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PRODUCTION AND QUALITY CONTROL OF SHRIMP FEED CONTAINING TURMERIC EXTRACT

Miss Jirisuda Suthiprapa

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Thesis Title	PRODUCTION AND QUALITY CONTROL OF SHRIMP FEED CONTAINING TURMERIC EXTRACT
By	Miss Jirisuda Suthiprapa
Field of Study	Pharmaceutics and Industrial Pharmacy
Thesis Advisor	Narueporn Sutanthavibul, Ph.D.
Thesis Co-Advisor	Jittima Chatchawalsaisin, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…… Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Pintip Pongpech, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Associate Professor Poj Kulvanich, Ph.D.)

…….……. Thesis Advisor
(Narueporn Sutanthavibul, Ph.D.)

…….. Thesis Co-Advisor
(Jittima Chatchawalsaisin, Ph.D.)

…….. Examiner
(Assistant Professor Pornchai Rojsittisak, Ph.D.)

…….. External Examiner
(Associate Professor Somlak Kongmuang, Ph.D.)

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การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อผลิตอาหารกุ้งผสมสารสกัดขมิ้นชันซึ่งมีสารประกอบเคอร์คูมินอยด์เป็นองค์ประกอบหลัก เคอร์คูมินอยด์มีตัวยาสำคัญในการออกฤทธิ์ 3 ชนิดคือ เคอร์คูมิน เดสเมทอกซีเคอร์คูมิน และ บิสเดสเมทอกซีเคอร์คูมิน จากการศึกษาก่อนการตั้งตำรับ ไม่พบความไม่เข้ากันของสารสกัดขมิ้นชันและอาหารกุ้งจากการวิเคราะห์ด้วยความร้อน อาหารกุ้งผสมสารสกัด ขมิ้นชันผลิตโดยการเตรียมสารละลายของสารสกัดขมิ้นชันเข้มข้นและ /หรือ ไมโครอิมัลชันของสารสกัดขมิ้นชันเข้มข้น ก่อนที่จะนำไปใช้ในกระบวนการอัดรีดและ /หรือกระบวนการเคลือบ จากนั้นจะนำอาหารกุ้งที่ผ่านกระบวนการผลิตไปศึกษาคุณลักษณะ ผลที่ได้มีดังนี้ กระบวนการ ผลิตมีผลต่อลักษณะทางกายภาพและรูปแบบการปลดปล่อยสารสกัดขมิ้นชันของ อาหารกุ้ง สารสกัดขมิ้นชัน ที่ผลิตได้ ซึ่ง อาหารกุ้ง ที่ผลิตโดยกระบวนการเคลือบจะมีลักษณะทางกายภาพดีเยี่ยม เมื่อเทียบกับการ ผลิตโดยกระบวนการ อัดรีด ผลที่ได้อาจเนื่องมาจากในระหว่างกระบวนการเคลือบแกนเม็ดของอาหารกุ้งไม่ถูกทำลาย ซึ่งเกิดขึ้นได้ใน กระบวนการ อัดรีด อย่างไรก็ตามอาหารกุ้ง ที่ผลิตโดยกระบวนการอัดรีด จะมีรูปแบบการปลดปล่อยสารสกัดขมิ้นชัน ที่ช้าเป็นที่น่าพอใจ อาจเนื่องมาจากสารสกัดขมิ้นชันเข้มข้นถูกรวมเป็นระบบเมทริกซ์ ในอาหารกุ้ง นอกจากนั้น ชนิดของสารสกัดขมิ้นชันเข้มข้นยังมีผลต่อความคงตัวของผ ลิตภัณฑ์ทั้งที่ผลิต ใหม่ และหลังจากเก็บ รักษาเป็นเวลา 6 เดือน ซึ่งอาจสรุปได้ว่าวัฏภาคน้ำมันในอิมัลชันของสารสกัดขมิ้นชันเข้มข้นสามารถป้องกัน การเสื่อมสลายของสารสกัดขมิ้นชันจากทั้งสภาวะในการเคลือบ สภาวะในการอัดรีด และระหว่างการเก็บรักษา

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The purpose of the present study was to produce shrimp feed containing turmeric extract which typically contains three major curcuminoid active compounds i.e., curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. Preformulation indicated that incompatibility of the initial shrimp feed and turmeric extract, evaluated by differential scanning calorimeter (DSC), did not occur. The shrimp feed containing turmeric extract were produced by first preparing turmeric extract concentrated solution and/or turmeric extract concentrated emulsion to be used in the coating and/or extrusion processes. Shrimp feeds containing turmeric extract after processed were characterized. The results indicated that processing methods affect the physical properties and release characteristics of shrimp feeds containing turmeric extract produced. Shrimp feed obtained by coating method showed excellent physical properties compared to extrusion method. This may be because initial shrimp feed core was not destroyed during the process as in extrusion method. However, extruded products resulted in a more desirable sustained release character due to an incorporation of turmeric extract within the feed matrix. The results also showed that types of liquid turmeric extract concentrate had significant influence on the chemical stability, both after processing and 6 months of storage. It can be postulated that the oil phase in turmeric extract concentrated microemulsion aid in protecting turmeric extract against degradation caused by coating or extrusion environment and during storage.

Department : Pharmaceutics and Industrial Pharmacy Student's Signature .....
Field of Study : Industrial Pharmacy Advisor's Signature .....
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LIST OF ABBREVIATIONS

%	percentage
<	less than
>	more than
≥	equal or less than
BHA	butylated hydroxy anisole
BHT	butylated hydroxy toluene
c.m.c.	critical micellization cocentration
CE	Turmeric extract concentrated microemulsion coated
cm	centimeter (s)
CNS	central nervous system
Conc.	concentration
COX-2	cyclooxygenase 2
CS	Turmeric extract concentrated solution coated
Cu	copper
d(3,2)	the surface weighted mean diameter
D(4,3)	the volume weighted mean diameter
d(v,0.1)	the diameter of particles of 10% volume percentile
d(v,0.5)	the diameter of particles of 50% volume percentile
d(v,0.9)	the diameter of particles of 90% volume percentile
DSC	differential scanning calorimetry
e.g.	exampli gratia (for example)
EBV	Epstein-Barr virus
EDTA	Ethylene Diamine Tetra Acetic Acid
EE	Turmeric extract concentrated microemulsion extrude
ES	Turmeric extract concentrated solution extrude
et al.	et alii (and others)
etc.	et cetera (and so on)
EU	European Union
FCR	feed conversion ratio
FTIR	Fourier Transform Infrared Spectrophotometer

g	gram (s)
HIV	human immunodeficiency virus
HLB	hydrophilic-lipophilic balance
hr	hour (s)
i.e.	for example
IgE	Immunoglobulin E
IgG	Immunoglobulin G
iNOS	Nitric Oxide Synthase
IP	intraperi- toneal
Kg	Kilogram(s)
kp	kilopound
kV	kilovolt (s)
LCT	long chain triglycerides
LD50	lethal dose at 50%
MCT	medium chain triglycerides
mg	milligram (s)
min	minute (s)
ml	milliliter (s)
mm	millimeter (s)
MT	Metric Ton
MW	molecular weight
NF	Nation Formulary
nm	nanometers
NO	nitric oxide
No.	number
O	oxygen
o/w	oil in water emulsion
^{o}C	degree celcius
PC	Phosphatidylcholine
PCS	photon correlation spectroscopy
pH	the negative logarithm of the hydrogen ion

psi	pound (s) per square inch
rpm	round per minute
RSD	Relative Standard Deviation
S.	*Staphylococcus* genus
SD	standard deviation
SEM	scanning electron microscope
Spp	species
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TNF	Tumor necrosis factor
USA	United States of America
USP	United States Pharmacopeia
UV	Ultra Violet
V	*Vibrio*s species
v/v	volume by volume
w /o	water in oil emulsion
w/v	weight by volume
w/w	weight by weight
α	alpha
γ	gamma
δ	delta
µg	microgram (s)
µl	microliter (s)
µm	micrometer (s)

CHAPTER I

INTRODUCTION

The shrimp farming industry plays a significant role in the economic development in many parts of the world, including Thailand. This industry is the economic strategy that has enormous potential to develop. Since shrimp culture industry has a record of impressive production and export revenue figures*(1)*. In recent years, most of the disease problems have been overcome also trends in consumer concern over the safety of food and drug additives have renewed. The nature produces are considered to be harmless. Though nature produces a variety of brilliant plants, only a few appear to be of commercial value for food drugs and pharmaceutical used. Turmeric has a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic*(2)*. Turmeric can also be applied topically in poultices to relieve pain and inflammation. Current research has focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties *(3-6)*. The most compelling and key rationale for the continuing traditional therapeutic use of turmeric and its main active substance also known as curcumin is extremely good safety profile. To date, no studies in either animals or humans have discovered any toxicity associated with the use of curcumin, and it is clear that curcumin is not toxic even at very high doses. However, the use of curcumin is thereby limited due to low water solubility under acidic or neutral conditions, high decomposition rate in alkaline media and photodegradation in organic solvents*(7, 8)*. Attempts to prepare water-soluble curcumin by complex formation with various macromolecules (e.g. gelatine, polysaccharides) and variety preparations such as emulsion and liposome have been reported*(9, 10)*. To our knowledge, the incorporation of turmeric to shrimp feed which is appropriate quality in agriculture application as antimicrobial instead has never been investigated. Therefore, this study were to investigate the ways to produce and control the quality of shrimp feed containing turmeric extract in order to

achieve a satisfiable physicochemical properties which is appropriate to use for shrimp feeding. Moreover, the stability of turmeric extract in the preparations was determined.

OBJECTIVES

The purpose of this study were to:

1. Develop shrimp feed containing turmeric extract which exhibit an appropriate quality.
2. Determine the physicochemical properties of shrimp feed containing turmeric extract after production.
3. Evaluate and control the quality of active ingredient in shrimp feed containing turmeric extract after production.
4. Investigate the stability of shrimp feed containing turmeric extract.

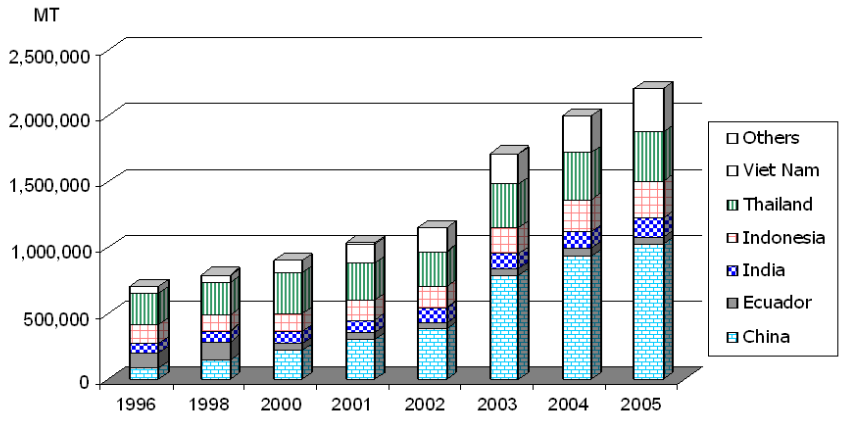
CHAPTER II

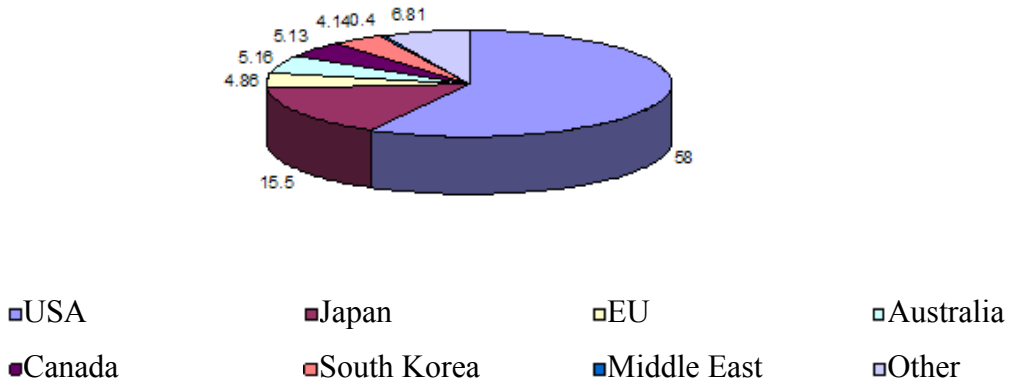
LITERATURE REVIEW

1. Shrimp

Thailand is regarded as an agricultural country. The agricultural sector has played an important role in the growth of the economy throughout Thai history. The country better known as the world's leading exporter of seafood products, shrimp is the main seafood commodity in terms of value. Thailand export shrimp approximately 25% in global market share*(11)*. Shrimp aquaculture accounting for over 87 % by weight of total production was exported as fresh chilled and frozen shrimp, dried shrimp, frozen cooked shrimp, processed shrimp and canned shrimp*(12)*, The main export markets are The United States of America (USA) 58 %, Japan 15.5 %, European Union (EU) 4.86% , Australia 5.16 % , Canada 5.13 %, Korea 4.14 % , and Middle East 0.40% as shown in Figures 1. The shrimp exports reached 346,416.48 tons, which is worth approximately 86,572.85 million bath income during 2006*(13)*. However, Thailand faces fierce competition from other developing-country shrimp suppliers in USA and the EU market such as, China, Indonesia, Ecuador, Vietnam, Brazil, India, Malasia, Philippines and Maxico.

Table 1 The global production volume of shrimp from aquaculture during 1996–2005*(14)*





Figures 1 The volume of Thai shrimp exports classified by major market in 2006*(15)*

Nowadays, the shrimp industry worldwide is presently facing a widespread crisis due to serious infection and chemical contamination. The diseases problem cause by various biological agents such as viruses, bacteria, fungi and parasites. Among the groups of microorganisms that cause serious losses in shrimp culture are bacteria, mainly due to *Vibrio* species*(16)*, have been reported in penaeid shrimp culture systems implicating at least 14 species and they are *V. harveyi, V. splendidus, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, V. campbelli, V. fischeri, V.damsella, V. pelagicus, V. orientalis, V. ordalii, V. mediterrani, V. logei* etc. *Vibrio spp*. is an opportunistic pathogens, vibriosis occur when shrimps are stressed. High mortalities usually occur in postlarvae and young juvenile shrimps. Adult shrimps suffering vibriosis may appear hypoxic, show reddening of the body with red to brown gills, reduce feeding, empty guts and may be observed swimming lethargically at the edges and surface of ponds. Systemic vibriosis typically results in the formation of septic nodules in the lymphoid organ, heart and connective tissues of the gills, hepatopancreas, antennal gland, nerve cord, telson and muscle. Six *Vibrio*s species, including *V. harveyi and V. splendidus* cause luminescence, which is readily visible at night, in infected postlarvae, juveniles and adults.

Table 2 Volume and values of various Thai shrimp product exports during 2002-2006*(13)*

Year / Shrimp Product	2002		2003		2004		2005		2006	
	Volume (tons)	Values (million bath)	Volume (tons)	Values (million bath)	Volume (tons)	Values (million bath)	Volume (tons)	Values (million bath)	Volume (tons)	Values (million bath)
Fresh chilled and frozen	99,223.46	34,406.25	118,921.43	35,921.21	122,517.93	32,536.07	157,985.64	37,731.48	178,246.74	42,835.24
Dried	336.63	92.08	363.20	101.48	490.22	126.31	538.89	125.91	759.94	168.81
frozen cooked	56.29	10.59	101.25	27.27	163.33	29.80	157.87	31.72	1,064.91	289.78
canned	7,446.11	2,161.47	6,435.79	1,549.35	5,354.66	1,348.13	4,259.47	1,096.58	4,487.30	1,155.25
Other processed	104,648.76	37,286.60	108,270.46	34,201.35	112,430.83	33,267.84	116,391.95	32,368.60	152,251.25	41,825.65
Total	211,711.23	73,956.99	234,092.14	71,800.67	240,956.98	67,308.14	279,333.83	71,354.29	336,810.14	86,274.73

(a) (b) (c)

Figures 2 Vibriosis in shrimp showing marine blood agar displaying strong haemolytic activity of *Vibrio*s species*(17)* (a), black nodules in shimp (b), luminescent colonies of *Vibrio* species in general marine agar plate*(18)*(c)

Various chemical has been used to treat bacterial infection in shrimp culture. The incidence after chemical and antibiotic treatment was found, drug-resistant bacteria and drug-residual antibiotic in shrimp has become a major problem in shrimp culture. In March 2002, There was reports about chemical contamination in Thai shrimp shipments released from the Netherlands, Thai shrimps were found contaminated with two chemicals known as chloramphenicol and nitrofurans, two antibiotics applied in shrimps now banned because of potential harmful effects on humans. For solving problems concerning The Ministry of Commerce coordinated with Ministry of Agriculture and Cooperatives and Food and Drug Administration has a strict regulation and control on chemical imports, focusing on 16 chemicals following Aristosia, Chloramphenicol, Chloroform, Chlorpomazine, Colchicine, Dapsone, Dimetridazole, Metronidazole, Nitrofurans, Ronidazole, Diethylstilberstrol, Ipronidazole, Nitroimidazoles, Sulfonamides, Fluoroquinolones and Glycopeptides*(19)*.

1.1 Shrimp feeds

An accounting for 50% to 70 % of the total variable cost of shrimp production is feed cost. Therefore, feed quality and cost are critical factors to determined the profitability of a shrimp farm. Like all animals, shrimp require energy sources (lipids,

protein, and carbohydrates), vitamins, minerals, oxygen, and water. So that, shrimp feed are usually contain a large number of nutrient to meet nutritional requirements**.** The marine animal protein sources are vary close to the dietary requirements of shrimp. The ingredients in most commercial shrimp feeds accounting for 20% – 50 % are derived from marine capture fisheries, includes fish meal, fish oil, shrimp/crustacean meal, squid meal and other miscellaneous products such as fish, fish silage, fish/squid liver meals and seaweed extracts*(20)*. However, shrimp nutrition is a very complex subject its must be specifically formulated for different states of the life cycle because the changing in nutritional requirements of shrimp in each stage.

a b c

Figures 3 The different kinds of shrimp feed fine powder (a) , crumble/flake (b), pellet (c)

1.1.1 Feed processing

Due to a slow and intermittent feeders of shrimp thus shrimp feed are required to be physically stable and bound firmly, they must be stay in water without disintegration for a longer period than most fish diets. Be cause of water pollution and feed conversion ratio (FCR) could be improved by the highly water stable feeds due to reduction of nutrients loss and maintenance of the feed physical characteristics in acceptable form.

1.1.2 Physical characteristics of shrimp feeds

Feed physical characteristics such as appearance, water stability, particle size or feed pellets size are also affected by processing and its ingredients.

a) Feed Size

Since shrimp feed was vary up to shrimp stage thus shrimp feed is also have a very wide size range. As shown in Table 3 size of shrimp feed classified by shrimp weight.

Table 3 Feed pellet size characteristics by shrimp weight*(21)*

Shrimp stage	**Feed size**
larvae	<50, 50-125, 250, 500 μm, according to larval substage
postlarvae	flakes, fine crumbles (500 μm)
juveniles to 2-3 g	medium crumble (1mm) to coarse crumble (2mm)
3-6 g	short pellet (3/32 x 2-4 mm)
6-10g	medium pellet (3/32 x 6 mm)
10-16 g	long pellet (3/32 x 10 mm)
over 16 g	1/8 inch. diameter, various lengths

b) Feed Color

The color of a pellet is not related to attractability or consumption behavior, but is just indicated of ingredient composition and quality of manufacturing. Most commercial shrimp feeds are dark brown its mainly due to the heat of processing and feed ingredient color (most are relatively dark in color). However, the exposure to long-term excessive heat and direct sunlight may causing lighter in color of shrimp feeds.

c) Feed Water Stability

Binding characteristics of commercial shrimp feeds should be allow about 4-6 hours shrimp feeds stability. The increased in shrimp feeds stability may increase economical value of feeds since most feed attractants are lost within this amount of time*(21)*.

2. Curcuma longa (Turmeric)

2.1 Characteristics

Curcuma longa L. (Turmeric), is a member of Zingiberaceae family along with the other note worthy members like ginger, cardamom and galangal, the genus Curcuma that consists of hundreds of species of plants that possess rhizomes and underground root like stems. Turmeric is herbal plant which grown in warm, rainy regions of the world such as China, India, Indonesia, Jamaica, Peru included Thailand. It is commonly know as Curuma, Haridra, Indian Saffron, Haldi (Hindi)*(22)*. and Khamin in Thai. Taxonomic position of Turmeric is show following.

