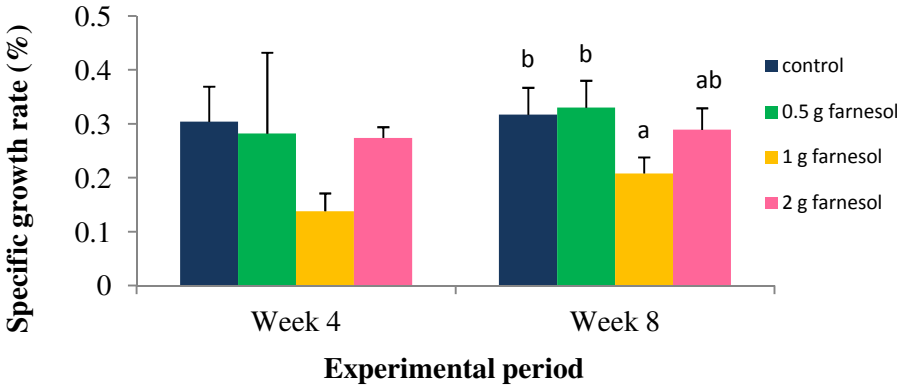

Figure 4.4 The percentage of specific growth rate

The effect of farnesol on growth among four treatments, the weight gain showed significantly negative regression ($p<0.05$) when increased concentration of farnesol (as mentioned in Table 4.3). The highest percentage of weight gain showed in 0.5 g farnesol 22.06±3.09 %, conversely in 1 g farnesol had the lowest percentage 13.36±1.62. The specific growth rate significantly decrease ($p<0.05$) when raised of farnesol titers that exhibited the highest in 0.5g of farnesol (0.33±0.06 %) whereas treatment 3 expressed the similarly lowest of the percentage (0.208±0.03 %). This study supported similar results on fed MF diet on larval freshwater prawn (Abdu *et al*., 1998a) that exhibited the fastest growth in control group (basal diets) and the lowest concentration of MF (0.21 µg/ml) while the highest concentration of MF (0.59 µg/ml) was significantly growth slower than that of the control group ($p<0.001$). The retardation in larval growth found in this study that higher MF levels caused earlier retardation of late larval growth, and the highest dose retarded larval development.

Besides, MF administered to *M. rosenbergii* larvae via artemia vector caused retardation of larval development, as manifested by larval stage. When larvae fed on Artemia that were enriched with higher doses of MF (0.11 and 0.2 µg MF/ml) also slower development than the control group and the highest dose (0.2 µg/ml) inhibited development after day 8 (Abdu *et al*., 1998b). According with the *Libinia emarginata* suggested that the different of MF levels caused delay metamorphosis when compared with the control (sea water) (Borst et al., 1987).

On the other hand, length gain showed collapse dramatically on 2 g farnesol at 2.48±1.47% whereas treatment 2 was appeared the highest of length gain 4.72±0.948 % (as shown in Table 4.4). In consistent, the tadpole shrimp, *Triops longicaudatus*, shown MF inhibited adult metamorphosis. Higher dose of MF at10 µg per gram of diet reduced somatic growth both body weights and lengths (Tsukimura *et al*., 2006). Since the farnesol was used in various titers, the highest titers caused inhibition crustacean growth and development that expressed in treatment 4 but in treatment 2 was still raised of weight and length gain. Tuberty and Mckenney (2005) indicated that juvenile hormone analogues (pyriproxyfen, methoprene and fenoxycarb) reduced metamorphic success lower concentrations (≥8 µg/L) in *Rhithropanopeus harrisii* and *Palaemonetes pugio*. From our study concluded that the effect of MF or farnesol on growth profile of *M. rosenbergii* depended on concentration of MF. The X-organ sinus gland complex plays a role in control of development in crustaceans but MF control of morphogenesis in crustaceans is not well understood. Regulation of MF synthesis is negatively controlled by MOIH, and in part through the inhibition of the enzyme farnesoic acid O-methyltransferase (*FAMeT*) that catalyses the final step in the MF biosynthetic pathway (Gunawardene *et al*., 2002). In insect species, juvenile hormone acts in conjunction with the molting hormone (20-hydroxyecdysone) to regulate metamorphosis. The JH titer is high during the larval molts, and then decreases in order to permit pupal development and formation of the adult (Homola and Chang., 1997).