Class Liliopsida
Subclass Commelinids
Order Zingiberales
Family Zingiberaceae
Genus *Curcuma*
Species *Curcuma longa*

Figures 4 Characteristic of *Curcuma longa* L.(Turmeric)*(23)*

"Botanical Characteristic of turmeric occur as ovate, oblong, or pear-shaped primary rhizomes, also know as bulb or round turmeric, about 3 cm in diameter and 4-5 cm long, and showing transverse annular leaf scars, and as cylindrical, sometimes

short- branched secondary rhizomes, also know as finger or long turmeric, about 1 cm in diameter and 2 to 7 cm long, and showing scars of lateral branches. The cured and dried turmeric of commerce is bright yellow to dull yellow in appearance, with a rough or polished surface, and a characteristic aromatic odor. The texture is hard and uneasily broken, and the fracture is smooth and finely granular. Internally it is orange-yellow to orange, showing a cortex separated from a central cylinder by distinct endodermis" *(24)*.

The results from studying of twelve turmeric cultivars indicated that active constituents of turmeric are curcuminoids 1.8-5.4 % and volatile oils 2.5-7.2 %. These oils consist of a 60 % of the sesquiterpene lactone turmerone. They also contain zingiberene 25 %, α- and γ-atlantone, bisabolene, guaiane, germacrene, 1,8-cineole, borneol, δ-sabinene, caprilic acid, dehydroturmerone, limonene, linalol, eugenol, curcumenol, curcumenone, curlone and phelandrene. Other constituents include sugars, proteins, and resins*(25)*.

"Curcuminoids is a partially purified natural complex diaryl heptanoids derivatives isolated from turmeric"*(24)*. Curcuminoids also contain three related compounds, the major compound curcumin, two minor constituents desmethoxycurcumin and bisdesmethoxycurcumin. Chemical of curcuminoids is show following.

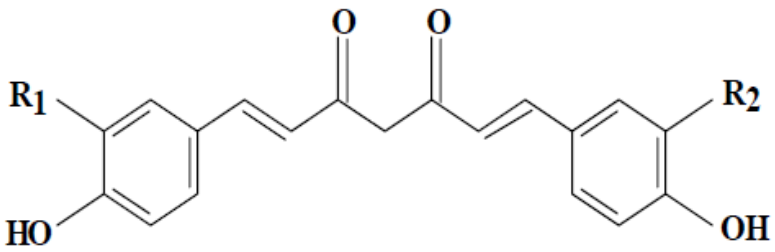


Figure 5 Schematic drawing of curcuminoids structure*(26)*

Table 4 Chemical of curcuminoids

	Curcumin	***Demethoxycurcumin***	***Bisdemethoxycurcumin***
R 1	OCH3	H	H
R2	OCH3	OCH3	H
Chemical Formula	C_{21} H_{20} O_6	C_{20} H_{18} O_5	C_{19} H_{16} O_4
Molecular Weight	368.4	338.4	308.4

As we known curcumin, [1,7-bis(4-hydroxy-3-methoxy phenyl) -l,6-heptadiene -3,5-dione] was the major active compounds of turmeric which irregular shape. Color shade is lemon yellow at pH3. At the pH above 9 a color changes from yellow to orange*(8)*. The solubility of curcumin is very different in various solvents, It is soluble in vegetable oils, sparely soluble in water at acidic or neutral pH. However, soluble in alkali. At pH above neutral the compound becomes dissociate rapid hydrolytic degradation occurred*(27)*. The main decomposition products in other studies have been identified as feruloyl methane, ferulic acid and vanillin*(28)*. The secondary degradation product formed by hydrolysis of feruolyl methane was investigated in latter study*(9)*. The coloured condensation products was formed. Moreover, decomposition of curcumin in organic solvent when exposure to light will be form several degradation products such as cyclisation product which formed by loss of two hydrogen atoms from the curcumin molecule.

The stability of curcumin was strongly improved by lowering the pH, adding glutathione, N-acetyl-L-cysteine, ascorbic acid, cyclodextrin*(9)* and rat liver microsomes*(29)*.

2.2 Pharmacokinetic

In previously studies indicate that giving of cucumin orally or intraperitoneally to rats, is dominantly present in the faeces and a little in the urine. Only traces of curcumin are found in the blood, liver and kidney. The major biliary metabolites of curcumin are glucuronides of tetrahydrocurcumin and hexahydrocurcumin. Curcumin, after metabolism is mainly excreted through bile*(30)*.

2.3 Pharmacodynamic

Turmeric is the phytoconstituent which widely used in food industry, traditional medicine in Southeast Asia since the second millenium. More recently, many studies have demonstrated that Turmeric and it's main active substance also known as curcumin exhibits various biological effect as presented below *(25, 31-34)*.

2.3.1 Effect on gastrointestinal system

Stomach: Turmeric powder is effective for use as gastroprotectant due to increases of rabbits mucin secretion and against irritants

Intestine: Curcumin has antispasmodic and antiflatulent activity both *in vivo* and *in vitro* investigation. Moreover it also enhances intestinal lipase, sucrase and maltase activity.

Pancreas: Curcumin also increases bicarbonate level, activity of pancreatic lipase, amylase, trypsin and chymotrypsin.

2.3.2 Effect on lipid metabolism

Curcumin significantly reduces low density lipoprotein and very low density lipoprotein and total cholesterol level. In addition, it also increase of *a*-tocopherol level in rat plasma.

2.3.3 Antioxidant effect

Many studies illustrated that curcumin is a powerful antioxidant, it can be inhibits lipid peroxidation, scavenger of oxygen free radicals, preventing of nitrate-induced oxidation and repair both oxidative and reductive damage in hemoglobin. It acts as a scavenger of oxygen free radicals such as. Curcumin derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect.

2.3.4 Anticarcinogenic effect

The anticarcinogenic effect of curcumin, from the comparative studied of curcumin, chlorogenic acid, caffeic acid and ferulic acid effect on tumour promotion in mouse skin, curcumin shown higher effective. The induction of apoptosis is the major mechanisms in curcumin anticarcinogenic effect. Curcumin could inhibits produce of Inducible nitric oxide synthase (iNOS) and cyclooxygenase2 (COX-2), thus tumour growth through nitric oxide (NO) was suppressed. Moreover, curcumin has also inhibit protein kinase C, glutathione depleation cause decrease in activation and oligonucleosomal.

2.3.5 Pro/antimutagenic effect

An antimutagenic effects of curcumin was to reduce amount of aberrant cells in cyclophosphamide-induced chromosomal aberration and prevention of mutation in urethane (powerful mutagen) models.

2.3.6 Anticoagulant effect

Anticoagulant activity of curcumin by inhibiting collagen and adrenaline-induced platelet aggregation also investigated both in vitro and in vivo in rat thoracic aorta shown a preferable effect.

2.3.7 Antifertility effect

Antifertility effect of turmeric orally fed and implantation in rats both petroleum ether and aqueous extracts show 100% effective. Curcumin also inhibits 5*a*-reductase, therefore convertion of testosterone to 5*a*-dihydrotestosterone could not be occur. In addition, curcumin also show human sperm motility inhibitor activity.

2.3.8 Antidiabetic effect

At a very low doses of curcumin also inhibits galactose-induced cataract formation in diabetes mellitus. In many studies indicated that turmeric and curcumin

also shown blood sugar lowering effect in diabetes rat induced by alloxan. Moreover, curcumin also suppress advanced glycation in diabetes mellitus.

2.3.9 Antibacterial activity

Both curcumin and the oil fraction of curcumin could be inhibits growth of several bacteria like *Streptococcus spp*., *Staphylococcus albus, Staphylococcus aureus, Bacillus typhosus*. *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, S. aureus, Escherichia coli*, *Pseudomonas aeruginosa*, etc. at different concentrations.

2.3.10 Antifungal effect

Many studies indicated that ether extract, chloroform extracts, ethanol extract and oil of turmeric inhibits growth of several fungi such as *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum* in various concentrations.

2.3.11 Antiprotozoal effect

Ethanol extract of turmeric also show anti-*Entamoeba histolytica* activity. In addition in previous studies found that curcumin has anti-*Leishmania* and anti-*Plasmodium falciparum* activity and its synthetic derivatives has anti-*L. amazonensis* effect.

2.3.12 Antiviral effect

The antiviral activities of curcumin have been reported. Its show an efficient inhibitor of Epstein-Barr virus (EBV) and anti-HIV (human immunodeficiency virus) activity. The report indicated that anti-HIV activity of by curcumin inhibits UV light induced HIV gene expression.

2.3.13 Antifibrotic effect

Curcumin 300 mg/kg/day take orally shown inhibits effect of increase in total cell counts and biomarkers of inflammatory responses induced by bleomycin.

Morevoer, significant suppression of TNF-*a*, superoxide and nitric oxide production by bleomycin induced. Therefore, curcumin is a potent antifibrotic agent.

2.3.14 Antihistamine effect

Ethylacetate extract of turmeric could be inhibits histamine release and type I allergy. The report indicated that turmeric extract suppressed IgE and IgG antibody response and shown the significantly induced of interferon in serum *(35)*.

2.3.15 Anti-inflammatory activity

Curcumin 200 mg orally suppressed inflammation induced by diethyl nitrosamine and hyperplasia in rats. The effect due to curcumin can reduced the granuloma growth and inflammation factor such as lipoxygenase*(6)*.

2.3.16 Other activities

The in vivo study in mice indicated that aqueous extracts of turmeric also shown antidepressant effects

2.4 Toxicity and safety profile

Safety evaluation with turmeric and curcumin(36)

2.4.1 Turmeric: The study reported no toxic symptoms were observed in administration of turmeric by Asians varies from 0.5 to 1.5 g/day/person. In addition no adverse effects were observed in turmeric 500 mg/kg/day monkeys fed for 9 months. At the higher doses of turmeric (2.5 g/kg body weight) fed in male and female wistar rats, guinea pigs and monkeys also shown no pathological, behavioural abnormalities and mortality was observed. Moreover, no significantly changes were observed in the investigation of kidney, liver and heart weight.

2.4.2 Curcumin: There are no pathological, behavioural abnormalities, adverse effects and mortality was observed in study of curcumin at a dose of 300 mg/kg body weight fed in both sexes of wistar rats, guinea pigs and monkeys. The oral LD_{50} in

mice of curcumin is higher than 2g and no toxicity was observed at doses of 1–8 g/day and 10 g/day in human.

3. Emulsion

3.1 Definition and general characteristics

Emulsion is generally define as a heterogeneous system consist of two immiscible liquids, oil and water. The internal phase uniformly dispersed as small droplets in external phase. Emulsion in pharmaceutical used were orally, parenterally, and topically (mainly in cosmetics), and for diagnostic purposes. The most common types of pharmaceutical emulsion can be define as following, If the oil is dispersed as droplets in the aqueous phase, the emulsion is termed oil-in-water (o/w) emulsion. If the oil is the continuous phase, the emulsion is the water-in-oil (w/o) as shown in Figure 6. On the other hand, the classification of emulsion may included macroemulsions, microemulsions, multiple emulsions, microemusions, gel emulsions, vesicle, and liquid crystalline systems.

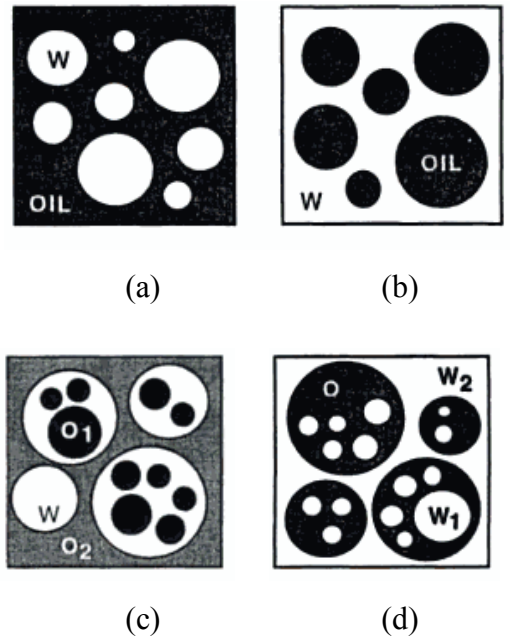


Figure 6 The structure of emulsion*(37)* structure of water in oil emulsions(a), structure of oil in water emulsions(b), structure of oil in water in oil multiple emulsions(c), structure of water in oil in water multiple emulsions(d)

Microemulsions are more stable emulsion systems which surface of the two immiscible liquids contain of surfactant and the cosurfactant layer. The desirable characteristics of microemulsions is using small amount of surfactant, cosurfactant, thermodynamically stable, transparent and spontaneusly forms. The most characteristic difference between an macroemulsion and a microemulsion are presented in Table 5.

Table 5 Characteristics of macroemulsions and microemulsions*(38)*

	Macroemulsion	**Microemulsion**
Appearrance	Turbid	Transparent
Size(μm)	0.15 -100	0.0015-0.15
Formation	Mechanical or Chemical energy	Spontaneous
Thermodynamic Stability	No	Yes(No)

The microemulsions is the advantages system compare to macroemulsions or other solutions because improving of stability or solubility characteristics. The microemulsions can be solubilize both lipophilic and hydrophilic agents. It also were used in pharmaceutical emulsions such as to solubilize cyclosporin A and stabilize of ascorbic acid. Moreover, phospholipid-based microemulsion can solubilize large molecules. Major problem in the preparation of microemulsions is to find suitable components or condition*(39)*.

4. Coating

Coating is the old pharmaceutical processes developed in mid-18th. This process is the one part of dosage form development process. Coating process is to apply a coating material onto the surface of a solid substrate such as tablets, capsules, pellets, granules or particles. The first purpose of coating in pharmaceutical science is masking unpleasant tastes and imparting a more elegant appearance. However, the changes in raw materials and processing technologies of coating have been interested over the last decade. Nowadays the purpose of coating as the following categories.

- Protection of drugs from environment factors such as light, moisture and air, in order to improve chemical and physical stability.
- Increased mechanical stability during manufacture, packing and shipment.
- Modification of product appearance to enhance marketability or hide undesirable color changes of the substrate.
- Masking of unpleasant taste, texture or odor.
- Increasing drug safety by better identification.
- Controlled or modified release of drugs.
- A mechanical barrier to avoidance of side effects or the interaction of incompatible ingredients.
- Active agent(S) layering on non-pariel core for instantaneous release

4.1 Type of Coating

Coating categories can be distinguished as following.

4.1.1. Coating with sucrose and other sugar

This type of coating is to apply sugar mass onto the core surface. It is widely used in the manufacture of pharmaceuticals and confectionery.

4.1.2. Hot melt coating

The raw materials in this type of coating are fats, mostly cocoa fat and the sugar/ alcohol mixture xylitol/ sorbitol. The temperature is necessary for used in the process.

4.1.3. Film coating

This type of coating need lower amount of coating agent. It is form a thin membranes over the surface of the substrate. The film formers may affect the partly on pH-dependent solubility and selective permeability of coatings.

4.1.4 Powder coating

The substrate are directly coated with polymer powder (film former). The temperature is necessary for the coalescence of the film formers.

4.1.5 Compression coating

Compression coating is the alternative techniques because of this technique spending time lower than in sugar coating. The process is to compress coating material around the core by using special machines. In addition temperature is not necessary for the process.

The three different kind of coated tablets shown in Figure 7.

(a) (b) (c)

Figure 7 Coated tablets sugar coated tablet (a), film coated tablet (b), compression coated tablet*(40)* (c)

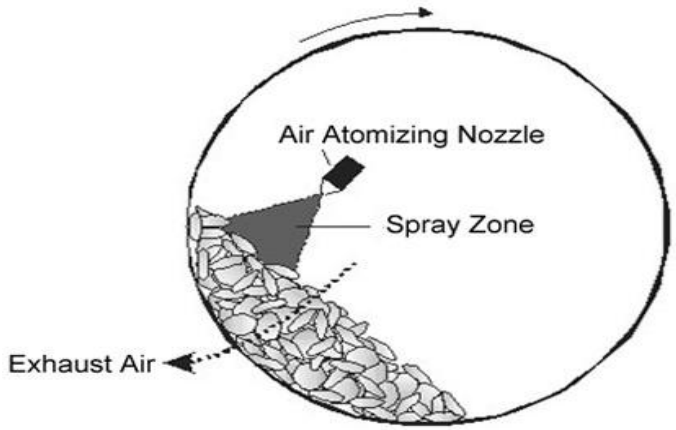


Figure 8 Coating process*(41)*

5. Extrusion

Extrusion is processing technology that has been developed in many industrial fields. Extrusion can be defined as the process of forming a new material call extrudate by forcing a material through an orifice or die under controlled conditions such as temperature, shear, pressure and types of equipment (extruders)*(42)*.

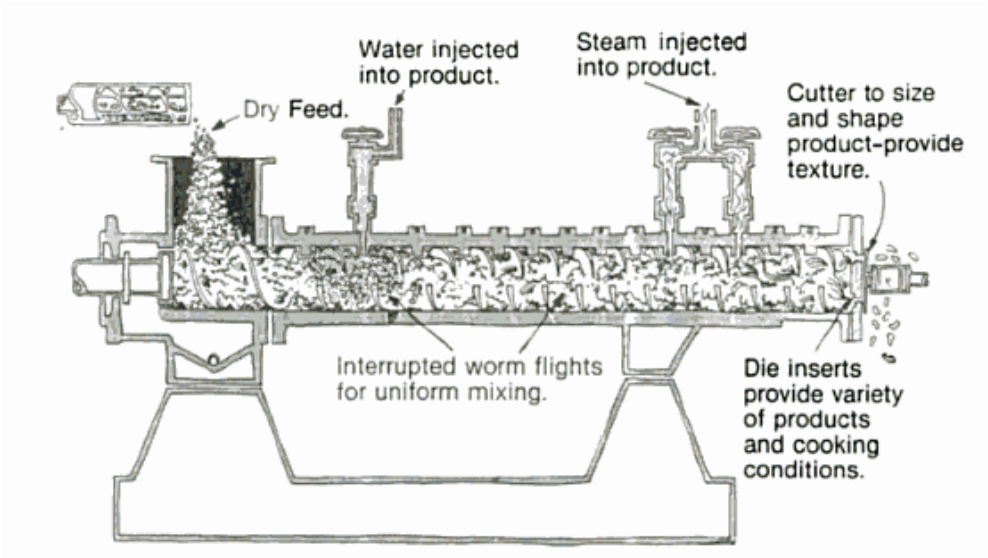


Figure 9 Extrusion process*(43)*

5.1 Type of extruders

Extruder is the term of a machine with Archimedean screw characteristics. Extruders may be designed to include various part of operations such as grinding, mixing, homogenizing, cooking, cooling, vacuumizing, shaping, cutting, and filling. Thus, the types of extruders available on the market are varieties. The two primary types of extruders are single screw extruders and twin screw extruders

5.1.1 Single screw-extruders

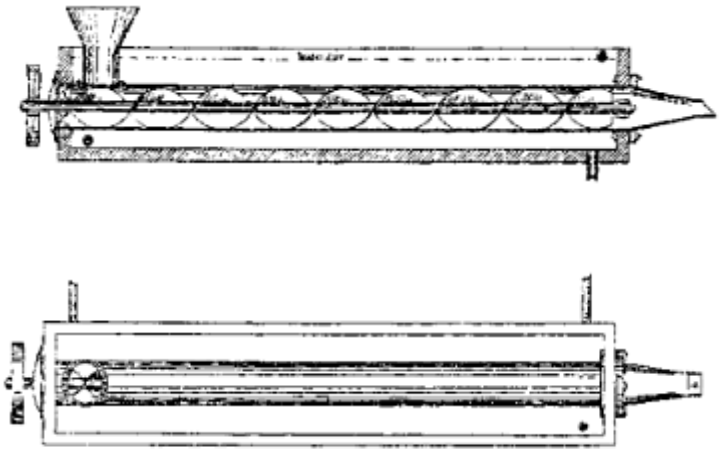
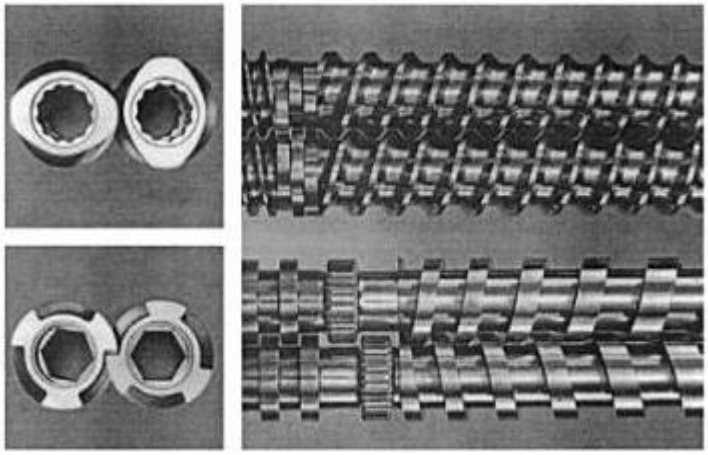


Figure 10 The structure of single screw extruders*(42)*

The basic processing of single-screw extruders is using one screw. However, the difference in their characteristics lead to classify of single-screw extruders to many sub types. The classification of single-screw extruders sub types usually base on wet vs dry, segmented vs. solid screw, extent of shear generated and source of heat generation.

5.1.2 Twin Screw Extruders

The twin screw extruders use two screw which arranged side by side. The positive coveying and effective mixing are advantages of twin screw extruders when compared with single-screw extruders. The two primary types of twin screw extruders are counterrotating and corotating. Corotating twin screw extruders is defined when the two screw rotate in same direction and counterrotating twin screw extruders is defined when the screw rotate in opposite directions.



(a) Corotating twin screw extruders

(b) Counterrotating twin screw extruders

Figure 11 The structure of twin screw extruders*(42, 43)*

a) Corotating twin screw extruders

Corotating twin screw extruders can be made variety of products which provide high degree of heat transfer but conveying characteristics is too low. The advantages of this type of extruders are pumping efficiency, good control over residence time distribution, self cleaning mechanism, and uniformity of processing. These type of extruders widely used in the food and snack food industry.

b) Counterrotating twin screw extruders

The counterrotating twin screw extruders show very positive material feed and conveying characteristics. Moreover, the resident time and temperature of material uniform. This extruder design are exhibited very high shear process and good for dispersing particles. Disadvantages of counterrotating twin screw extruders are

potential air entrapment, high pressure generations, and low maximum screw speeds and output.

5.2 Function of an extruder

The extruder can be generated many functions that very variety used in pharmaceutical, food, feed and industrial applications which are show following.

5.2.1 Agglomeration: Ingredients can be compacted and agglomerated into discrete pieces with an extruder.

5.2.2 Degassing: Ingredient that contain gas pockets can be degassed by extrusion processing.

5.2.3 Dehydration: During normal extrusion processing, a moisture loss of 4-5% can occur.

5.2.4 Expansion: Product density (i.e., floating and sinking) can be controlled by extruder operation conditions and configuration.

5.2.5 Gelatinization: Extrusion cooking improves starch gelatinization.

5.2.6 Grinding: Ingredients can be ground in the extruder barrel during processing.

5.2.7 Homogenization: An extruder can homogenize by restructuring unattractive ingredients into more acceptable forms.

5.2.8 Mixing: A variety of screws are available which can cause the desired amount of mixing action in the extruder barrel.

5.2.9 Pasteurization and sterilization: Ingredients can be pasteurized or sterilized using extrusion technology for different applications.

5.2.10 Protein denaturation: Animal and plant protein can be denatured by extrusion cooking.

5.2.11 Shaping: An extruder can make any desired shape of product by changing a die at the end of the extruder barrel.

5.2.12 Shearing: A special configuration within the extruder barrel can create the desired shearing action for the particular product.

5.2.13 Texture alteration: The physical and chemical textures can be altered in the extrusion system.

5.2.14 Thermal cooking: The desired cooking effect can be achieved in the extruder.

5.2.15 Unitizing: Different ingredient lines can be combined into one product to produce special characteristics by using an extruder.

CHAPTER III

EXPERIMENTS

1. Materials

The following materials obtained from initial sources were used.

1.1 Model drug

Turmeric extract powder (Batch No. NP510020, purchased from GPO, Thailand)

1.2 Other components

- Initial shrimp feed (Neo Feed 204) purchased from Inteqc Feed Co., Ltd., Thailand
- Deoiled enzyme-modified soy lecithin (Batch no. R550000629, Decatur Plant Solae, USA)
- Absolute ethanol (ethyl alcohol 95 % Lot no. 51SP1208, Liquor Distillery Organization, Excise Department, Thailand)
- Squid liver oil (K.D. Mulsan Co., Ltd., Korea)
- Methanol HPLC grade (Batch no.10071753, Burdick and Jackson, USA)
- Acetic acid analar grade (Lot no.K32754317349, BDH Chemicals. Poole England)
- Acetonitrile HPLC grade (Batch no.10071743, Burdick and Jackson, USA)

2. Equipment

- Analytical balance (Model XP205, Mettler Toledo, Switzerland and Model A200s, Sartorius Gbh, Germany
- Conventional coating pan equipped with atomizer (Thai Coater®), Thailand)
- Differential scanning calorimeter (Model 822^e, Mettler Toledo, Switzerland)
- Digital camera (model Coolpix 4500, Nikon, Japan)
- Disintegration apparatus (Model ZT 31, ERWEKA, Germany)
- Dissolution apparatus (Model VK7000, Vankel, USA)
- Extruder (Model EXKS-1, Fuji Paudal Co.,Ltd., Japan)
- Hardness tester (Model Schleuniger 2E, Switzerland)
- High performance liquid chromatography (Model SCL-20ADVP, Shimadzu, Japan) with
    - System controller (Model SCL-20AD VP, Shimadzu, Japan)
    - Liquid chromatography (Model LC-20AD VP, Shimadzu, Japan)
    - Degasser (Model DGU-20A_3, Shimadzu, Japan)
    - Auto injector (Model SIL-20AD VP, Shimadzu, Japan)
    - Column oven (Model CTO-20AS VP, Shimadzu, Japan)
    - UV-VIS detector (Model SPD-M20A VP, Shimadzu, Japan)
- Hot air oven (Model UL80, Memmert, Germany)
- Hot stage (Model PF 90, Mettler Toledo, Switzerland)

- Hotplate magnetic stirrer (Model M6, CAT, Germany)
- Jolting volumeter (Modified by Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)
- Laser light scattering particle size analyzer (Mastersizer 2000MU, Ltd., UK)
- Microscope (Model CSHG, Eclipse E200, Nikon, Japan)
- Moisture balance (Model HR83, Mettler Toledo, Switzerland)
- Oscillating granulator (Model AR400 Erweka, USA)
- Peristaltic pump, (Model 503S, Watson-Marlow, England)
- pH meter (Model 210A+, Thermo Orion, Germany)
- Planetary mixer (Model 5K5SS, Kitchen Aid, USA)
- Polarized lens (Nikon, Japan)
- Powder X-Ray diffractometer (Model D8 Bruker AXS, Karlsruhe, Germany)
- Scanning electron microscope (Model JSM-5800LV, Joel Ltd., Tokyo Japan)
- Analytical sieves (Filtra, Spain)
- Thermogravimetric analyzer (Model 851^{e}, Mettler Toledo, Switzerland)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Germany)
- Vernier caliper (150X100 mm, China)
- Zetasizer Nano ZS (Malvern Instruments Ltd., UK)

Methods

1. Preformulation Study

1.1 Determination of turmeric extract powder physical properties

1.1.1 Morphology

The shape and surface topography of turmeric extract powder were determined by Scanning Electron Microscope (SEM) model JSM-5800LV from Joel Ltd., Tokyo Japan. The samples were prepared by gold sputtering technique prior to SEM examination. Photomicrographs of turmeric extract powder were taken using magnifications of x100, x200, x1000 and x3500 at 15 kV.

1.1.2 Particle size and size distribution

The particle size and size distribution of turmeric extract powder were determined by laser light scattering technique using Mastersizer 2000MU from Malvern Instruments Ltd., UK. The sample was dispersed in water and the pump speed was set at 15 rpm. The sample was determined in triplicate and the average of the mean diameters was calculated.

1.1.3 Solid State Characterization

a. Polarized light microscopy (PMC)

Crystal habit of turmeric extract powder were observed under a microscope Model CSHG, Eclipse E200, Nikon, Japan equipped with a 35-mm digital camera Nikon Coolpix 4500 from Japan and Nikon polarized lens. The sample was placed over a glass slide and covered with a cover slip. The birefringence of the crystals were evaluated to qualitatively characterized the crystallinity of turmeric extract powder under magnification of x100.

b. Powder X-ray diffraction (*XRD*)

Powder X-ray diffraction pattern of turmeric extract powder was acquired on powder X-Ray diffractometer using CuKα radiation (tube operated at 40 kV, 30 mA). Data was collected over an angular range from 5 to 40^{o} 2θ in continuous scan mode using a step size of 0.018^{o} 2θ/sec at room temperature.

c. Thermal analyses

- Differential scanning calorimetry (DSC)

The thermogram of turmeric extract powder was recorded on the DSC (Mettler, Toledo DSC 822^{e}, Switzerland). The temperature axis and cell constant of DSC cell were calibrated with indium. Heating rate of 10 ^{0}C/min was employed over a temperature range of 25–250 ^{0}C with nitrogen gas purging at 60 ml/min. Turmeric extract powder (1–5 mg) was accurately weighed into an aluminum 40 µl pan, sealed and analysed. An empty aluminum 40 µl pan with sealed lid was used as reference.

- Thermogravimetry (*TGA*)

Thermogravimetric analysis was carried out using TGA/SDTA 851^{e}. The water loss was determined by placing the turmeric extract powder (1–5 mg) in 70 µl alumina crucibles and heated from 25^{0}C to 250 ^{0}C at a rate of 10 ^{0}C/min under nitrogen gas purging (20 ml/min). To determine the weight loss due to moisture, crystal water, volatiles or degradation turmeric extract powder.

- Hot-stage microscopy (*HSM*)

Thermal events were observed on a hot stage under a microscope Model CSHG, Eclipse E200, Nikon, Japan equipped with a 35-mm digital camera (Nikon model Coolpix 4500 from Tokyo, Japan) and Nikon polarized lens. The turmeric extract powder was placed over a drop of mineral oil, covered with a cover slip and heated from 25^{0}C to 250 ^{0}C at a rate of 10^{0}C/min. The temperature at which liberation of bubbles occur corresponded to the escape of vapor were observed.

1.1.4 Solubility study

An excess amount of turmeric extract powder were placed in glass vials with 5 ml of six different types on 37^{0}C of solvents (water, absolute ethanol, cremophor EL, mineral oil, 1.272 squid liver oil and isopropyl myristate) a shaker water bath at 100 rpm over night (15 hours) then analyzed using the HPLC method which will be described in the next section.

1.1.5 Identification and analysis of turmeric extract powder

The identification and the investigation of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin contents in turmeric extract powder was determined by means of calculating absorption peak area from high performance liquid chromatography (HPLC) method using the following condition~~system~~.

HPLC chromatographic condition:

Column	: Alltech Alltima ® C18 column (4.6 x 150 mm, 5µm)
Mobile phase	: 2% acetic acid : acetonitrile (60:40)
Flow rate	: 2.0 ml/min
Injection volume	: 20 µl
Detector	: UV 425 nm
Oven Temp.	: 34 ^{0}C
Run time	: 16 min

1.1.6 Compatibilily Testing

Differential scanning calorimetry (DSC) was used for the characterization and investigation of the physical and chemical interactions between the active ingredients and excipients in the expected formulations*(44, 45)*. Therefore, accurately weigh of 1-5 mg of the formulation to 40 µl aluminium pan and sealed. Scaning rate of 10 ^{0}C/min over a temperature range of 25–250 ^{0}C was used.

Characterization of initial shrimp feed

1.2.1 Morphology

Scanning electron microscope (SEM) was used to examine shape and surface topography of initial shrimp feed as same as turmeric extract powder which is described in 1.1.1 Photomicrographs of shrimp feed were taken in magnification of x1000 at 15 kV.

1.2.2 Particle size and size distribution

Size distribution of initial shrimp feed were classified by sieve analysis which consisted of a set of US standard sieves, ranging from sieve passing apertures of 1,400, 1,180, 850, 600 and 425 μm respectively and a collecting pan. One hundred grams of initial shrimp feed were accurately weighed and placed on the top of the sieves. A set of sieves were placed on the Analytical Sieves and allowed to shake for 10 minutes. The retained of initial shrimp feed on each sieve size were weighed and calculated for the percentage of weight retained on sieve by following equation:

$$\% \text{ Retained} = \frac{\text{Retained weight (g)}}{\text{Total shrimp feed pellet weight (g)}} \text{ x } 100$$

1.2.3 Moisture content

The moisture content of initial shrimp feed was determined by moisture balance Model HR83 from Mettler Toledo, Schwerzenbach, Switzerland. About 5 g of shrimp feed were accurately weighed and uniformly spread as thin layer on an aluminium plate. Then, they were exposed to high temperature of approximately 105 °C until constant weight was obtained. The moisture content in terms of loss on drying (%LOD) was calculated automatically. The results were obtained from an average of three determinations.

1.2.4 Bulk, tapped densities and compressibility index

The bulk density (ρ_b) of the initial shrimp feed was determined by pouring 20 g of the shrimp feed into a 50 ml graduate cylinder and measuring the volume of

shrimp feed. The graduate cylinder was tapped on a jolting volumeter until a constant volume was obtained. The tapped density (ρ_t) was then calculated. Both densities were average from three determinations. The Carr's compressibility, which expresses the flow property was calculated from the following equation.

$$\% \text{ Compressibility} = \frac{(\rho_t - \rho_b)}{\rho_t} x\,100$$

1.2.5 Flow rate

Accurately amount about 20g of initial shrimp feed were filled in a glass funnel with 1.5 cm internal stem diameter fixed on the clamp. When the shrimp feed started to flow until finished, the time was recorded. Flow rate was average from three determinations and reported in term of g/sec.

1.2.6 Angle of repose

The angle of repose was measured from a heap built up by falling of 20g of the initial shrimp feed samples through a glass funnel with 1.5 cm internal stem diameter fixed on the clamp at 10 cm height from the smooth surface. Average result from five determinations was reported. The angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} \frac{H}{R}$$

H and R are the height and radius of the powder cone

1.2.7 Diameter

The determination of initial shrimp feed thickness was carried out by measuring of twenty pellets of initial shrimp feed randomly sampling with vernier caliper. An average of twenty determinations (±SD) was calculated.

1.2.8 Hardness

Twenty pellets of initial shrimp feed randomly sampling were measured for its hardness individually on a hardness tester model Schleuniger 2E, Switzerland. An average value of hardness was calculated.

1.2.9 Immersion time

The shrimp feed weighing approximately 20 g were place into a beaker containing 500 ml distilled water with a temperature of 37±1°C from 15 cm hight the time at which all shrimp feed pellets settled to the bottom of the beaker after immersion was record as the immersion time (seconds).

2. Liquid turmeric extract concentrated formulation

2.1 Preparation of turmeric extract concentrated solution

Turmeric extract concentrated solution to be used as coating agent and wetting agent in the coating and extrusion processes were prepared. The 800 ml of turmeric extract concentrated solution was prepared by dissolving an accurate amount of turmeric extract powder 1.272 g in 800 ml of absolute alcohol solution. Stir by using magnetic stirrer Model M6, CAT, Germany for 45 minute to dissolve all solid content. In addition, dissolving chamber must be covered with aluminium foils.

2.2 Preparation of turmeric extract concentrated microemulsions

This part is the preparation of turmeric extract concentrated microemulsion to be used as a coating agent and a wetting agent for coating and extrusion processes. The 160 g of turmeric extract concentrate microemulsion was prepared by dissolving accurate amount of turmeric extract powder 1.272 g in absolute alcohol and cremophor El. Lecithin and squid liver oil were mix in various ratios (1:1.5, 1:2, 1:3, 1:4 and 1:5). Finally, the two separated phases were gradually mixed continuous under stirring of 45 minutes and the chamber was covered with aluminium foil. Varying amount of each phase in order to find a suitable microemulsion system are shown in the Table 6.

Table 6 Formulations for turmeric extract microemulsion preparation

Lecithin : Squid Liver Oil (1:1.5,1:2,1:3,1:4,1:5)	**Alcohol**	**Cremophor EL**
1.5	3	0.5
1.5	2.5	1
1.5	2	1.5
1.5	1.5	2
1.5	1	2.5
1.5	0.5	3
0.5	2.5	2
1	2	2
2	1	2
2.5	0.5	2
2.75	0.25	2

Amount of turmeric extract powder used was determined by calculating the following equation

$$\text{Amount of turmeric extract powder (g)} = \frac{\text{Total Curcuminioids in preperation (g)}}{\%\ \text{Curcuminoids content in turmeric extract powder}} \text{ x } 100$$

3. ~~The m~~Methods for the incorporation of turmeric extract to shrimp feed

There are two methods in the incorporation of active turmeric extract to the shrimp feed i.e. coating method and extrusion method.

3.1 coating method

a. Preliminary study on the coating process

Many parameters on the coating process haves been known to influenced many characteristics of the final coated product. Therefore, the investigation of the parameters which effect the characteristics of shrimp feed when coated with turmeric extract concentrated solution using conventional coating pan equipped with spraying atomizer were done. The two-level full factorial design was used to screen the variables. Drug concentrations (X1), pump speed (X2) and inlet air temperature (X3) were selected as the independent variables for the study. The dependent variables were homogeneity of coating (Y1) (illustrated by Relative Standard Deviation (RSD) of near zero) and drug content (Y2) (illustrated by percentage of near 100%). Program design expert 7.1.1trail version was used in the evaluation.

Experimental design

The pan load, atomizing air pressure, pan speed, and spray distance wre kept constant at 1,000 g, 2.5 (kgf/cm^2), 40 rpm, and 15 cm, respectively. Eight experimental runs with different coating conditions werewas designed. First, two different concentrations of coating solutions were prepared. The Ccoating solution (X1) was prepared by dissolving accurately weighed amount of turmeric extract powder (0.634 g for 500 µg/g of the final coated product and 1.272 g for 1,000 µg/g of final coated product) in 800 milliliters of absolute ethanol (Table 7). Both coating agents were stirred continuously using magnetic stirrer at 50 rpm speed for —1 hour to obtain clear solution at room temperature before use. An atomized spray of the final solution from Thai coater® atomizer was applied onto the surface of initial shrimp feed in conventional coating pan. The different inlet air temperature was 25± 2°C and 60 ± 2°C. Then, the coating agent was sprayed with a constant atomizing air pressures at 2.5 (kgf/cm^2) and different feed rates of 5 and 10 rpm. The coated shrimp feed was post-heated in a conventional coating pan for 5-7 min at 25°C and 60 °C. A three-factor, two-level full factorial design was used for the screening procedure. The independent variables were coded and levels were assigned as shown in Table 8.

Table 7 Ingredients used in the preparation of 1 kilogram of coated shrimp feed containing turmeric extract for the preliminary coating evaluation

Amount / **Concentration**	**Turmeric extract pPowder (g)**	**Absolute ethanolAlcohol (ml)**
Low concentration (500µg/mg)	0.634	800
High concentration (1,000µg/mg)	1.272	800

- Curcuminoids content in the turmeric extract was 82.60% (data from supplier)

Table 8 Coating condition in the preliminary coating evaluation

Experiments	**Coating condition**		
	Drug concentrations (µg/g)	Feed pump speed (rpm)	Temp (^{0}C)
1	500	5	25
2		5	60
3		10	25
4		10	60
5	1000	5	25
6		5	60
7		10	25
8	***B***	10	60

b. Preparation of shrimp feed containing turmeric by coating process

An appropriate 1 kg weight of the initial shrimp feed wereere place in the conventional coating pan equipped with Thai Coater® atomizer. The atomizing air pressure, pan speed, spraying distance, inlet air temperature and feed pump speed wereas kept constant at 2.5 (kgf/cm^2) 40 rpm, 15 cm, 25±2 °C and 5 rpm, respectively. The coating agent was prepared as in previous section. Amount of 800 ml was atomized onto the surface of the shrimp feed in conventional coating pan. The coated shrimp feeds were post driedy in a rotating pan for further 5-7 min at 25°C.

3.2 Extrusion process

The initial shrimp feeds were ground and passed through pores of 60 mesh sieve by using oscillating granulator. Appropriate 1 kg weigh of grounded shrimp feeds and both liquid turmeric extract concentrates were gradually mixed rain a planetary mixer at the lowest speed for 20 minutes. After that, the wet mass were extruded by a radial single screw extruder with a die of 1 mm diameter and 1 mm length. The obtained extrudates were driedy in hot air oven for ——5 hour at 45°C.

4. Characterization of shrimp feed containing turmeric extract

4.1 Homogeneity of Mixing (uniformity of dosage unit)

The turmeric extract content in shrimp feed was quantitatively determined by weighing 1 g of fine powder of shrimp feed. Sample were taken by random sampling at 6 points. Each 1 g sample from each point was placed into a 25 ml volumetric flask, add 15 ml of methanol (HPLC grade) and sonicate for 30 minutes. Add 10 ml of mobile phase, mixed well and centrifuged at 4,000 rpm for 10 min. The sample passed through a nylon filter having 0.45 µm porosity. Filtered solutions were injected to the HPLC column. The turmeric extract content was quantitatively calculated by means of absorption peak areas obtained from high performance liquid chromatography (HPLC) method.