4.3.2 The effect of farnesol on survival rate

After 4 week, the result showed significant difference survival rate ($p<0.05$). The highest survival prawns in treatment 2 as administered of farnesol 0.5g (95.55±3.85%) and the lowest expressed 82.22±3.8% in control (as shown in Table 4.6 and Figure 4.5). There was no significant difference ($p>0.05$) after 8 week on survival rates for all diets. Marsden *et al* (2008) found no significant difference ($p>0.05$) on survival rate of *Penaeus monodon* for application MF diets and all diets were showed very high percentage of survival rate from 96% to 100%.

Table 4.6 Survival rate on *M. rosenbergii* in various treatments after 8 week

Treatment	Week 0	Week 4	Week 8
1	100±0	82.22±3.8[a]	73.22±6.67
2	100±0	95.55±3.85[b]	86.67±6.67
3	100±0	91.11±7.69[a,b]	77.78±10.18
4	100±0	86.67±6.67[a,b]	77.78±10.18

Note: Different superscripts within columns indicate significant ($p<0.05$) differences between diets treatment.

4.3.3 Effect of farnesol on molting cycle

Molting is one of the most important physiological processes for crustacean growth since increase body size occurs in several steps associated with changing of the old exoskeleton. This is a dynamic event in life cycle of crustacean that was a mechanical associated with selective water and salt absorption. In this study, the average molt cycle was range from 15.92 to 18.6 days during 8 weeks of rearing. It also observed that molting duration was shortening as the farnesol increased. Molt interval was significantly shorten ($p<0.05$) in treatment 4 (2 g farnesol) with molting duration 15.92 ±1.66 day than if was in treatment 1 17.13 ±1.33 day, treatment 2 at 17.69 ±0.28 day and treatment 3 at 18.596 ±1.23 day.

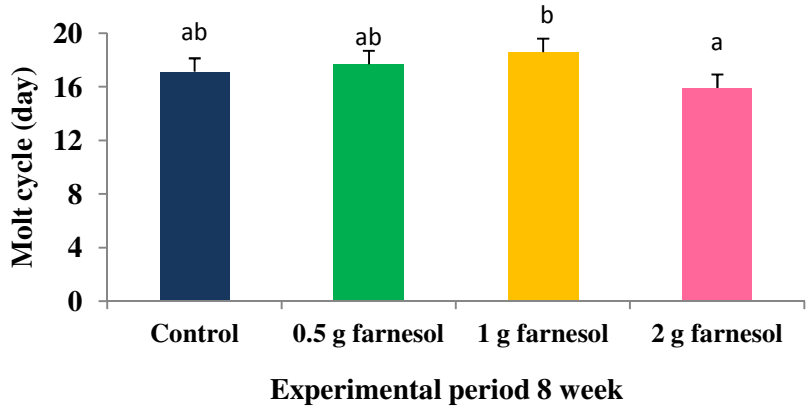

Figure 4.5 Molting cycle (days) of female freshwater prawns during 8 week

In *M. rosenbergii*, MF levels in the hemolymph rise during the premolt stage and decline in the postmolt stage that had seem like the ecdysteroid levels. Administration of MF also caused molt acceletation in the crayfish, *C. quadricrinatus* (Abdu *et al*, 2001), crayfish *P.clarkii* (Laufer *et al*., 2005). Furthermore, Hui *et al* (2007) studied on the function of *LvFAMeT* gene in molting of white shrimp, *Litopenaeus vannamei*, the RNAinterference (RNAi) technique was used. The wide distribution of *LvFAMeT* expression may be related to its role in growth and regulation of molting that injection of double stranded RNA (dsRNA) for *LvFAMeT* knocked down the expression of *LvFAMeT* in shrimp for at least 3 days and the shrimp did not advance to the final stage of molt cycle. Therefore, an important enzyme for the conversion of FA to MF, RNAi injection knocked down the expression is *LvFAMeT* which could potentially result in a decreasing of MF production and subsequently, could affect the molting process.