4.2 Percent Assay

The drug content of turmeric extract in shrimp feed was quantitatively determined by HPLC method. Random sampling 6 points of 5 g and pooled all samples. Transfer an accurately weighed quantity of the feed equivalent to 1 g of turmeric extract to a 25 ml volumetric flask. Dissolved 15 ml of methanol (HPLC grade) and sonicated for 30 minutes. Add about 10 ml of mobile phase and centrifuged at 4,000 rpm for 10 min. Passed the sample through a nylon filter having 0.45µm pore size. Filtered solutions were injected to the HPLC column. The percentage of labeled content was quantitatively calculated averaging the peak area obtained from HPLC method.

4.3 Morphology

The morphology of shrimp feed containing turmeric extract was characterized as described in section 1.2.

4.4 The pPhysical properties

Physical properties of shrimp feed containing turmeric extract were evaluated. The moisture content, morphology, bulk and tapped densities, percent compressibility, flow rate, angle of repose, thickness, hardness and immersion time were characterized as according to the method described in section 1.2.

4.5 Leaching Test

The cPondition used for determination leaching of turmeric extract from shrimp feed containing turmeric extract by USP Dissolution Apparatus II (Paddle) is shown in Table 9.

Table 9 Leaching test conditions for shrimp feed containing turmeric extract

Parameter	Condition
Medium	900 ml
Rotating speed (rpm)	20
Temperature (°C)	37 ± 0.5
Total time (hours)	1
Sampling interval (min)	10

One gram of shrimp feed was accurately weighed and filled in vessel of USP Dissolution Apparatus II (Paddle). Vessel filled with 900 ml water at 37^0C ± 0.5 and the rotating speed was kept 20 rpm. Recover all solid sample remaining at time intervals of 10, 20, 30, 40, 50, 60 minutes. Fresh medium was exchanged with the same quantity of medium immediately after sampling at every time interval. The solid sample was diluted to a suitable concentration (1 g shrimp feed was diluted with 5 ml methanol and 5 ml mobile phase to obtain turmeric extract concentration of about 100 µg). Sample mixture was transferred to a centrifuge tube, sonicated for 20 minutes and centrifuged at 4,000 rpm for 10 minutes. The clear supernatant were filtered with 0.45 µm nylon filter to be assayed for drug content by HPLC. The amounts of turmeric extract powder released at various time interval were calculated from the standard curve. Correlation was plotted as a function of percent amounts of turmeric extract remaining in shrimp feed and leaching time.

5. Stability study

Stability study of shrimp feed containing turmeric extract was performed according to ICH Guideline on Stability Testing of Drug Products *(46)*. The samples were packed in closed aluminium foil bags and stored under ambient conditions

(30 $\pm$ 2^0C and 65$\pm$ %RH) for 6 months and randomly sampled for analyzing the percent remaining of turmeric extract contents by HPLC method. The sample preparations were done as described in Appendix C. Moreover, the physical appearances and physical characterization of shrimp feed were observed every month and at the end of the storage period, except for the test of uniformity of dosage unit was not done.

CHAPTER IV

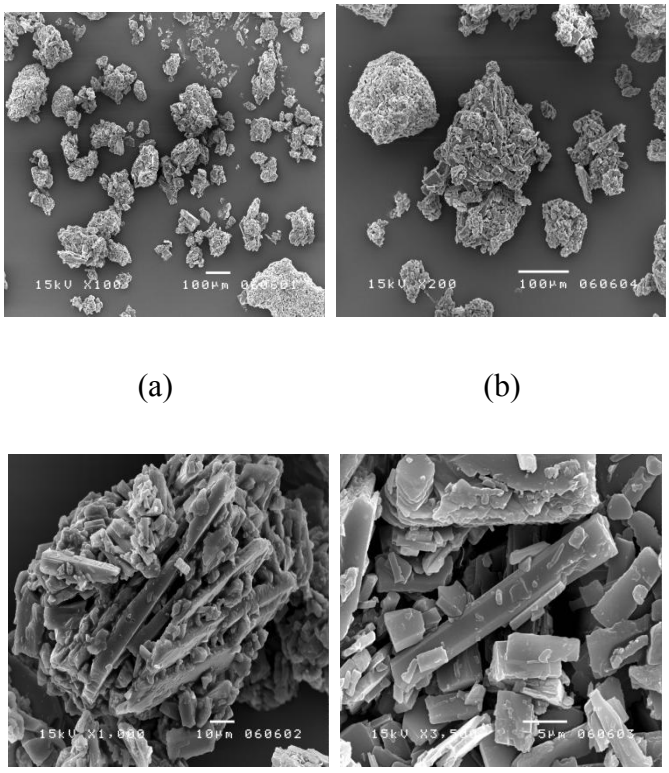
RESULT AND DISCUSSION

1. Preformulation Study

1.1 Determination of turmeric extract powder physical properties

1.1.1 Morphology

The morphology of turmeric extract powder examined by scanning electron microscope (SEM) with magnifications of x100, x200, x1000 and x3500 at 15 kV are presented in Figure 12.



(a) (b)

(c) (d)

Figure 12 Scanning electron photomicrographs of turmeric extract powder at 15 kV with the magnifications of (a) x100, (b) x200,(c) x1,000 and (d) x3500

The scanning electron photomicrographs illustrate that most of turmeric extract powder are rod shape, with a various particle sizes (10-100 μm) with agglomerates.

1.1.2 Particle size and size distribution

The particle size and size distribution of turmeric extract powder was determined by laser light scattering technique as shown in Table 10 and Appendix A.

Table 10 The particle size and size distribution of turmeric extract powder

Physical properties	**Turmeric extract powder**
d (v,0.1), μm	3.71
d (v,0.5), μm	22.15
d (v,0.9), μm	54.20
Volume weighted mean diameter (μm)	25.96
Particle size range (μm)	0.3-100

From Table 10 illustrates that particle size distribution of turmeric extract powder is broad. Variation smallest size to the largest to (100μm).This result agreed well with the results obtained from scanning electron microscopy wich also showed a wide particle size distribution from less than 5 μm to over 100 μm.

1.1.3 Solid State Characterization

a. Polarized light microscopy (PMC)

Crystal habit of turmeric extract powder observed under a microscope equipped with a 35-mm digital camera and polarized lens at magnification of x100 is shown in Figure 13.

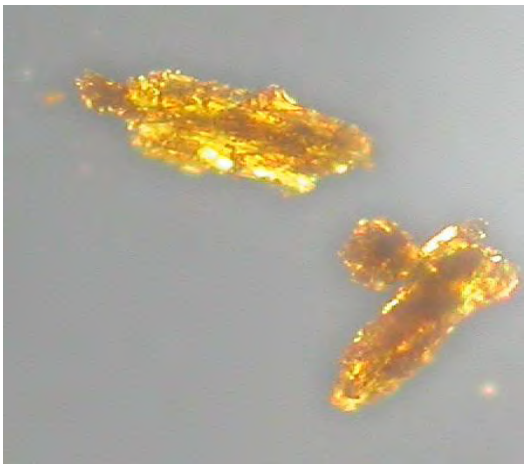


Figure 13 The photomicrograph of turmeric extract powder observed under a polarized microscope at the magnification of x100

The photomicrograph of turmeric extract powder under magnification of x100 illustrates irregular habit with agglomeration. It was also shown the birefringence of particles under polarizing lens. Thus, crystallinity of turmeric extract powder could be determined using these technique.

b. Powder X-ray diffraction (XRD)

Powder X-ray diffraction pattern of turmeric extract powder was acquired on powder X-Ray diffractometer and are shown in Figure 14.

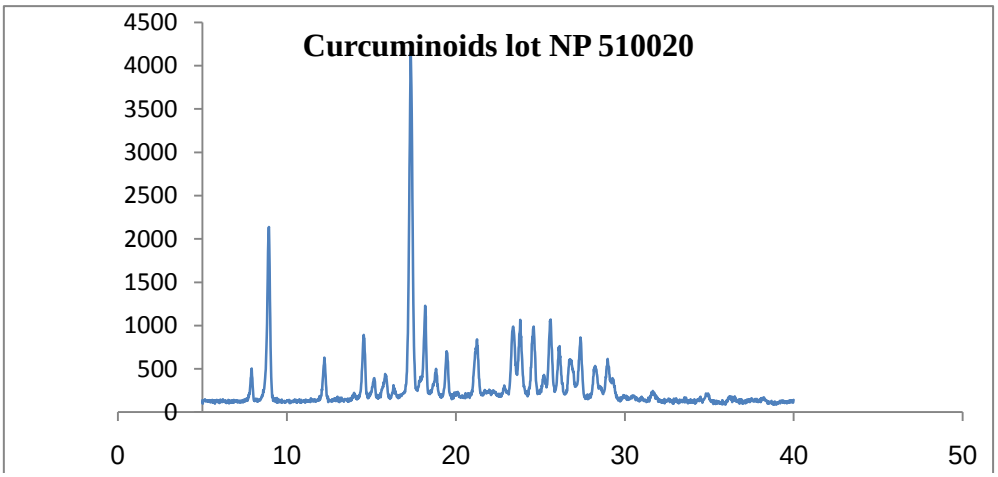


Figure 14 Powder X-ray diffraction pattern of turmeric extract powder

The powder diffraction fingerprint of turmeric extract powder showed a unique pattern that can be used as reference to identify the presence of solid turmeric extract within a processed sample. Moreover, the sharp narrow diffraction peaks and the high intensities quantitatively represent high crystallinity of turmeric extract powder *(47)*.

c. Thermal analyses

- Differential scanning calorimetry (DSC)

Thermogram of turmeric extract powder obtained by DSC over a temperature range of 25–250 ^{0}C is shown in Figure 15.

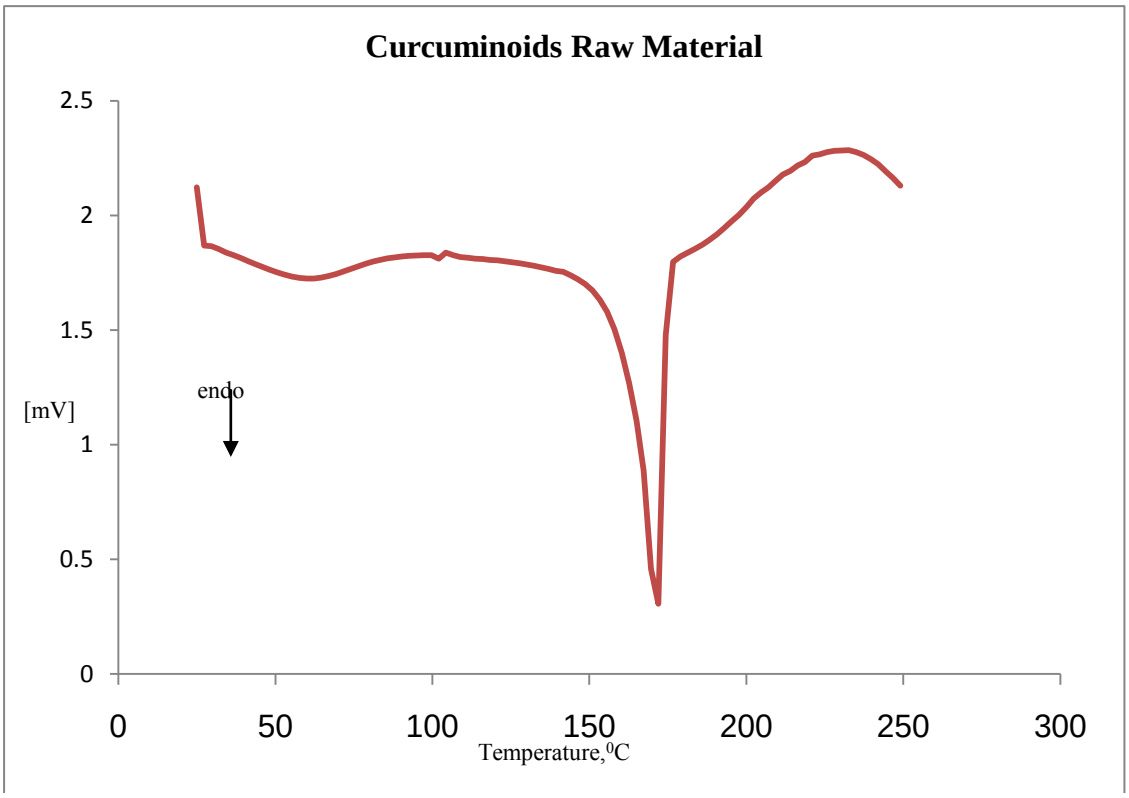


Figure 15 DSC thermogram of turmeric extract powder at scanning rate 10 ^{0}C/min heating up 25^{0}C to 250 ^{0}C

The DSC thermal characteristics of turmeric extract powder at a scanning rate of 10 ^{0}C/min from 25^{0}C to 250 ^{0}C shows two endotherms. The first endotherm is a broad endothermic event in the range of 40-90 ^{0}C corresponding to loss of adsorbed moisture. The second endotherm within the range of 150^{0}C-175^{0}C may be due to melting of the 3 active components in turmeric extract powder. However, the respected melting endotherm is usually broad to represent only melting phenomenon. This broad character may be due to simultaneous degradation of the extract at the same time of melting as supported by irregular baseline shift after 175^{0}C where degradation occurs.

- *Thermogravimetric analysis (TGA)*

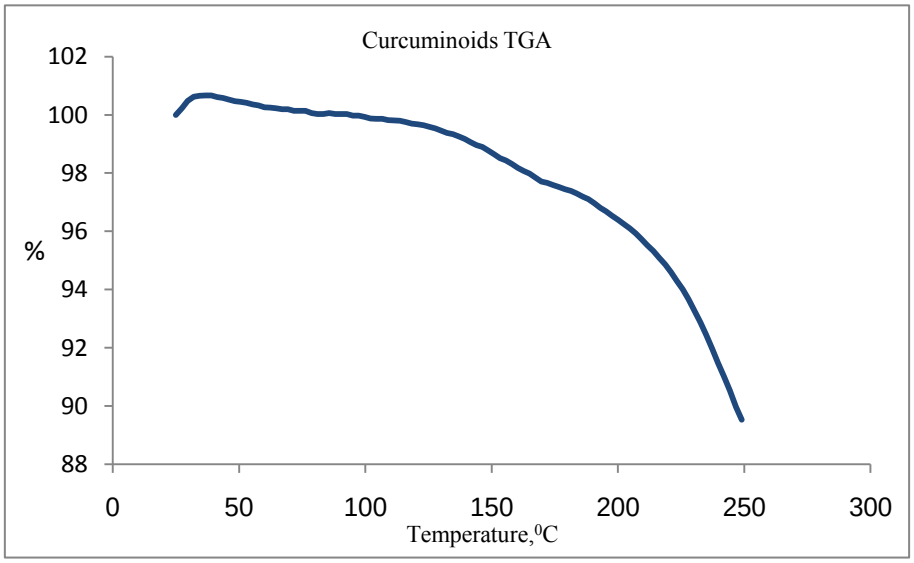
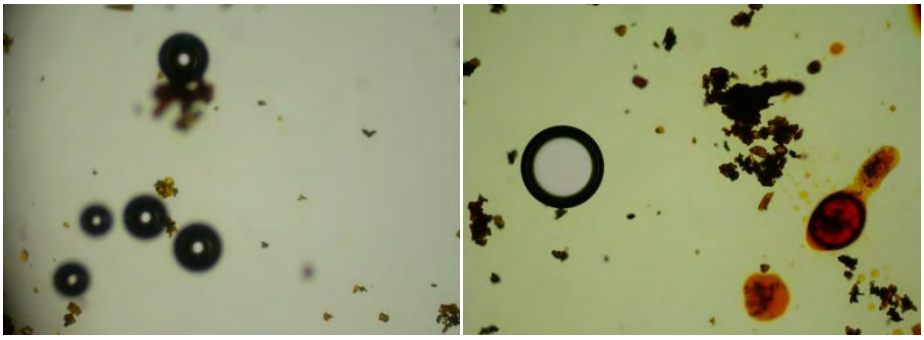


Figure 16 TGA thermogram of turmeric extract powder at scanning rate 10 ^{0}C/min from 25^{0}C to 250 ^{0}C

TGA analysis of turmeric extract powder at scanning rate 10 ^{0}C /min from 25^{0}C to 250 ^{0}C shows minor step of 1.03 %w/w weight loss corresponding to the loss of adsorbed moisture from 40-90 ^{0}C. Significant weight loss corresponding to degradation was found to overlap in some part with melting after 150 ^{0}C onwards when compared to DSC result. This finding corresponds well with the result obtained by DSC.

- *Hot-stage microscopy (HSM)*

Thermal events according to DSC and TGA were visually observed on a hot stage under a microscope equipped with a 35-mm digital camera and polarized lens and are shown in Figure 17.



(a) (b)

Figure 17 Thermal events of turmeric extract powder as seen from using hot stage microscope at a scanning rate of 10 ^{0}C/min from 25^{0}C to 250 ^{0}C at 137^{0}C (a) and 157^{0}C (b)

Thermal events of turmeric extract powder observed by hot stage microscope confirmed the results obtained by DSC and TGA. It was found that the generation of bubbles started at aproximately 137 ^{0}C (Figure 17(a)) signifying an increase in volatile degradation product. While, the temperature continued to increase up to 157 ^{0}C, turmeric extract powder was found to melt with bubbles becoming larger in size simultaneously with chared discolored solids indicative of degradation (Figure 17(a)).

1.1.4 Solubility

Solubility of turmeric extract powder investigated in six different solvents (water, absolute ethanol, squid liver oil, isopropyl myristate, mineral oil and cremophor EL) are shown in Table 11.

Table 11 Saturated solubility data of turmeric extract powder in various solvents at temperature 37 ^{0}C

Solvent	Turmeric extract Solubility (µg/ml)			Mean (SD)
	1	2	3	
Water	56.95	57.34	57.47	57.3(0.03)
Absolute ethanol	1002.77	996.648	1009.01	1002.8(0.63)
Squid liver oil	2813.36	2745.11	2713.29	2757.3(5.11)
Isopropyl myristate	491.13	489.55	493.79	491.5(0.21)
Cremophor EL	13379.32	13120.00	12482.51	12993.9(46.15)
Mineral oil	328.89	330.08	330.83	329.9(0.10)

The solubility study is a useful method to select an appropriate solvent and excipients in liquid turmeric concentrated preparations. The different sources of raw materials usually resulted in variation in curcuminoids content which eventually affects many pharmaceutical properties. In previous studies, the type of extraction solvents and extraction conditions (such as temperature and solid/liquid ratio) affect curcuminoids content, total phenolic content and antioxidant activities of dry turmeric*(48)*. Therefore, the solubility study of turmeric extract powder must be performed on every lot of material obtained. The solubility study of turmeric extract powder was performed in six different solvents. The result illustrates very poor solubility of turmeric extract powder in water at 37 °C. Whereas, the solubility increased in mineral oil 329.9µg/ml, isopropyl myristate 491.5µg/ml, absolute ethanol 1000.28µg/ml, squid liver oil 2757.3µg/ml and cremophor EL 12993.9µg/ml. It was probably due to the non polar nature of curcuminoids in turmeric extract powder resulting in limited solubility of turmeric extract in polar solvents and well dissolved in non polar solvents*(49)*. The solubility of turmeric extract powder in various

solvents from this study can be ranged as cremophor EL > squid liver oil > ethyl alcohol (absolute) > isopropyl meristate > mineral oil > water.

From the results, Cremophor EL, squid liver oil and ethyl alcohol (absolute) were chosen to prepare liquid curcuminoids concentrates in the future study.

1.1.5 Identification and analysis of turmeric powder

The result for the identification and determination of content of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in turmeric extract powder using high performance liquid chromatography (HPLC) (Alltech Alltima® C18 column (4.6 x 150 mm, 5µm) flow rate : 2.0 ml/min and 2% Acetic acid : Acetonitrile (60:40) as mobile phase) are shown in Figure 18.

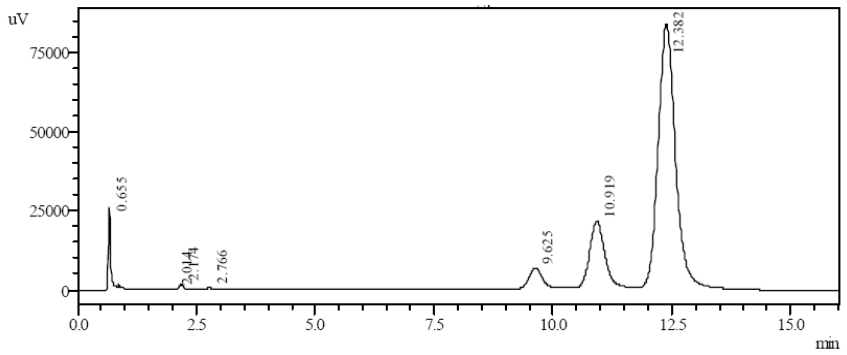


Figure 18 HPLC chromatogram of turmeric extract powder indicating curcumin, desmethoxycurcumin and bisdesmethoxy curcumin at 12.3, 10.9 and 9.6 minutes respectively

The retention times of bisdesmethoxycurcumin, desmethoxycurcumin and curcumin were 9.6, 10.9 and 12.3 minutes, respectively. Area under the curve of each compound in turmeric powder batch No. NP510020 are shown in Table 12.