Table 4.7 Summarize the effect of farnesol in reproduction and molting cycle on *M. rosenbergii* during 8 weeks of culture.

Treatment	Number of females spawned (%)	Egg weight (g)	Moult cycle (days)	Spawning period (day)
1 (control)	78.37 ± 7.29[a]	2.01 ± 0.32[a]	17.13 ± 1.33[a,b]	19.33±0.73
2 (Farnesol 0.5 g)	61.56 ± 6.66[a,b]	1.76 ± 0.3[a]	17.69 ± 0.28[a,b]	17.18±2.29
3 (Farnesol 1 g)	60.94 ±12.91[a,b]	1.2 ± 0.11[b]	18.596 ± 1.23[a]	20.95±4.46
4 (Farnesol 2 g)	58.33 ± 7.56[b]	1.07 ± 0.19[b]	15.92 ± 1.66[b]	21.05±5.40

4.3.4 The effect of farnesol on female reproductive system

For 8 weeks of rearing, the addition of farnesol diet significantly reduced both spawning and egg biomass of female prawn. Treatment 4 (2g of farnesol) gave the lowest of egg production weight 1.07±0.19 g (Figure 4.7) and reduced number of female spawns also significant became lowest to 58.33±7.56% (Figure 4.8). Moreover, treatment 4 showed the highest of prawn spawning period at 21.05±5.40 day (Figure 4.9).

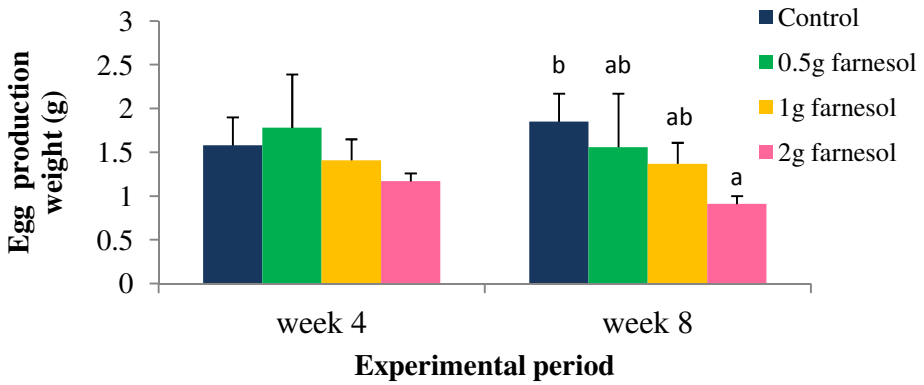

Figure 4.6 Individual female egg production weight (g).

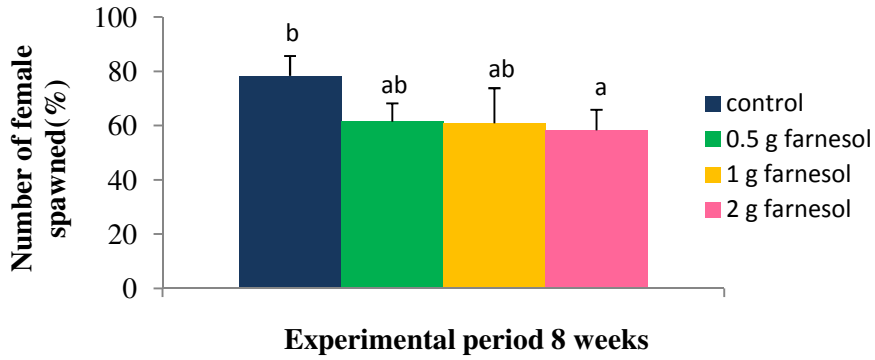

Figure 4.7 Number of females spawned (%) during 8 weeks of experiment.

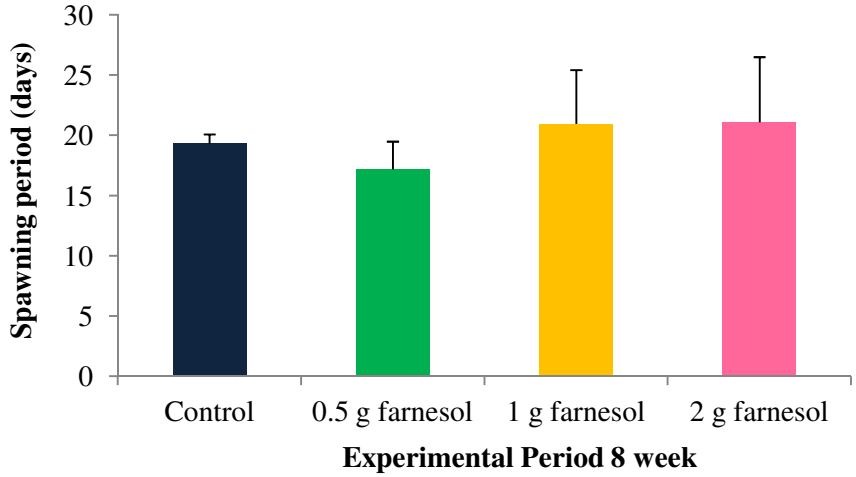

Figure 4.8 Spawning period (days) during 8 weeks of rearing.

In this study, farnesol used as a precursor of MF could reduce aspect of maturation in adult female freshwater prawn that also decreased egg production and percent number of female spawning. Previous studies showed that orally administered MF to ablate *Penaeus monodon* at a concentration of 5.5 µg / g diets could inhibit late stage ovary development and reduced fecundity when compared with the artificial diet without MF. Addition of MF in diet significantly reduced the number of spawner an average of 3±0.4 in a control group to 1.8±0.3 (addition MF) and reduced average fecundity of the first three spawns from 4.1 thousand to 3.2 thousand eggs per gram of prawn in ablated *P. monodon* (Marsden *et al*., 2008). While lower concentration of MF at 0.75-3.8 µg /g MF decrease fecundity and ovarian weight. But in higher

concentrations at 10 µg /g of MF reduced somatic growth (body weight and length) when administered MF coat food pellet to the juvenile of *Triops longicaudatus* (Tsukimura *et al*., 2006).

MF as a juvenile hormone analogue has an influence on reproduction, molt and juvenile development. The reproductive process of crustaceans is under hormonal regulation and neurohormones play an important role in this regulation. Among the neurohormones involved in ovarian growth, the role of the gonad-inhibiting hormone, secreted by the X-organ sinus gland complex in the eyestalks has been well documented (Charmantier *et al*. 1997; Fingerman 1997). One of the effects for this hormone seems to be an inhibition of vitellogenin uptake by the oocytes (Charniaux Cotton and Payen., 1988). The effect of JH analogue (metroprene) was inhibited vitellogenesis in xanthid crab *Rhithropanopeus harrisii* which the vitellogenic oocyte could not be detected in ovaries of either exposed or non exposed females. Methoprene does not seem to affect the beginning of oogenesis, that occurs as normally as in controls but seem to promote vitellogenesis in spider crab *Libinia emerginata* (Payen *et al*., 1977). In crayfish, *Cherax quadricarinatus*, during reproductive period MF did not cause any increment of vitellogenesis in hepatopancrease and had a significant ($p<0.05$) decreased vitellogenin content when detected MF at the highest concentration. But in pre-reproductive period MF significant accumulation was seen in ovaries treated with 15 µM. Therefore, this hormone (MF) was not able to stimulated vitellogenin synthesis in the ovary during the reproductive period (Medesani *et al*., 2011). Tokishita *et al* (2006) studies on juvenile hormone mimics (pyriproxyfen and fenoxycarb) repressed a vitellogenin *DmagVTG1* gene in *Daphnia magna* that the pyriproxyfen inhibited accumulation of *DmagVTG1* mRNA. A concentration of 0.2 nM, 50% inhibition was achieved at 120 h. Further, the fenoxycarb showed almost the same inhibitory activity as pyriproxyfen (99% inhibition at 15.6 nM). Interestingly, four sequences homologous with the JH-responsive element (JHRE) involved in transcriptional repression of *D. magna* vitellogenin genes. Therefore, the reproductive roles of MF in crustaceans were not universal which depended on the size of MOs that specific produced in MF titers, the optimal concentration of MF and suitable time of MF application with also the sex, animal stage and species of crustaceans.