Table 12 Area under the curve and content of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in turmeric extract powder batch No. NP510020

	curcumin	**desmethoxycurcumin**	**bisdesmethoxycurcumin**
Area	1764934	396541	96268
	1764906	395836	96504
	1766154	396251	96309
Content (mg/g)	632.72	156.48	31.94
	632.71	156.24	32.00
	633.14	156.39	31.95
Mean	632.86	156.38	31.96
SD	0.02	0.01	0.00
RSD (%)	**0.03**	**0.08**	**0.09**

It is shown that total curcuminoids content in turmeric extract powder batch No. NP510020 is 82.12%. Whereas curcumin content is 63.29%, desmethoxycurcumin content is 15.64% and bisdesmethoxycurcumin content were 3.20%. The result indicated that curcumin content of this batch is less than the lower limit in USP forum 33*(24)* where curcumin content must not be less than 70%. Although, total curcuminoids content showed higher amount than the lower limit specified in USP forum 33 (total curcuminoids content not less than 80%) but the change in ratios of the curcuminoidsmay affect the pharmacological outcome of turmeric extract powder.

1.1.6 Compatibilily Testing

The compatibility of turmeric extract powder and initial shrimp feed were evaluatedby differential scanning calorimetry (DSC) and are shown in Figure 19.

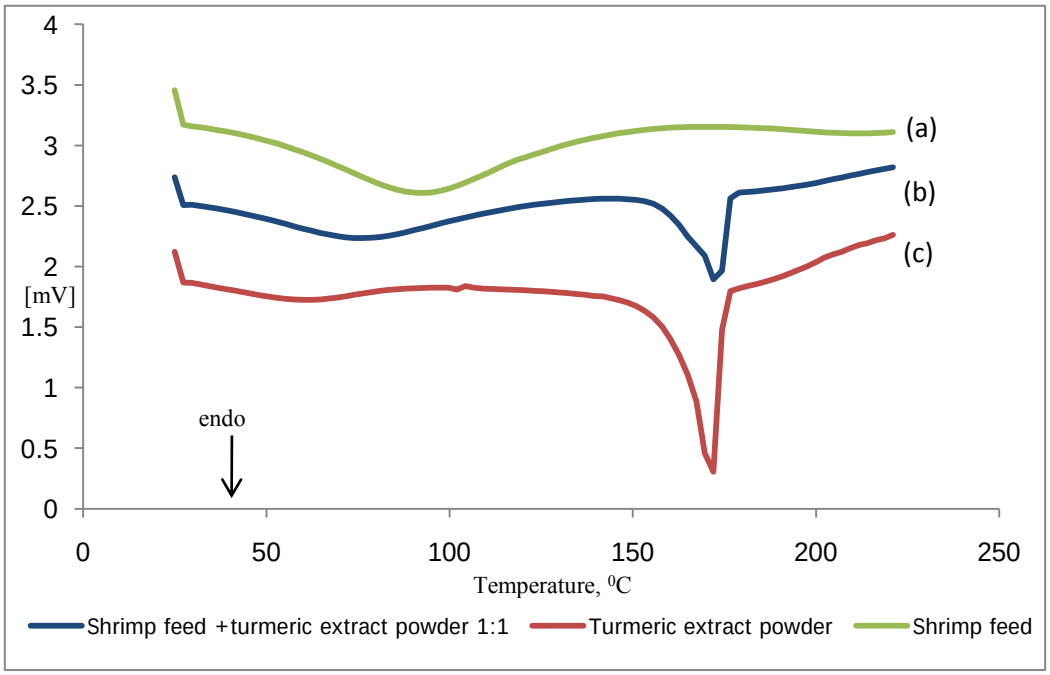


Figure 19 Differential scanning calorimetry (DSC) thermograms of raw materials, initial shrimp feed (a), and physical mixture of shrimp feed : turmeric extract (1:1) (b) and turmeric extract powder (c)

From the DSC thermograms shown in Figure 19, the endothermicevents of 1:1 physical mixture of initial shrimp feed and turmeric extract powder is practically unchanged. It is similar with turmeric extract powder raw material and the initial shrimp feed used. The result illustrated that incompatibility of the turmeric extract and initial shrimp feed when induced thermally did not occur**.**

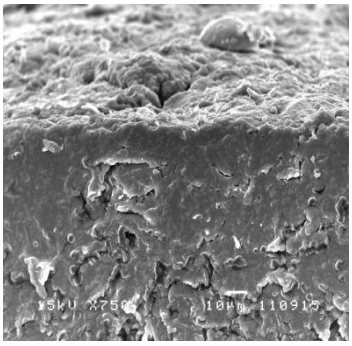
Characterization of initial shrimp feed

The initial shrimp feed from commercial source was characterized.

1.2.1 Morphology

Scanning electron microscope (SEM) was used to examine the morphology, size, shape and surface topography of initial shrimp feed similar to turmeric extract powder described previously. Photograph and photomicrographs of shrimp feed are shown in Figure 24.

(a) (b)



(c)

Figure 20 Photograph of shrimp feed (a), scanning electron photomicrographs of the initial shrimp feed (x1000) (b) and cross section of the initial shrimp feed (x750) (c)

As shown in Figure 20 initial shrimp feed had cylindrical shape with an almost smooth surface and the cross section of shrimp feed were dense and show practically no cavities.

1.2.2 Particle size and size distribution

Sieve analysis was used to evaluate size distribution of initial shrimp feed. The percent of weight retained on each sieve was calculated and plotted against sieve size and shown in Figure 21. Approximately 70% to 80 % of initial shrimp feed are within the range of 1180-1400µm. This highest fraction of initial shrimp feed was characterized and used in further development process. However, due to the fact that shrimp feed are cylindrical, thus clarification of size using standard sieved can only clarify though the longitudinal axis of the particles. However, the lack of sieves having pore size larger than1400µm lead to an incomplete evaluation of initial shrimp feed size distribution especially in the larger size distribution range. Percent of initial shrimp feed retained on each sieve size is shown in Figure 21. Thus, the size was identified by measuring the diameter of the cylindrical shrimp feed instead and shown in the next section.

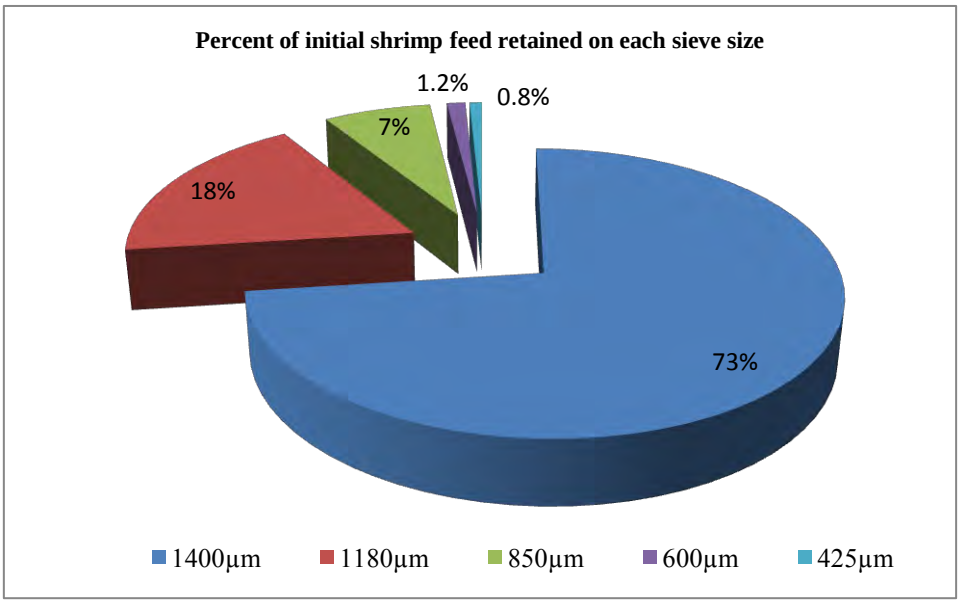


Figure 21 Particle size distribution of initial shrimp feed evaluated by sieve analysis

1.2.3 Physical properties of initial shrimp feed

Moisture content, bulk density, tapped density, compressibility index, flow rate, angle of repose, diameter, hardness, and immersion time of initial shrimp feed

were characterized as the methods described in chapter III. The physical properties of initial shrimp feed are shown in Table 13.

Table 13 Physical properties of initial shrimp feed [mean (SD)]

Physical properties	Initial shrimp feed
Moisture content (%LOD)	10.54(0.01)
Bulk density (g/ml)	0.42(0.00)
Tapped density (g/ml)	0.45(0.00)
%Compressibility	6.67(0.00)
Flow rate (g/sec)	17.18(0.06)
Angle of repose (deg)	24.85(0.05)
Diameter (mm)	1.3(0.01)
Hardness (kp)	6.18(0.08)
Immersion time (sec)	42(0.00)

The moisture content of initial shrimp feed was determined by moisture balance Model HR83, Mettler Toledo. Moisture content of shrimp feed is presented in terms of percent loss on drying (%LOD) as shown in Table 13. Percent LOD of the initial shrimp feed is 10.54(0.01)%. It is less than 11 % which is the limit of shrimp feed acceptable in Thai Industrial Standard *(50)*.

Bulk density of initial shrimp feed 0.42 g/ml, tapped density 0.45 g/ml resulted in the compressibility index of6.67 percent. The small difference in bulk and tapped density resulted in low compressibility index value (<10%), indicating a free flowing behavior and good packing*(51, 52)* of initial shrimp feed. This result may be due to its hard cylindrical shape and almost smooth surface.

The flow rate and angle of repose of initial shrimp feed measured by the funnel method were 24.85 degrees and 17.18 g/sec, respectively. The initial shrimp feed could not flow pass the funnel with internal stem diameter less than 1.5 cm, because of its cylindrical shape. The results shown that the initial shrimp feed have excellent flow property when compared to values in Appendix A.

As describe in literature review section shrimp feed pellets has many size range depending on the shrimp stage. In this study, the investigated the shrimp feed had average diameter value and hardness of 1.3mm and 6.18kp, respectively.

Immersion time of initial shrimp feed takes 42seconds. Immersion time is the property required for using shrimp feed in the farm environment due to water immersion significantly affect the attractability of the shrimp diet*(53)*. It also implies that the faster the diet immersed under water the more preferable.

2. Liquid turmeric extract concentrated formulations

2.1.1 Preparation of turmeric extract concentrated solution

Due to the limit in turmeric extract powder solubility.ethyl alcohol, the stirring time for the preparation of 800 ml turmeric extract concentrated solution to be used as a coating agent and wetting agent took over 45 minutes to dissolved all solid content. Moreover, mixing vessel must be covered with aluminium foils to prevent alcohol evaporation and photo-degradation of turmeric extract which could easily occurred*(7)*. Final solution obtained was clear yellow to orange color with pH of 6.76-6.82 and the appearance is shown Figure 22(a).

2.1.2 Preparation of turmeric extract concentrated microemulsion

Microemulsion system was chosen instead of using typical oily solution of turmeric extract because of its potential in protecting turmeric extract against degradation during protection process. It was postulated that turmeric extract will be incorporated in the internal oil phase within microemulsion. Thus, during coating or extrusion process, turmeric extract in the internal oil phase protected from the harsh processing environment. However, after coating and extrusion process, alcohol will

all be evaporated and microemulsion system will non longer exist. The only barrier against degradation during storage is the oil medium which is hoped to be enough to protect the turmeric extract during mild normal storage condition. The turmeric extract powder 1.272 g in 160 g of turmeric extract concentrated microemulsion was prepared. Suitable microemulsion system contained absolute alcohol, cremophor EL and various ratios of lecithin:squid liver oil (1:1.5,1:2,1:3,1:4 and 1:5). The stirring time took over 45 minutes. To prevent photo-degradation of turmeric extract, the mixing vessel mustbe covered with aluminium foil. Finally, accurate amount of BHT 0.01% w/w 0.016g was added as antioxidant and preservative in the microemulsion system. The appearance of final emulsion system is shown in Figure 22(b).

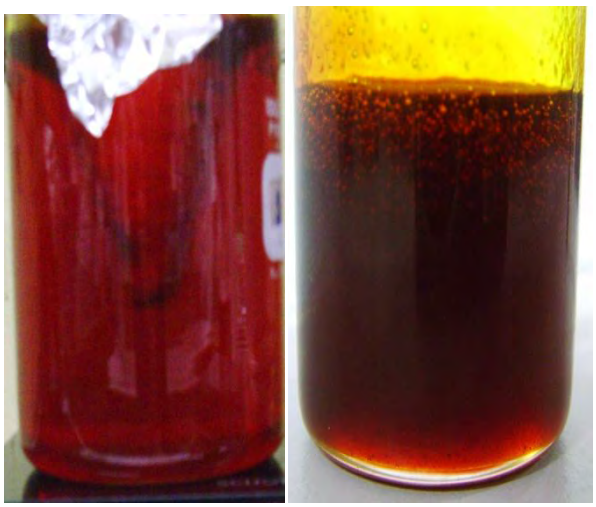
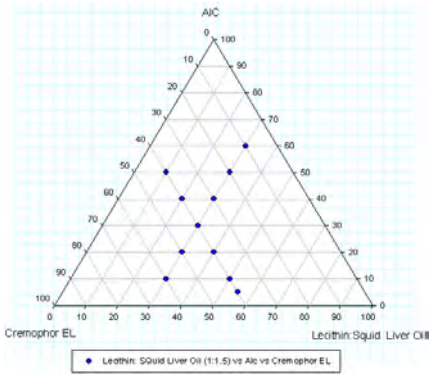
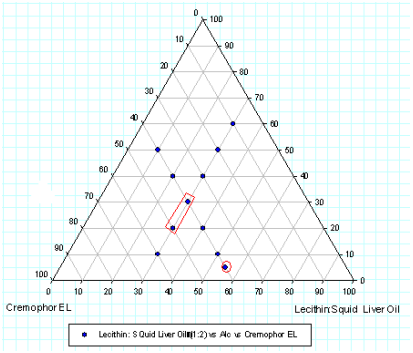


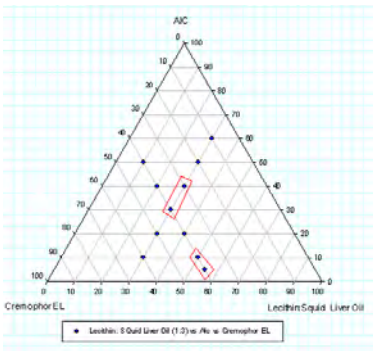
Figure 22 The appearance of final turmeric concentrated solution(a) and turmeric extract concentrated microemulsion(b)



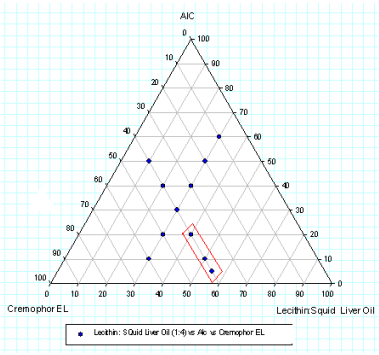
(a)



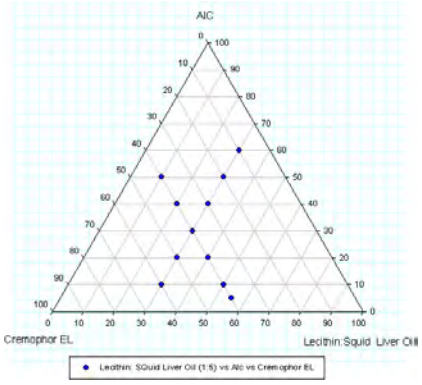
(b)



(c)



(d)



(e)

Figure 23 Ternary phase diagram of turmeric extractconcentrated microemulsion illustrating systems containing lecithin:squid liver oil ratios 1:1.5(a), 1:2(b), 1:3(c), 1:4(d) and 1:5(e)

*Red outline are regions of spontaneous formation of microemulsion

Turmeric extract concentrated microemulsion were prepared by an emulsion technique where the spontaneously occurring clear microemulsion was seen only in the red regions in the ternary phase diagrams in Figure 23. It implied that the amount of aqueous phase (absolute alcohol) lipid phase (squid liver oil), coemulsifier (lecithin) and emulsifier (cremophor EL) had extreme influence on the formation of the microemulsion. Proper formulations were obtained by varying absolute alcohol concentrations from 60%-10% w/w, cremophor EL from 10%-60% w/w while keeping the amount of lecithin:squid liver oil constant at 30% w/w. Another study was done by varying absolute alcohol concentrations from 50%-5% w/w, and lecithin:squid liver oil at fixed ratio from 10%-55% w/w while keeping the amount of cremophor EL constant at 40% w/w. The ratios of lecithin:squid liver oil used were 1:1.5,1:2,1:3,1:4 and 1:5. The results indicated that the spontaneous microemulsion did not occur when lecithin:squid liver oil ratios of 1:1.5 and 1:5 are used. With other ratios formation of microemulsions are possible and shown in Table 14.

From the data obtained, clear microemulsion containing absolute alcohol 20%w/w, cremophor EL concentration 40%w/w and squid liver oil: lecithin (1:3) 40%w/w was selected to be used as coating agent in the coating process and wetting agent in the extrusion process. On account of another study reported that lecithin in the formulation could promote antioxidative action*(54)*. Therefore higher lecithin concentration system was preferable. However, at higher concentrations of lecithin such as 1:4, viscosity of the microemulsion were too great causing difficulty in spreading and may affect the content homogeneity in the final product as seen in the results from other studies*(55)*.

Cremophor EL is normally used as an emulsifying agent in many animal feed formulations. The amount of turmeric extract powder incorporated in the microemulsion system depend on the amount of cremophor EL used. The result may be related to the solubility of turmeric extract powder in cremophor EL. It was found in this study that turmeric extract powder 1.272 g can be incorporated in 160 g of selected microemulsion system.

Table 14 Percentage of each component which shows for microemulsion formations

Total amount of lecithin and squid liver oil	**Absolute ethanaol**	**Cremophor EL**	**Ratios of lecithin: squid liver oil which form microemulsion**
30.00	60.00	10.00	-
30.00	50.00	20.00	-
30.00	40.00	30.00	1:3
30.00	30.00	40.00	1:2, 1:3
30.00	20.00	50.00	1:2
30.00	10.00	60.00	-
10.00	50.00	40.00	1:4
20.00	40.00	40.00	-
40.00	20.00	40.00	-
50.00	10.00	40.00	1:3, 1:4
55.00	5.00	40.00	1:2, 1:3, 1:4

Squid liver oil is suitable to be used as oil phase in this formulation. In addition, squid liver oil is well known as flavoring agent in commercialshrimp feed*(56)*. By adding more squid liver oil in the formula the more shrimp will become attracted to the feed. BHT was used as an antioxidant and preservative in this microemulsion formulations.

3. The incorporation of turmeric extract to shrimp feed

In this study, shrimp feed containing turmeric extract powder were prepared by two different methods, coating and extrusion.

3.1 Coating process

a.The result from preliminary study on the coating process

The observed responses of eight different experimental coating conditions were homogeneity of coating (Y1) and drug content (Y2). The relationship was elucidated by using three dimensional response surface plots shown in Figure 24.