CHAPTER V

CONCLUSION

Encapsulated efficiency of farnesol showed the highest percentage of encapsulation efficiency before and after drying at 98.03±0.2 and 93±0.19 % which used 3 gram farnesol per alginate 3.33 (% w/v) but percentage extractable oil was low. While the ratio constant of alginate at 0.67 %w/v and used 1 gram of farnesol, showed both higher percent of encapsulated efficiency and amount of extractable oil at 82.6±1.21%, 83.41±0.23% and 63.8±0.66%.

The effect of farnesol fed on female prawns that expressed the highest percentage of weight gain 22.06±3.09 and specific growth rate 0.33±0.06 % in 0.5 g farnesol but 2 g farnesol showed the lowest percent of length gain at 2.48±1.47%. Ability of inhibit reproduction on female prawn was expressed 2 g of farnesol which showed the lowest of egg production weight at 1.07±0.19 gram, had a shorten molt duration at 15.92 ±1.66 days and was reveal to the longest spawning period at 21.05±5.40 days. No effect of farnesol supplemented diets on survival of female prawn.

The result of this study confirmed that MF can play a role in regulating prawn reproduction. For *M. rosenbergii*, MF exhibited active reduce individual female egg reproduction, delay number of female spawning and shortened molting duration. To achieved a predictable outcome for control of reproduction in cultured that is a require increasing of female weight gain for industry viability and expansion. Greater control of reproduction will be improved both male and female prawn to enable more efficient use of wild caught, raised total productivity, to be evaluated in the market or achieve full domestication in commercial scale closure of this species.

Recommendation

1. Study the effect of MF on reproduction and molting cycle with male freshwater prawn for size increase.
2. Study the optimum concentration of farnesol for increasing weight gain and reduced reproduction of female prawn.
3. Study the effect of farnesol had a longer period more than 2 month which the result on reproduction and molting cycle maybe clearly evident.
4. The farnesol titers should be confirmed by another method such as HPLC when fed diet on female prawn.

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APPENDICES

Appendix A

The maximum wave length(nm) of farnesol were estimated by spectrophotometer (Lambda 25, Perkin Elmer Instruments, USA) as showed in table 1.

Table 1A Maximum wave length (nm) of farnesol

Lampda (nm.)	Absorbance	Lampda (nm.)	Absorbance	Lampda (nm.)	Absorbance
300	0.9267	311	1.065	322	1.0487
301	0.9466	312	1.0685	323	1.0401
302	0.9646	313	1.0716	324	1.0317
303	0.9798	314	1.072	325	1.0224
304	0.9971	315	1.0742	326	1.0131
305	1.0104	316	1.0713	327	1.0487
306	1.0234	317	1.0684	328	0.8711
307	1.035	318	1.066	329	1.0875
308	1.0444	319	1.062	330	0.9366
309	1.0533	320	1.058	331	0.8191
310	1.0586	321	1.0533	332	0.8677

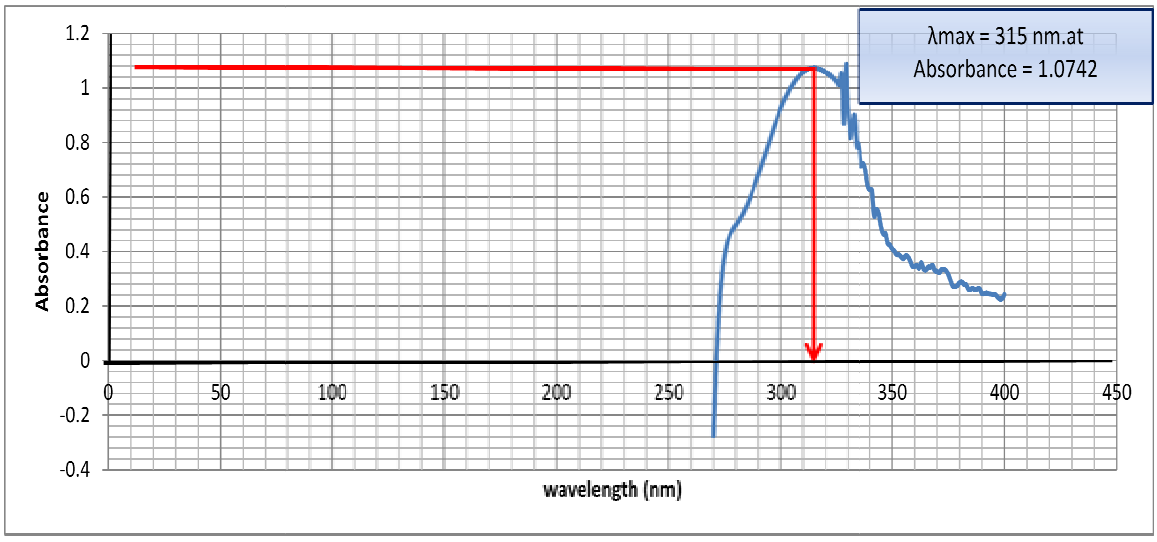

Figure 1A Maximum wave lengths (nm) of the farnesol

Preparation of farnesol stock solution

$$\textbf{Original stock formulated} = \frac{140.42 \text{ mg} \times \text{stock volume / tube (µl)}}{1{,}000 \text{ µl}}$$

= x mg. (Quantity of farnesol / tube)

$$\text{Tube 1} = \frac{(140.42 \text{ mg} \times 100 \text{ µl})}{1{,}000 \text{ µl}} = 14.042 \text{ mg}.$$

Table 2A The quantities of farnesol (mg) from upper formulation

Tube number	Farnesol Stock (µl)	Quantities of farnesol (mg)
Blank	0	0
1	100	14.042
2	200	28.084
3	300	42.126
4	400	56.17
5	500	70
6	600	84.25
7	800	112
8	1000	140.42

Appendix B

Water quality parameters

Table 1B Water quality

Tank Number	pH	Temperature (ºc)	Dissolve oxygen	Alkalinity	Ammonia	Nitrite
1	7-8	28.5-29	5.5-6	90	0.2	0.15-0.2
2	7-8	29-30	5.5-6	90	0.2	0.2-0.25
3	7.6-8	29-30	5.6-6	90	0.2	0.2
4	7.6-8	28-29	5.6-6	90	0.2	0.2
5	7-8	29-30	5.8-6	90	0.2	0.2
6	7-8	29-30	5.7-6	90	0.2	0.25

The water quality parameters in the experiment are shown in the table 2. Temperature, pH, dissolved oxygen, alkalinity, ammonia and nitrite were had rather constant which was optimum range for *M. rosenbergii* . Daily temperatures ranged from 28 to 30 ºc and pH fluctuated from 7-8; variations in the levels of total nitrite and dissolve oxygen were 0.15-0.25 and 5.5-6 mg L^{-1}, respectively. Moreover, total ammonia and alkalinity showed constantly 0.2 and 90 throughout the experimental period. Generally, the water quality was not difference between culture tanks and remained within the suitable range for the normal growth of *M. rosenbergii*.