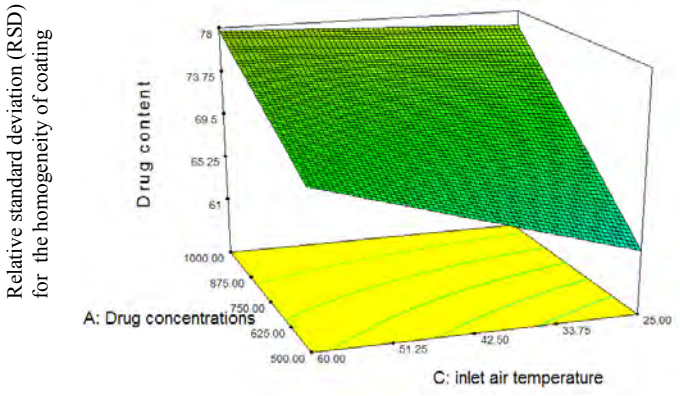


Figure 24 Response surface plot (3D) showing the effect of drug concentration (*X*1) and inlet air temperature (*X*3) on relative standard deviation for coating homogeneity (Y1)

The model indicated significant ($p < 0.05$, R^2=0.891) that drug concentration (X1) and inlet air temperature (X3) on the RSD of coating homogeneity. Feed pump speed (X2), however, did not show significant effect on this response. The increased in inlet air temperature from 25 ^{0}C to 60 ^{0}C, RSD of coating homogeneity increased from 6.53 to 12.03 (while drug concentration and feed pump speed were kept constant at 750 µg/g and 7.5 rpm, respectively). When the drug concentrations in the coating solution increased from 500 µg/g to 1,000 µg/g, the RSD decreased from 11.23 to 7.33 (inlet air temperatures and feed pump speed were kept constant at 42.5 ^{0}C and 7.5 rpm, respectively). The equation obtained from this relationship are shown below.

$$RSD = +9.28 - (1.95 * X1) + (2.75 * X3) - (1.35 * X1 * X3) \quad \textbf{(1)}$$

In this equation, X1, X2 and X3 are the factors studied; Y1 and Y2 are the measured response associated with combined factor levels.

Significant relationships ($p < 0.05$, $R^2 = 0.996$) between drug concentration, feed pump speed and inlet air temperature on drug content were elucidated using response surface plots as seen in Figures 25 and 26.

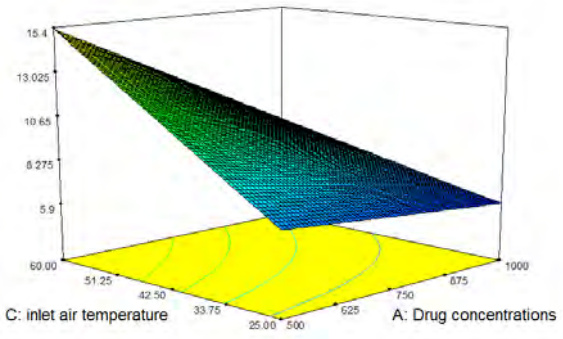


Figure 25 Three dimensional response surface plot showing the effects of drug concentrations (*X*1) and inlet air temperatures (*X*3) on drug content (Y2)

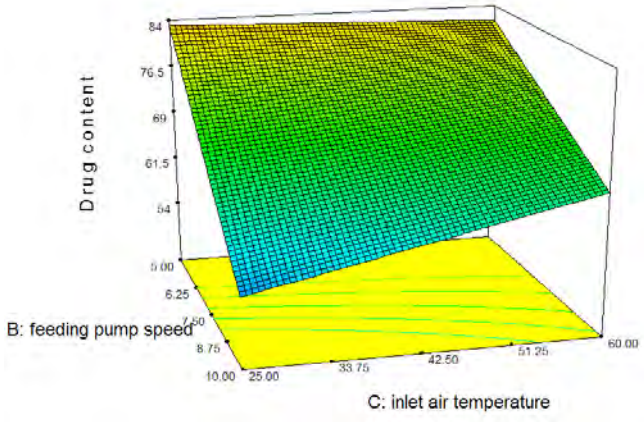


Figure 26 Three dimensional response surface plot showing the effects of feed pump speed (*X*2) and inlet air temperatures (*X*3) on drug content (Y2)

Figure 25 shows the effect of drug concentration and inlet air temperature on drug content. The drug concentration levels increased from 500 µg/g to 1,000 µg/g resulted in the drug content increased from 65.81 to 77.15% (feeding pump speed and inlet air temperature were held constant at 7.5 rpm and 42.5 ^{0}C, respectively). The

increased inlet air temperatures led to an increased in drug content. Meanwhile, Figure 26 shows the effect of inlet air temperature and feed pump speed on drug content. The increased inlet air temperature of 25 ^{0}C to 60 ^{0}C induced an increased of drug content from 69.03% to 73.94% (drug concentration and feed pump speed were held constant at 750 µg/g and 7.5 rpm, respectively). The increased feed pump speed from 5 rpm to10 rpm, the drug content decreased from 82.42% to 60.55% (drug concentration and inlet air temperature were held constant at 750 µg/g and 42.5 ^{0}C respectively). The equation obtained from this relationships are shown below.

Drug content = +71.48 + (5.67*X1) - (10.94*X2) + (2.45*X3-1.86*X1* X3)

+(3.22*X2* X3) **(2)**

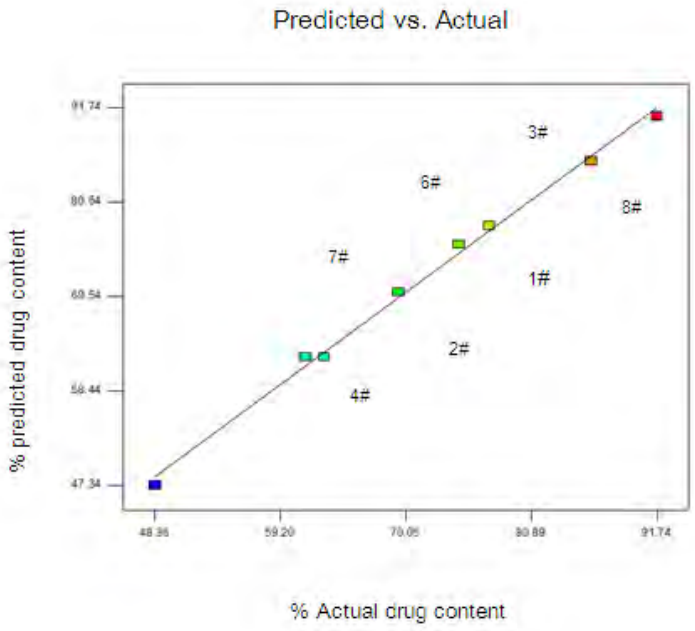


Figure 27 Percentage of actual and predicted drug content response for the incorporation of turmeric extract with commercial shrimp feed

The predicted percent drug content suggested by equation (2) are compared with the actual percent drug content for all the coating runs shown in Figure 27. The actual percent drug content was remarkably correlated to the predicted percent drug content obtained by equation 2 as seen by the very similar percentage and a slope of

near 1 The results illustrated that the experimental design used was effective for designating the coating process parameters.

Coating process variables with turmeric alcoholic solution has been evaluated to achieve proper coating homogeneity and drug content using the two-level full factorial design. Coating process has been conducted in conventional coating pan equiped with spraying atomizer. The quantitative effect of these factors at different levels on the homogeneity of coating and drug content could be predicted by using the above equations to obtain optimum response. Observed actual response were similar to the predicted values. The predict response with the highest drug content 90.72% and the lowest RSD 5.3 obtained from the equations when using $X3$ = 42.5 ^{0}C, $X1$ = 1000 μg/g and $X2$ = 5 rpm but the limiting of the equipment cause $X3$ = 42.5 ^{0}C cannot set up . Therefore, the condition which selected to be used in coating process were $X3$ = 25 ^{0}C, $X1$ = 1000 μg/g and $X2$ = 5 rpm.

b. Preparation of shrimp feed containing turmeric extract by coating process

In previous section, optimal inlet air temperature, concentration and feed rate of coating solution were obtained. Therefore, shrimp feed containing turmeric extract prepared by coating process using the coating condition from the preliminary study above. The pan load, atomizing air pressure, pan speed, spray distance, inlet air temperature, drug concentration and feed pump speed were set at 1,000 g, 2.5 kgf/cm^2, 40 rpm, 15 cm, 25 ^{0}C, 1000 μg/g and 5 rpm, respectively. The properties of processed shrimp feed were further assessed.

3.2 Extrusion process

Preparation of shrimp feed containing turmeric extract by extrusion process

Shrimp feed containing turmeric extract was prepared by extrusion process. First the particles size of initial shrimp feed was reduced. Initial shrimp feed was placed in an oscillating granulator equipped with sieve number 60 mesh for crushing to the required size. The particles after reduction were transfered to the mixer for wet mass production using liquid turmeric concentrate (solution and microemulsion) as

wetting agent. The amounts of turmeric concentrate has an extreme effects on wetted mass properties. The wetting agent is the most critical factor in the formulation to turn powder into a plastic mass that could be easily extruded. During extrusion liquid content must be high enough to bring about the optimum surface plasticity required in the extrusion process*(57)*. If the liquid content was less than the lower limit, dust will be released during the process resulting in large yield of fines. Exceeding the range of liquid content leads to an over wetted mass and agglomeration of the individual shrimp feed extrudates due to excess of water at the surface of particles as described by Harrison et al*(58)*. The amounts of turmeric concentrated solution used to wet the initial shrimp feed powder was more than the microemulsion used because of the difference in turmeric concentrations. The turmeric concentrated microemulsion contained oily material causing limited amounts which can be added to give appropiate texture and final *(59)*. In this study, the amounts of turmeric extract concentrated microemulsion [79.50 mg/ml] and turmeric extract concentrated solution [15.90 mg/ml] used were 160 g and 800 ml per 1,000 g of initial shrimp feed powder respectively. The final step were to dry extrudate in hot air oven at 50 ^{0}C for 5 hours.

The turmeric extract concentrated microemulsion contained oil and other greasy materials which will usually produced a crumbly extrudate and was not dried. While, the extrudate prepared by turmeric extract concentrated solution resulted in brittle extrudate.

4. Characterization of prepared shrimp feed containing turmeric extract

Shrimp feed containing turmeric extract produced by two different methods were characterized as follow.

4.1 Homogeneity of Mixing (uniformity of dosage unit)

The content of curcuminoids in shrimp feed produced was quantitatively determined by high performance liquid chromatography (HPLC) as mention in detail in Appendix C.

The uniformity of curcuminoids in shrimp feed was immediately determined after preparation is illustrated as the percent labeled amount and the relative standard deviation (RSD). The results obtained for shrimp feeds coated with solution (CS), emulsion(CE) and shrimp feeds extruded with solution(ES), emulsion(EE) were 89.95, 93.31, 95.23 and 90.65, respectively as shown in Table 15. Percent labeled amount was within range of 90.0-110.0% specified in USP forum 33 except for CS. RSD in CE and turmeric ES were undesirably more than 6. The result indicated that CS and EE passed the specification of USP 32 monograph *(24)* of having RSD less than 6.

Table 15 Content uniformity of turmeric extract in shrimp feed produced by coating and extrusion methods

	% Labeled amount	SD	%RSD
CS	89.95	5. 01	5.57
CE	93.31	7.34	7. 87
ES	95.23	8.39	8. 81
EE	90.65	5. 01	5.53

From Table 15, one can compare the two methods of turmeric shrimp feed production, coating and extrusion. Homogeneiity or uniformity of spray coated products highly depended on the droplet size of the coating liquid. In this experiment, turmeric extract concentrated microemulsion is very viscous giving very large droplet of low spreadability resulting in product with high RSD value seen in CE. As for extrusion process, well blended wet-massing process is required. Alcohol in turmeric extract concentrated solution was easily evaporated during wet massing thus, deposition of turmeric extract on powder feed beds was not evenly distributed. As a result, higher RSD value was obtained.

4.2 Percent Assay

Assay of total curcuminoids in shrimp feed containing turmeric extract are shown in Table 18. The ideal content of turmeric extract in shrimp feed assayed immediately after preparation was 1,000 µg/g of shrimp feed. Percent labeled amount for CS, CE, ES and EE were 90.71%, 93.09%, 93.99% and 94.83% respectively. The curcuminoid contents in all preparations complied with the specification in USP 33 Forum of 90.0 – 110.0 % labeled amount.

Methods of preparation and types of turmeric extract concentrated used significantly affect (p<0.05) in percent content of processed shrimp feeds. The percent labeled amount of turmeric extract in shrimp feed produced by extrusion method was slightly higher than coating method. It was probably due to the shorter time used and the duration of air contact proned to oxidation was less for extrusion process. In addition, the microemulsion preparations exhibited higher turmeric extract content for both coating and extrusion methods due to protective property of microemulsion for environmentally labeled drugs.

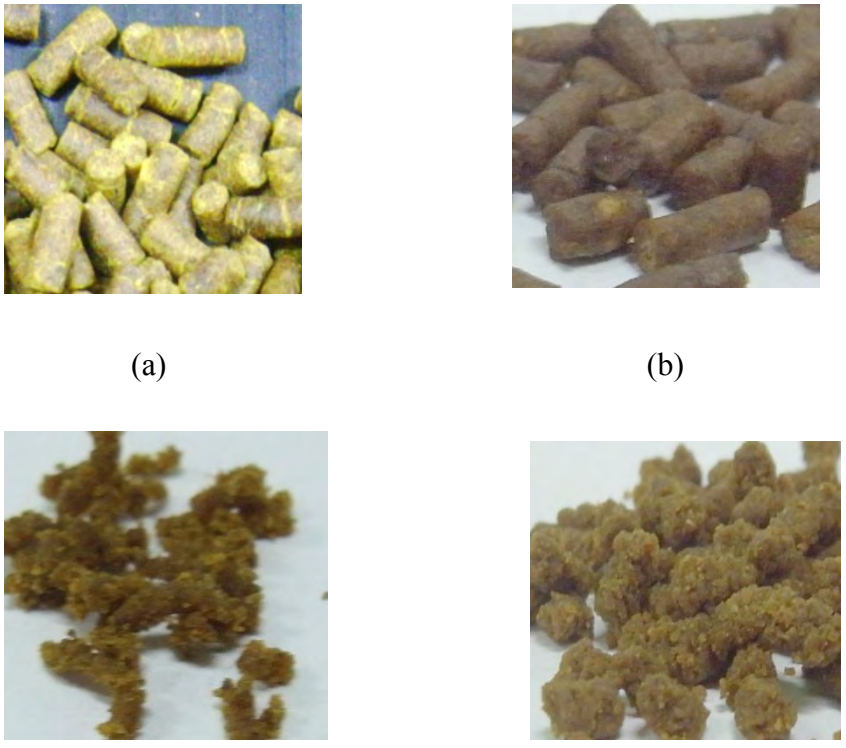
Table 16 Curcuminoids contents in shrimp feed containing turmeric extract

Method	**Shrimp feed**	**Contents (%)**			
		Sample 1	**Sample 2**	**Sample 3**	**Mean (SD)**
Coating *	CS *	90.43	90.71	90.97	90.71 (0.27)
	* CE	94.10	92.37	92.80	93.09(0.90)
* Extrusion	ES *	92.83	94.06	95.08	93.99(1.12)
	EE	94.08	95.36	95.06	94.83(0.67)

* Significantly different (p<0.05)

4.3 Morphology

Scanning electron microscope (SEM) was used to examined the morphology, size, shape and surface topography of shrimp feed containing turmeric extract similar to what was done for the initial shrimp feed and turmeric powder described in Chapter III. Photographs and photomicrographs of shrimp feed produced are shown in Figures 28 and 29 respectively.



(a) (b)

(c) (d)

Figure 28 Photographs of shrimp feed CS(a), CE(b), ES(c) and EE(d)

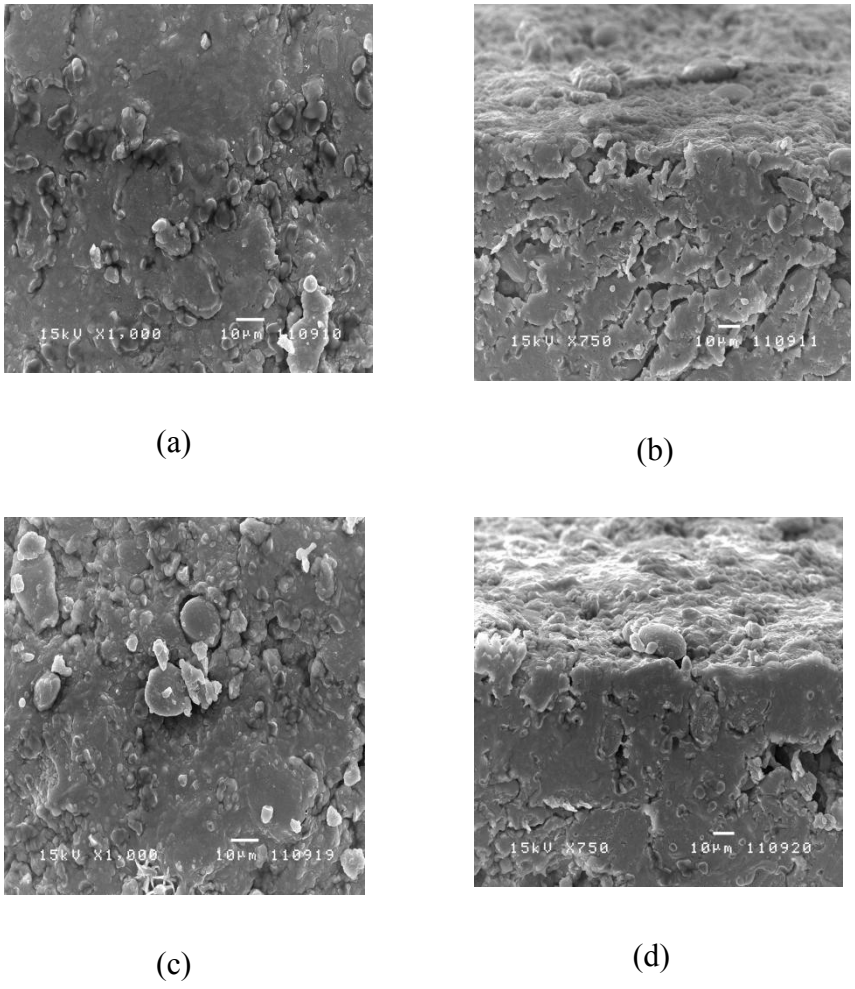


Figure 29 Scanning electron photomicrographs of the surfaces of CS (a), and its cross section (b), CE (c) and its cross section (d) taken at the magnification of x1000

The result illustrated that the processed shrimp feed obtained by coating method retained its cylindrical shape with almost smooth surface. The cross section of shrimp feed were dense, minimal cavities and no significant difference compared to the initial commercial shrimp feed. However, shapes of both extruded shrimp feeds were more irregular. The surface imperfections similar to bread crumbs and flakes were observed. These defects may result from the appropiate of liquid concentrate used, length of die, velocity of throughput and liquid content of the formulation*(60)*. Moreover, in another study showed that preferable extrudates resulted from optimum composition, die length and ram speed*(61)*.

Cross section photomicrographs of shrimp feed obtained by coating method showed no definite interface between the core shrimp feed pellets and the coated materials. It was possible that the coating layer was washed away by methanol during the gold sputtering process or the coating layer is very thin and could not be observed.

4.4 Physical properties shrimp feed containing turmeric extract

The physical properties of processed shrimp feed were characterized and the data shown in Table 17.

Moisture content (%LOD) of CS and CE were 10.45 and 10.87 % which was lower than initial shrimp feed. ES and EE were 8.12 and 9.96 %, respectively. It also indicated that moisture content of shrimp feed after processing were within specification in Thai Industrial Standard the similar to the initial shrimp feed*(50)*.

The angle of repose of coated shrimp feed both CS and CE were less than 25 degree which indicated that shrimp feed had excellent flowability. Moreover, ES seem to flow better than EE as seen by values for angle of repose of 18.70 and 41.62, respectively.

Flow rates, bulk and tapped densities were mainly affected by method of production and types of liquid turmeric extract concentrate used. Bulk and tapped densities were strongly affected by methods used. The values of bulk and tab densities of ES and EE were lower than the initial shrimp feed, CS and CE. However, types of turmeric extract concentrate used played major role on the percent compressibility of coated shrimp feed for bothcoating and extrusion processes. Percent compressibility is an excellent indication of uniformity in size, shape and moisture content. CS and ES particles are highly brittle and did not compact well while CE and EE were soft, moist and compact better due to residual oil from using microemulsion.

Table 17 Physical properties of shrimp feed containing turmeric extract [mean (SD)]

Physical properties	CS	CE	ES	EE
Moisture content (%LOD)	10.45(0.01)	10.87(0.01)	8.12(0.03)	9.96(0.01)
Bulk density (g/ml)	0.47(0.00)	0.44(0.01)	0.33(0.01)	0.29(0.02)
Tapped density (g/ml)	0.48(0.00)	0.46(0.02)	0.37(0.01)	0.33(0.01)
%Compressibility	2.08(0.00)	5.05(1.04)	10.91(0.17)	15.69(1.70)
Flow rate (g/sec)	19.18(0.03)	12.74(0.06)	10.06(0.09)	8.61(0.12)
Angle of repose (deg)	23.21(0.02)	37.62(0.09)	18.70(0.01)	41.62(0.03)
Diameter (mm)	1.3(0.01)	1.29(0.01)	0.89(0.01)	0.92(0.01)
Hardness(kp)	6.26(0.08)	5.98(0.17)	2.83(0.89)	-
Immersion time (sec)	38.00(1.00)	40.33(2.08)	56.67(1.53)	45.33(1.53)

The result indicated that flowability of processed shrimp feed was lower than initial shrimp feed in case of extrusion, while it was higher than initial shrimp feed in case of coating. It may be caused by attrition force during the coating process. The flow rate of CS was higher than CE and ES was higher than EE. Possible explanations for this behavior were the liquid concentrate and viscosity of emulsion composition may resist the flowability. The extrusion process exhibited products with high percent of cohesiveness, low bulk and tapped densities. This result were mainly due to irregularly shaped particles. There was significant different densities between coated product and extruded product. The coated products were more dense due to the retention of its original commercial shape.