Appendix C

Animal feed proximate analysis (AOAC, 1980)

Table 1C The percentage of proximate compositions values of diet ingredients

Treatment	Proximate compositions values of diet ingredients			
	Protein	Lipid	Humidity	Ash
1	42.84±0.17	11.04±0.15	31.40±0.10	11.65±0.30
2	42.57±0.40	11.70±0.10	44.23±0.11	11.53±0.13
3	43.39±0.47	11.43±0.11	25.06±0.10	11.24±0.21
4	43.99±0.30	11.28±0.12	24.17±0.14	11.10±0.26

After the prawn pellets were analyzed the proximate compositions values by animal feed proximate analysis (AOAC, 1980) that showed resemble of each compositions value (as showed in table 4.10). Total nitrogen was determined by the micro-Kjeldahl method. The quantity of crude protein was calculated from the total nitrogen (N × 6.25) and extracted with sulfuric acid. The quantity of lipid was determined by Soxtherm automatic S-11, Gerhardt machine and extract lipid by petroleum ether solution. However, in some feeds, neutral fats may make up only a small percentage of the total ether extract. Humid was determined according to the method of analysis of the AOAC (1980). Ash was determined by burning 2 g of prawn pellets in a porcelain crucible at 550 ºC for 5 hours in a muffler furnace according to the method of analysis of the AOAC.

Table 2C Composition of protein and lipid (%) in the ingredients*

Ingredient of basal diets	Protein (%)	Lipid (%)
Fish meal	67.03	8.25
Shrimp meal	51.12	7.5
Soy bean	49.9	0.5
Wheat flour	13.4	1.4
Wheat gluten	65	2
Lecithin	36	8
Fish oil	0	100

* Total of protein and lipid (%) in the pellet: 41.96 and 10.04 %.

Appendix D

Statistical analysis

Table 1D Difference statistic analysis of each diet treatments on growth rate, survival rate, egg production and molt duration on female prawns for 8 weeks.

- Survival rate after feed dietary on female prawns for 8 weeks

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Survival0m	.	3	.	.
Survival1m	.977	3	8	.450
Survival2m	.479	3	8	.706

Survival 1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
1	3	82.22333	
4	3	86.66667	86.66667
3	3	91.11333	91.11333
2	3		95.55333
Sig.		.108	.108

Survival 2m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
1	3	73.22333
3	3	77.78000
4	3	77.78000
2	3	86.66667
Sig.		.110

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

- Specific growth rate after feed 4 treatment diets on female fresh water prawns for 8 weeks

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
SGR1m	7.763	3	8	.009
SGR2m	.370	3	8	.777

Table of Duncan's New Multiple Range Test

SGR 1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
3	3	.13800
4	3	.27367
2	3	.28200
1	3	.30367
Sig.		.053

Treatment	N	Subset for alpha = 0.05	
		1	2
3	3	.20800	
4	3	.28900	.28900
1	3		.31723
2	3		.33000
Sig.		.081	.362

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000

- Weight and length average after feed 4 treatment diets on female fresh water prawns for 8 weeks

  Weight average

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Weightaverage1m	.532	3	8	.673
Weightaverage2m	.235	3	8	.870

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weightaverage1m	Between Groups	2.324	3	.775	.775	.540
	Within Groups	7.998	8	1.000		
	Total	10.322	11			
Weightaverage2m	Between Groups	4.085	3	1.362	.780	.537
	Within Groups	13.961	8	1.745		
	Total	18.045	11			

Table of Duncan's New Multiple Range Test

Weightaverage1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
3	3	16.98333
2	3	17.64367
1	3	17.72333
4	3	18.22000
Sig.		.192

- **Length average**

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Lengthaverage1m	1.804	3	8	.224
Lengthaverage2m	1.299	3	8	.340

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lengthaverage1m	Between Groups	.063	3	.021	.317	.813
	Within Groups	.532	8	.067		

	Total	.596	11			
Lengthaverage2m	Between Groups	.050	3	.017	.156	.923
	Within Groups	.863	8	.108		
	Total	.914	11			

Table of Duncan's New Multiple Range Test

Lengthaverage1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
3	3	11.55267
2	3	11.64467
4	3	11.71333
1	3	11.74100
Sig.		.423

Lengthaverage2m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
3	3	11.88500
4	3	11.93833
2	3	11.96567
1	3	12.06367
Sig.		.547

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000

- **Weight and length gain after feed 4 treatment diets on female fresh water prawns for 8 weeks**

- **Weight gain**

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Weightgain1m	8.162	3	8	.008
Weightgain2m	.750	3	8	.552

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weightgain1m	Between Groups	51.378	3	17.126	2.278	.156
	Within Groups	60.137	8	7.517		
	Total	111.514	11			
Weightgain2m	Between Groups	82.803	3	27.601	4.546	.039
	Within Groups	48.575	8	6.072		
	Total	131.378	11			

Table of Duncan's New Multiple Range Test

Weightgain1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
3	3	4.23667
4	3	8.55000
2	3	8.90333
1	3	9.42800
Sig.		.061

Weightgain2m

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
3	3	11.76767	
4	3	15.90900	15.90900
2	3		17.57000
1	3		18.68633
Sig.		.074	.222

- **Length gain**

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Lengthgain1m	.154	3	8	.924
Lengthgain2m	.811	3	8	.523

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lengthgain1m	Between Groups	1.343	3	.448	.290	.831
	Within Groups	12.344	8	1.543		
	Total	13.687	11			
Lengthgain2m	Between Groups	8.431	3	2.810	.661	.599
	Within Groups	34.016	8	4.252		
	Total	42.447	11			

Table of Duncan's New Multiple Range Test

Lengthgain2m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
4	3	2.48000
1	3	4.13500
3	3	4.14667
2	3	4.72533
Sig.		.245

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

- Molt cycle after feed 4 treatment diets on female fresh water prawns in 8 weeks

Test of Homogeneity of Variances

moultcycle

Levene Statistic	df1	df2	Sig.
2.201	3	8	.166

ANOVA

moultcycle

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.270	3	3.757	2.454	.138
Within Groups	12.245	8	1.531		
Total	23.515	11			

Table of Duncan's New Multiple Range Test

moultcycle

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
MF 2 g	3	15.9213333	
control	3	17.1303333	17.1303333
MF 0.5 g	3	17.6880000	17.6880000
MF 1 g	3		18.5966667
Sig.		.132	.201

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- Percentage of sprawn after feed 4 treatment diets on female fresh water prawns for 8 weeks

Test of Homogeneity of Variances

fecundity

Levene Statistic	df1	df2	Sig.
1.467	3	8	.295

ANOVA

% Number of female spawns

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	918.307	3	306.102	3.807	.058
Within Groups	643.203	8	80.400		
Total	1561.510	11			

Table of Duncan's New Multiple Range Test

% Number of female spawns

Duncan[a]

treatment	N	Subset for alpha = 0.05	
		1	2
MF 2 g	3	54.8267	
MF 1 g	3	60.9400	60.9400
MF 0.5 g	3	61.5667	61.5667
control	3		78.3767
Sig.		.403	.052

- Egg production weight after fed 4 treatment diets on female freshwater prawns during 8 weeks.

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Eggweight1m	3.553	3	8	.067
Eggweight2m	4.026	3	8	.051

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Eggweight1m	Between Groups	.603	3	.201	1.499	.287
	Within Groups	1.072	8	.134		
	Total	1.675	11			
Eggweight2m	Between Groups	1.416	3	.472	3.601	.065
	Within Groups	1.049	8	.131		
	Total	2.464	11			

Eggweight1m

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
4	3	1.17000
3	3	1.41000
1	3	1.58000
2	3	1.78000
Sig.		.092

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Eggweight2m

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
4	3	.91000	
3	3	1.37000	1.37000
2	3	1.55667	1.55667
1	3		1.85667
Sig.		.069	.153

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

BIOGRAPHY

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