Diameter of coated shrimp feed was similar to the initial shrimp feed. While the diameter of ES and EE were 0.89 and 0.92 mm which was less than the initial shrimp feed. These results corespond to the small diameter of the die in the extruder. Moreover, hardness of shrimp feed after processing varied between 2.83-6.26 kp. Extrusion products exhibited very low hardness and was unable to measure the hardness of EE. Wetting time of CS and CE were 38.00 and 40.33 minutes which took less time than the initial shrimp feed. ES and EE. As can be seen, shrimp feed produced from turmeric extract concentrated microemulsion retarded the wetting time due to its oil component.

4.5 *Fourier Transformed Infrared Spectrophotometer (FTIR) and Powder X-ray diffraction (XRD) data*

Fourier Transformed Infrared Spectrophotometer (FTIR) and Powder X-ray diffraction (XRD) was used to confirm the existance of turmeric extract after coated. Results are shown in Figures 30 and 31.

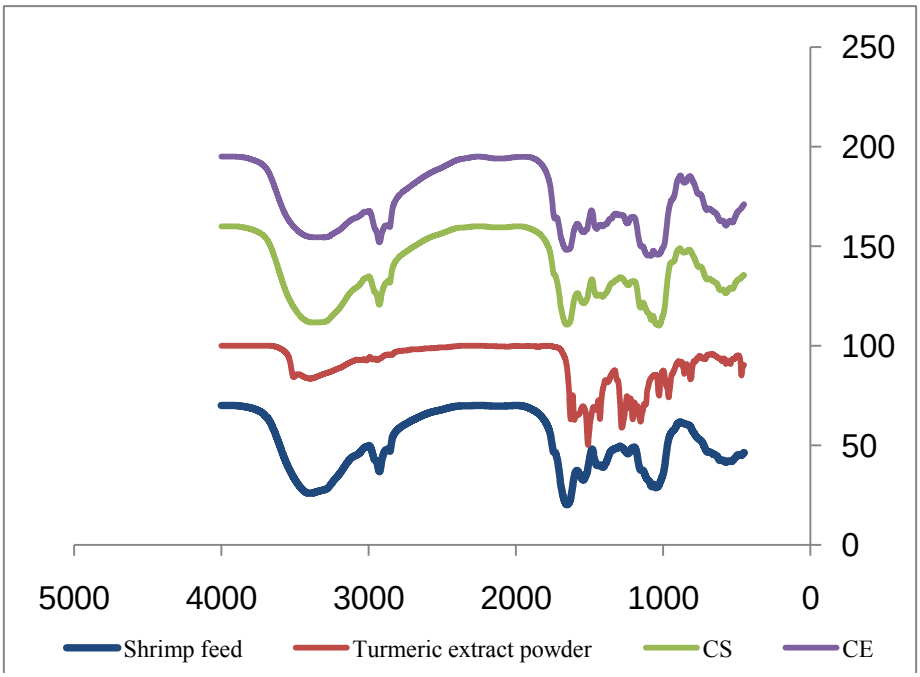


Figure 30 Fourier Transformed Infrared Spectroscopy (FTIR) spectra of shrimp feeds produced by coating method, initial shrimp feed and turmeric extract powder

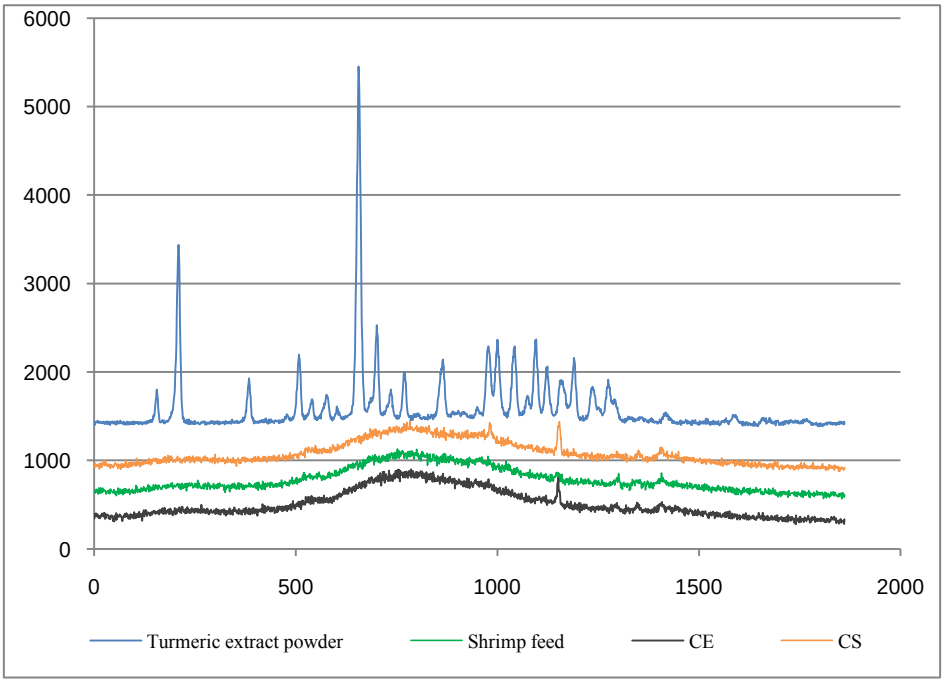


Figure 31 Powder X-ray diffraction (XRD) patterns of shrimp feed produced by coating method, initial shrimp feed and turmeric extract powder

From the Infrared FTIR spectra and Powder X-ray diffraction (XRD) patterns of turmeric extract powder, initial shrimp feed, CS and CE indicated that the patterns did not show the unique powder diffraction fingerprint or IR pattern of turmeric extract powder. This was due to the fact that amount of turmeric extract content in the shrimp feed was very low (1.03%), below the detection limit of both analytical methods. Thus, the existance of turmeric extract solid composition in shrimp feed after coating cannot be investigated by these methods.

4.6 Leaching Test

Leaching profile of processed shrimp feed was evaluate by USP Dissolution Apparatus II (Paddle). The results are shown in Figure 32. The amount of total curcuminoids released at various time were calculated from the standard curve. The correlation was plotted as a function of percent of turmeric extract released from the shrimp feed against time.

Table 18 Conditions used for leaching test of shrimp feed produced

Parameter	**Condition**
Medium	900.0 ml
Rotating speed (rpm)	20
Temperature (°C)	37.0 ± 0.5
Total time (hr)	1
Sampling interval (min)	10
wavelength (nm)	425

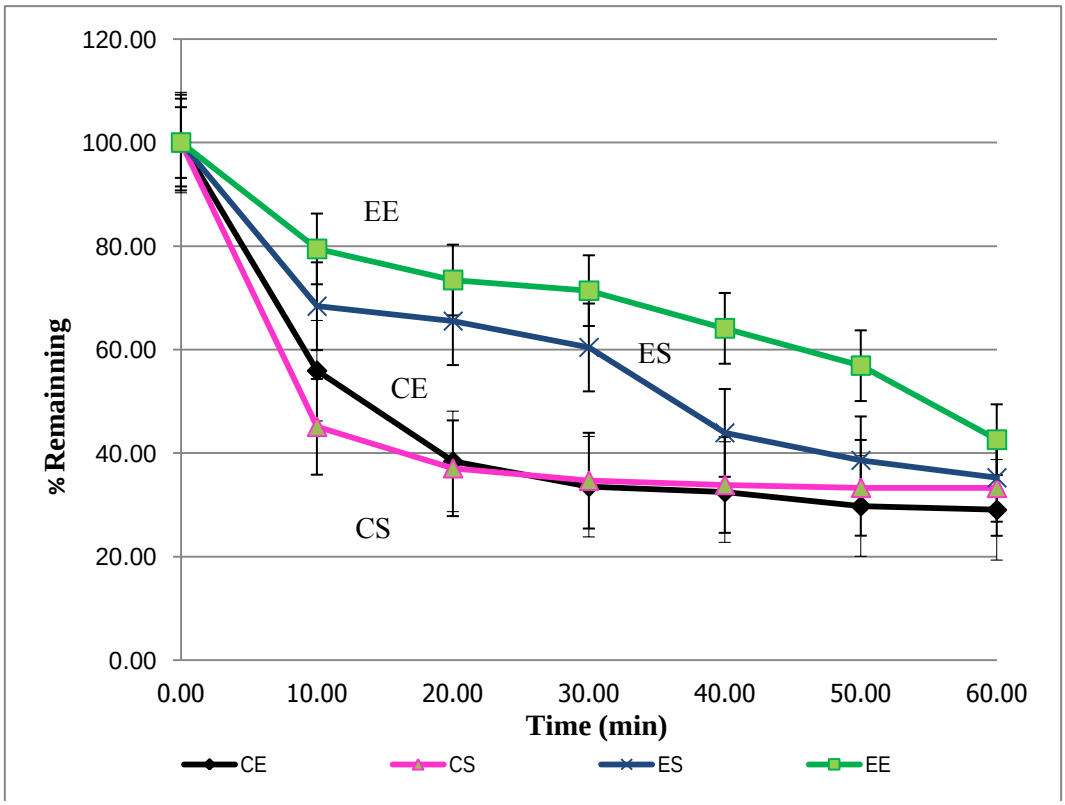


Figure 32 Release profiles of shrimp feeds containing turmeric extract

As shown in Figure 34, more than 50% of turmeric extract was released in from the coated products within 30 minutes. The coated products were not suitable for shrimp consumption due to this rapid release. In general, shrimp slowly and continuously feed on their food, hence, shrimp feeds should not have nutrients leached from the feed prior to shrimp consumption*(62)*. Turmeric extract released from CS, CE, ES and EE at 60 minutes were 33.28%, 29.05%, 35.23% and 40.60%, respectively. This could be implied that the release profile of the CS was higher than ES. In addition, release profile of CE was higher than EE. These results demonstrated that turmeric extract release rates were significantly affected by the preparation method (p<0.05) as described in other studies where types of extrusion affect properties of the extrudate*(63)*

In addition, significant difference (p<0.05) was seen between the release profiles of CS and CE. This result demonstrated that liquid turmeric extract concentrate preparations have significant effect on turmeric extract released in the

case of coating. It also agreed with the previous study which lipidi substance was added to extend the release of extruded and spheronized product*(64)*. Nevertheless, in another study found that the release profile of extruded andspheronized product could be enhanced by cremophor*(65)*. Therefore, CE did not show the extend release characteristic as expected. Moreover, there was significant difference (p<0.05) between the releas profiles of ES and EE. These results demonstrated that turmeric concentrate preparations had significant effect on turmeric extract release profile when extrusion was used. Therefore, the release profile of EE was slowest.

5. Stability study

Stability study of shrimp feed containing turmeric extract was performed at ambient condition (30^0C $\pm$ 2^0C and 65% RH $\pm$ 5%RH) according to ICH Guideline*(46)*.

The physical properties of processed shrimp feed after storage at ambient condition for 6 months were characterized and the data are shown in Table 19.

Table 19 Physical properties of shrimp feed containing turmeric extract after storage for 6 months at ambient condition [mean (SD)]

Physical properties	CS	CE	ES	EE
Moisture content (%LOD)	11.68(0.03)	11.02(0.04)	8.69(0.21)	10.40(0.14)
Bulk density (g/ml)	0.48(0.00)	0.44(0.01)	0.37(0.02)	0.32(0.02)
Tapped density (g/ml)	0.49(0.01)	0.49(0.01)	0.41(0.01)	0.36(0.01)
%Compressibility	2.69(1.13)	8.90(1.13)	9.80(2.68)	10.19(3.21)
Flow rate (g/sec)	20.07(0.04)	13.98(0.26)	11.29(0.26)	9.31(0.16)
Angle of repose (deg)	22.46(0.06)	39.55(0.45)	17.50(0.37)	39.83(0.80)
Diameter (mm)	1.30(0.02)	1.29(0.01)	0.89(0.01)	0.92(0.01)
Hardness(kp)	6.27(0.06)	6.23(0.42)	2.83(0.58)	-
Immersion time (sec)	34.33(2.08)	40.00(1.00)	55.33(1.53)	45.00(1.00)

The result illustrated that some of shrimp feed physical properties after storage for 6 months showed significant differance from the freshly prepared shrimp feeds (Table 21). Moisture contents of all shrimp feed after storage were higher than the freshly preparedshrimp feed resulting in an increased in percent compressibility, flow rate, bulk and tapped densities*(66)*.

. The moisture contens of CS, CE, ES and EE were 11.68%, 11.02%, 8.69% and 10.40%, respectively. Possible explanation was moisture content that increase in each product after storage for 6 months may depend on moisture sorption characteristic of the material and moisture protection properties of primary packaging material.

However, the immersion time and hardness of all shrimp feed after storage seem to be slower than from freshly prepare shrimp feed. Possibly due to an increased in moisture content. Whereas, diameter of all shrimp feed after storage remained unchanged.

The percent content of active constituent were calculated by comparing the corresponding values with the initial. The percent of active curcuminoids remainedin CS, CE, ES and EE after storage for 6 months were 75.23%, 76.76%, 75.07% and 84.20%, respectively. The results are summarized in Figure 33.

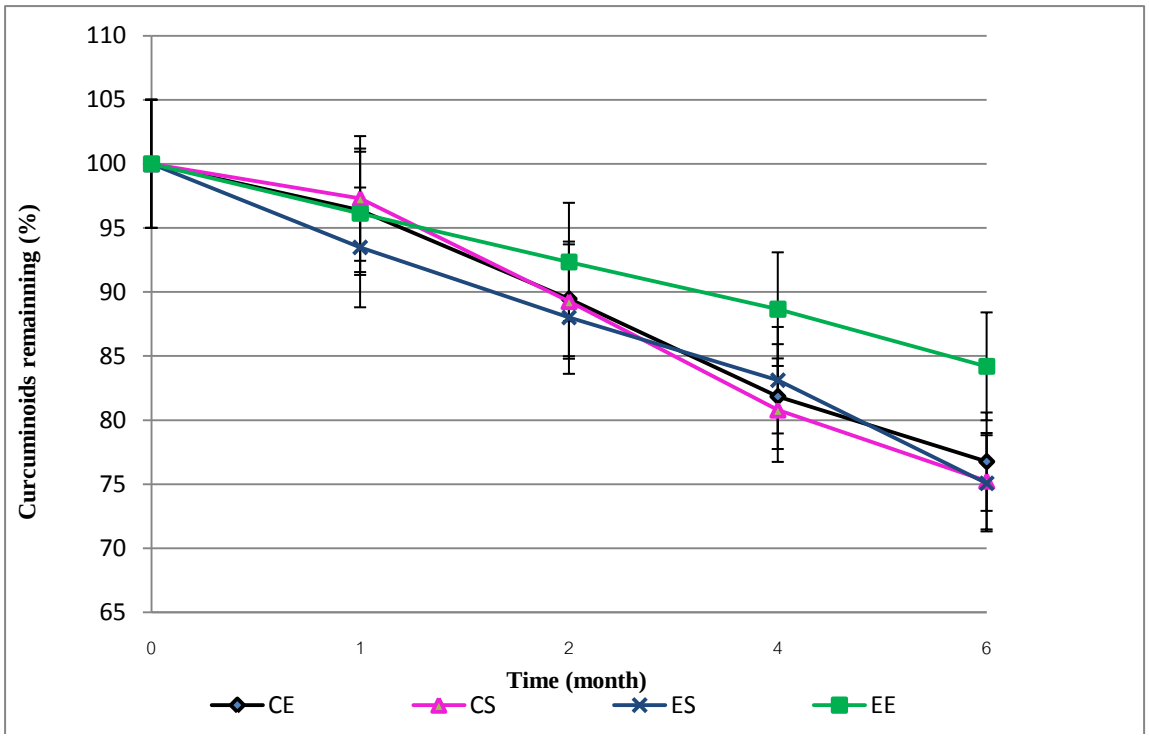


Figure 33 Stability of shrimp feed containing turmeric

From the data obtained, stability of shrimp feeds containing turmeric extract were not stable at ambient condition for 6 months. On the contrary, turmeric concentrate emulsion extrusion formulations were stable at ambient condition (30^0C $\pm$ 2^0C and 65% RH $\pm$5%RH) for 6 months*(67)*. However, EE was most stable at ambient condition for 6 months more than 80% remaining. Possible explanation is that components in microemulsion may prevent the degradation of curcumin especially when it was incorporated inside the shrimp feed matrix*(68)*. Moreover, it may be due to the antioxidant effect of lecithin*(54, 69)*. On the other hand, significant difference(p<0.05) was seen between products obtained by different methods i.e., coated and extrusion. This may be due to shrimp feed coated with turmeric extract have more opportunity for oxygen exposure which caused rapid oxidation and bleaching *(70)*.

Leaching Test

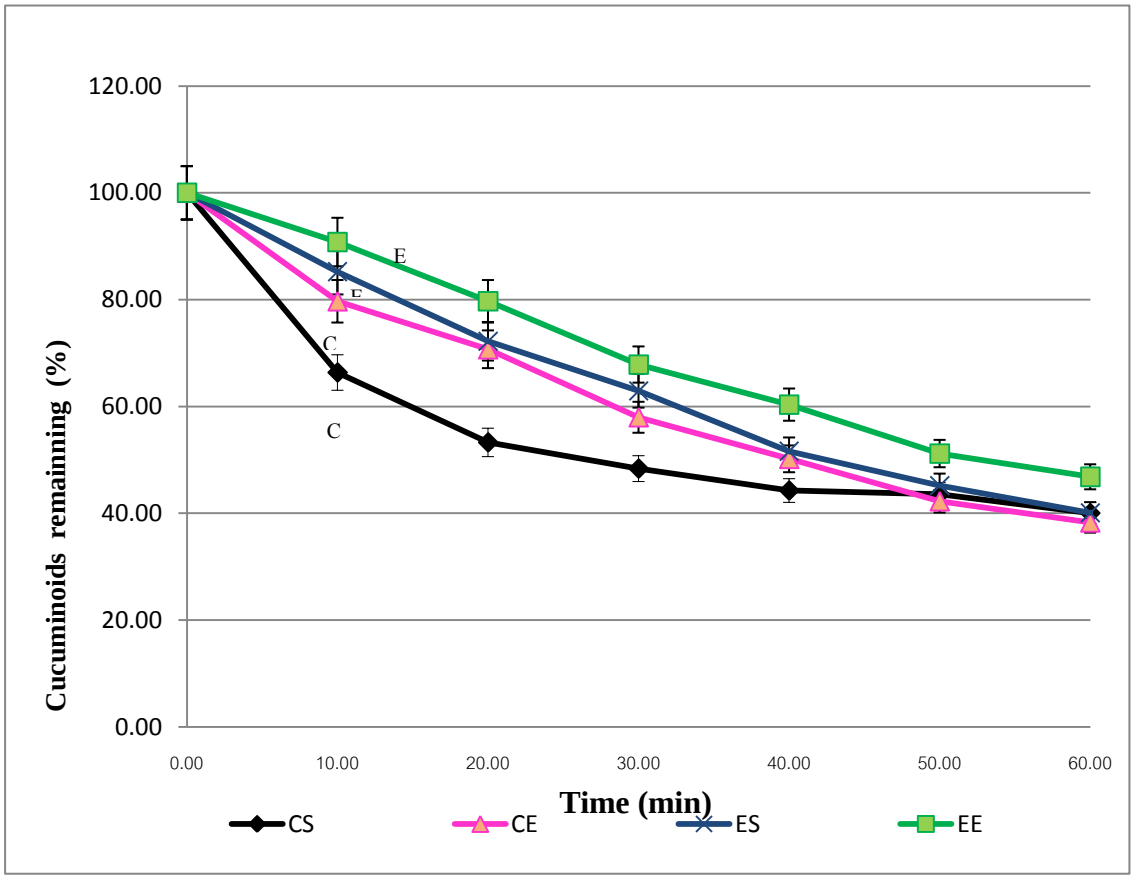


Figure 34 Release profiles of shrimp feed containing turmeric extract after storage at ambient condition (30^0C $\pm$ 2^0C and 65% RH $\pm$5%RH) for 6 months

Figure 34 the results demonstrated that percent of turmeric extract released in for CS, CE, ES and EE at 60 minutes after storage at ambient condition (30^0C $\pm$ 2^0C and 65% RH $\pm$5%RH) for 6 months were 40.02%, 38.28%, 40.09% and 46.83%, respectively. It was noticed that every preparations (CS, ES and EE) show similar release patterns and relative sequence as with freshy prepared shrimp feeds containing turmeric extract, except CE. CE after storage for 6 months indicates slower release than initial CE This incident may be due to the fact that CE after coated with microemulsion remained moist with oily liquid. As time passed, the oily liquid

migrated in to the shrimp feed core, thus resulting in matrix-type release as with ES and EE.

CHAPTER V
CONCLUSIONS

The present study was to produce shrimp feed containing turmeric extract which typically contains three major curcuminoid active compounds, i.e. curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. Shrimp feeds containing turmeric extract were prepared by coating and extrusion methods. Turmeric extract concentrated solution or/and turmeric extract concentrated emulsion were used as coating agents in the coating process and/or as wetting agents for the extrusion process. Shrimp feed after produced were characterized and assayed for its curcuminoids content. From the data obtained it can be concluded that:

The coated shrimp feed exhibited preferable physical properties than shrimp feed extruded. Coating with turmeric extract concentrated microemulsion (CE) did not show mark difference from those coated with turmeric extract concentrated solution (CS). Using turmeric extract concentrated solution in the extrusion method to produced shrimp feed (ES) resulted in harder and more brittle shrimp feed compared to shrimp feed extruded with turmeric extract concentrated microemulsion (EE).

The preparation methods showed extreme influence on turmeric extract release profile or leaching (investigated within the first 60 minutes). The shrimp feed prepared by extrusion method exhibited slower turmeric extract release than the coating method especially when turmeric extract concentrated microemulsion was used due to the incorporation of the turmeric extract in to the feed matrix by extrusion process. Moreover, the effect of turmeric extract concentrate type illustrated marked difference in the release profiles, ES release profile was higher than EE whereas, CS release profile was lower than CE.

Preformulation study on incompatibility of turmeric extract and initial shrimp feed was evaluated by differential scanning calorimetry (DSC) and was shown that no interactions occurred.

The stability of shrimp feed containing turmeric extract affected by differences in types of turmeric extract concentrate used. It was observed that, among the results by the same preparation method, the content of turmeric extract in shrimp feed produced from turmeric extract concentrated emulsion was likely to be higher than turmeric extract concentrated solution after storage at ambient condition over the period of 6 months. In addition, the result indicated that EE exhibited higher chemical stability than ES. Similarly, CE had higher stability than CS.

The shrimp feed containing turmeric extract is the new approach in agricultural field in order to replace antimicrobial used. No systematic study was done on the incorporation of turmeric extract to shrimp feed. As with several compounds, the variation of curcuminoid contents and limitation of their stability made it difficult to produce a homogenous product with sufficient quality without the degradation of the active constituents. In this study, although EE exhibited poorest physical properties, but it showed most preferable slow release profile in water (40.60% remaining after 60 minutes) and the highest chemical stability (82.40% remaining). In conclusion, extrusion technique may be potential method to slow down the turmeric extract release rate, however the physical properties must be improved in the future studies. Nevertheless, the relationship between the properties of shrimp feed containing turmeric extract obtained by varying the extrusion conditions have not yet been established.

The future studies to produce shrimp feed containing turmeric extract to be applied for apply in agriculture field could be as follows:

1. Investigation of the difference in equipment or techniques to produce shrimp feed by extrusion method using turmeric extract concentrated solution and turmeric extract concentrated emulsion should be carried out.

2. Investigation on the effect of adding polymer or high viscosity insoluble liquid in turmeric concentrated emulsion as coating agent to retard turmeric extract release profiles.

3. It is necessary to investigate the shrimp preference and consumption because of variation in feed properties greatly affect shrimp consumption behavior and eventually the shrimp growth and marketability.

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APPENDICES

APPENDIX A

Characterization

The particle size and size distribution of curcuminoids extract powder

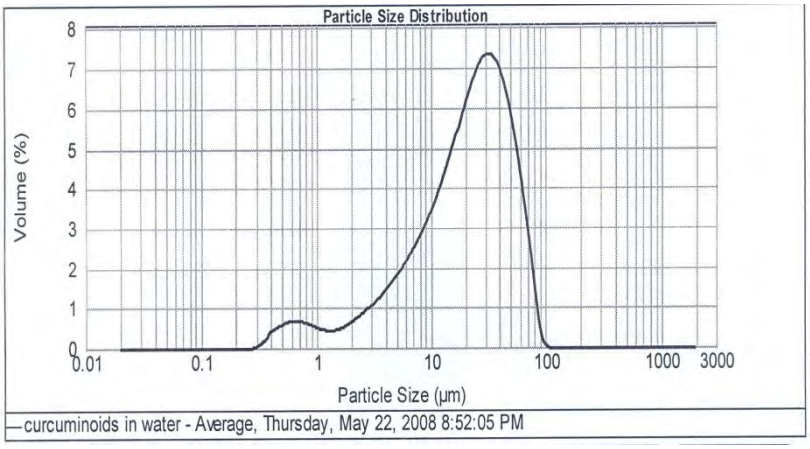


Figure 1A The particle size and size distribution profiles of curcuminoids extract powder without dispersant

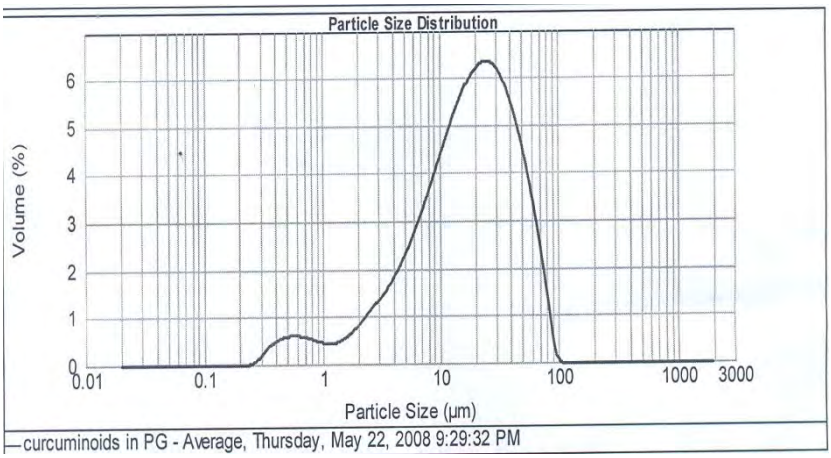


Figure 2A The particle size and size distribution profiles of curcuminoids extract powder using PEG 400 as dispersant

Table 1A Volume weighted mean diameter, d(0.1), d(0.5), d(0.9) and particle size range of curcuminoids extract powder

Parameter	Without dispersant	PEG 400
d(0.1)	3.71	3.26
d(0.5)	22.15	18.02
d(0.9)	54.20	51.23
Volume weighted mean diameter (μm) D[4,3]	25.96	23.13
Particle size range (μm)	0.5-100	0.5-100

d (0.5) is the size at which 50% of the sample is smaller and 50% is larger (mass median diameter). d (0.1) and d (0.9) are the size of particle below which 10% and 90% of the sample lies respectively. D[4,3] is the volume mean diameter

Table 2A Flow properties and corresponding flowability parameters *(24)*

Flow character	Angle of Repose (degree)	Compressibility index (%)
Excellent	25-30	≤ 10
Good	31-35	11-15
Fair	36-40	16-20
Passable	41-45	21-25
Poor	46-55	26-31
Very poor	56-65	32-37
Very, very poor	> 66	> 38

Table 3A Physical properties of commercial shrimp feed [mean (SD)]

Physical properties	**Initial shrimp feed**	**CS**	**CE**	**ES**	**EE**
Moisture content (%LOD)	10.54(0.19)	10.45(0.01)	10.87(0.01)	8.12(0.03)	9.96(0..01)
Bulk density (g/ml)	0.42(0.12)	0.47(0.00)	0.44(0.01)	0.33(0.01)	0.29(0.02)
Tapped density (g/ml)	0.45(0.00)	0.48(0.00)	0.46(0.02)	0.37(0.01)	0.33(0.01)
%Compressibility	6.67(0.16)	2.08(0.00)	5.05(1.04)	10.91(0.17)	15.69(1.70)
Flow rate (g/sec)	17.18(0.15)	19.18(0.03)	12.74(0.06)	10.06(0.09)	8.61(0.12)
Angle of repose (deg)	24.85(0.27)	23.21(0.02)	37.62(0.09)	18.70(0.01)	41.62(0.03)
Diameter (mm)	1.3(0.00)	1.3(0.01)	1.29(0.01)	0.89(0.01)	0.92(0.01)
Hardness(kp)	6.18(1.32)	6.26(0.08)	5.98(0.17)	2.83(0.89)	-
Watability(sec)	42(0.25)	38(1.00)	40.33(2.08)	56.67(1.53)	45.33(1.53)

APPENDIX B

Calculation of the amount of curcuminoids powder was used in the formulation

Amount of curcuminoids required in the formulation were curcuminoids : shrimp feed, 1,000 µg:g)

Theoretical curcuminoids content in shrimp feed 1 kg

1,000 x1,000 = 1,000,000 µg (1 g)

Added access amount of 105% curcuminoids content $= \frac{105}{100} \times 100$

Therefore total curcuminoids in preparation $= 1.05$ g

Amount of curcuminoids powder (g) $= \frac{\text{Total Curcuminio ds in preperation (g)}}{\text{\% Curcuminoi ds content in curcuminoi ds powder}} \times 100$

$= \frac{1.05}{82.60} \times 100$

curcurminoids powder = 1.272 g (curcurminoids content equivalent to 1.05 g)

Amount of curcuminoids required in the formulation 1 kilogram = 1.272 g

APPENDIX C
Validation of HPLC method

Validation of HPLC method

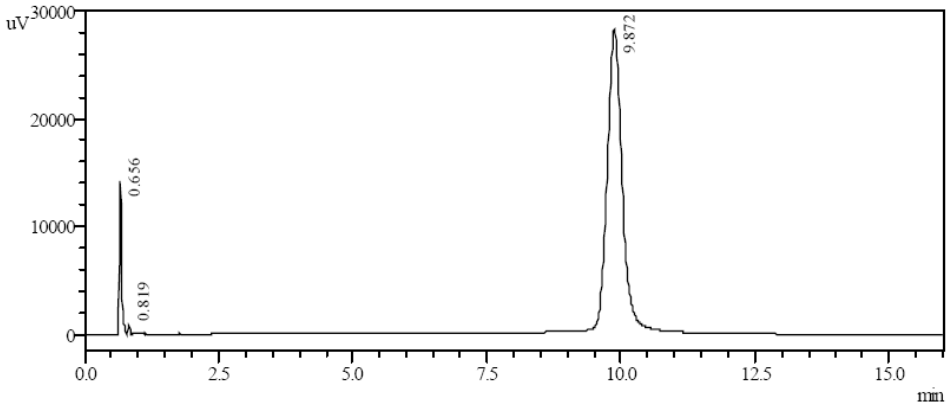
HPLC is the most suitable method for analysis of turmeric powder both raw material and pharmaceutical dosage form because of its high sensitivity, specificity and convenience for the research. In the available articles, HPLC gradient method was preferred to be utilized in this investigation as recommended by previous study*(71)*. The typical analytical characteristics used in method validation were specificity, accuracy, precision and linearity*(24)*.

Validation of HPLC method

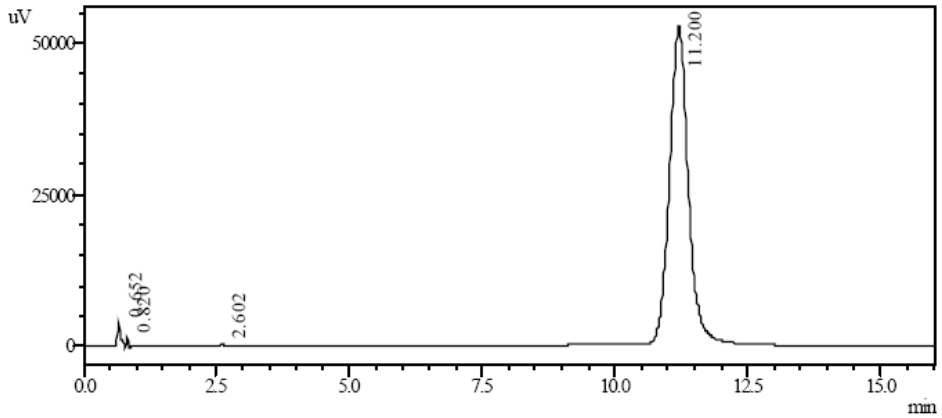
The typical analytical characteristics used in method validation were specificity, accuracy, precision, linearity and range.

Specificity

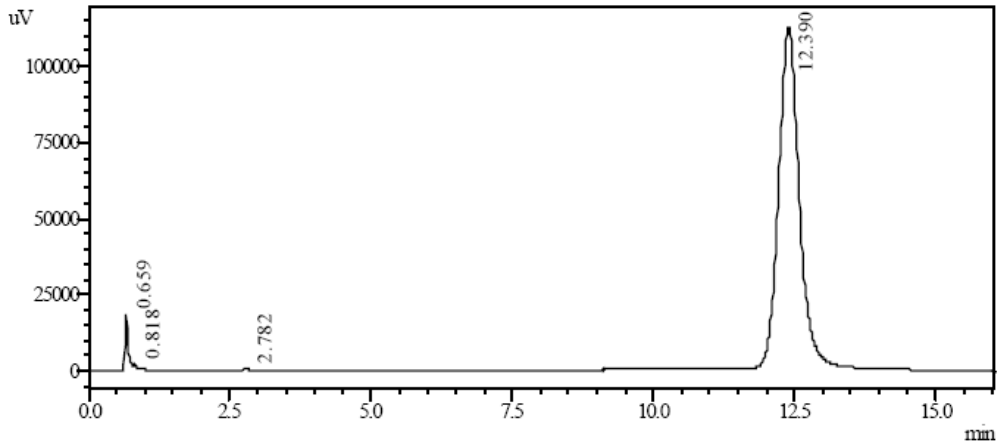
The specificity of an analytical method is the ability to assess the peak of turmeric extract powder standard solution from the sample without interfered by other components, presented in the sample. The excipients included initial shrimp feed, deoiled enzyme-modified soy lecithin, ethyl alcohol, squid liver oil and BHT. Their chromatograms were compared with the chromatogram for the standard solution of the turmeric extract powder standard. The data from an analytical method were shown bellow.



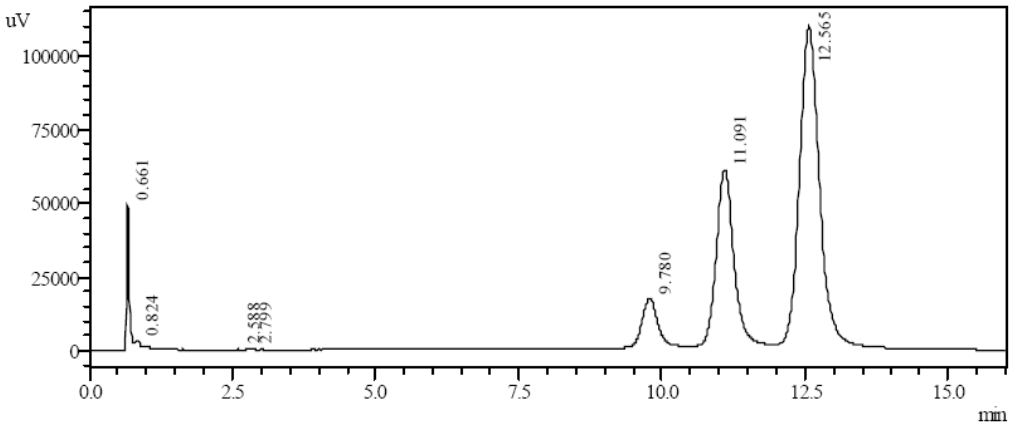
Figures 1C The chromatogram of references standard of bisdesmethoxycurcumin



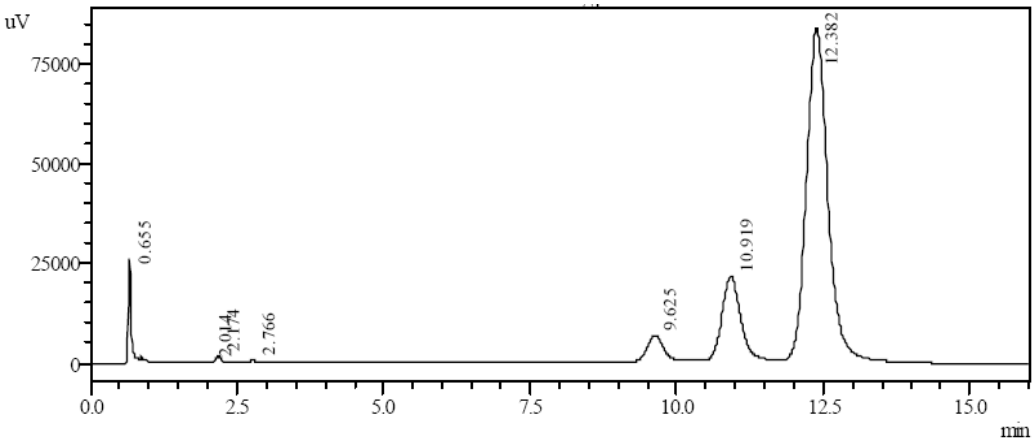
Figures 2C The chromatogram of references standard of desmethoxycurcumin



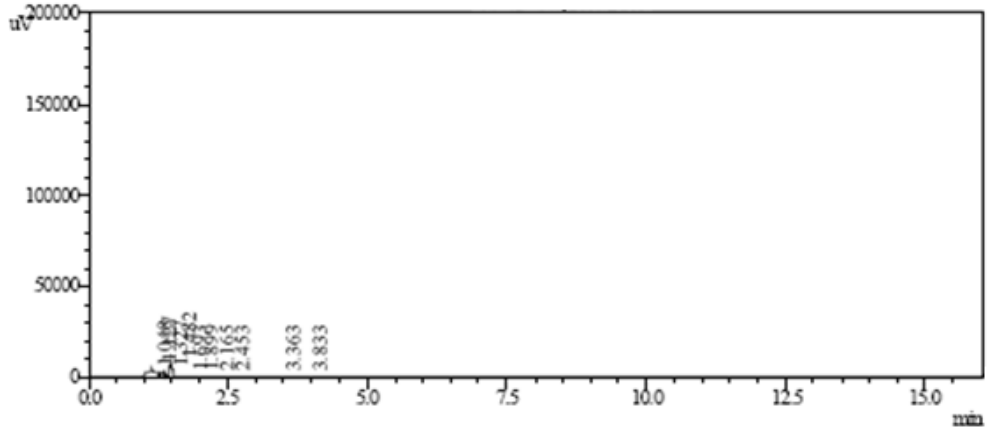
Figures 3C The chromatogram of references standard of curcumin



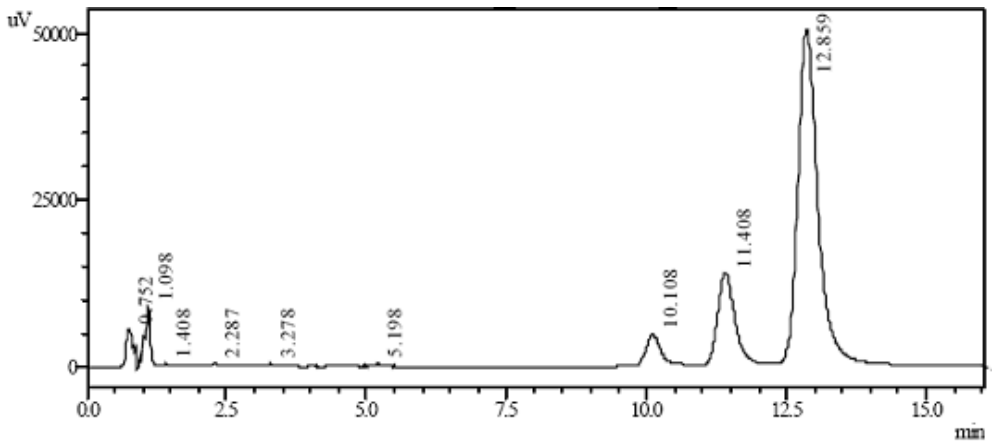
Figures 4C The chromatogram of standard mixture of bisdesmethoxycurcumin, desmethoxycurcumin, curcumin



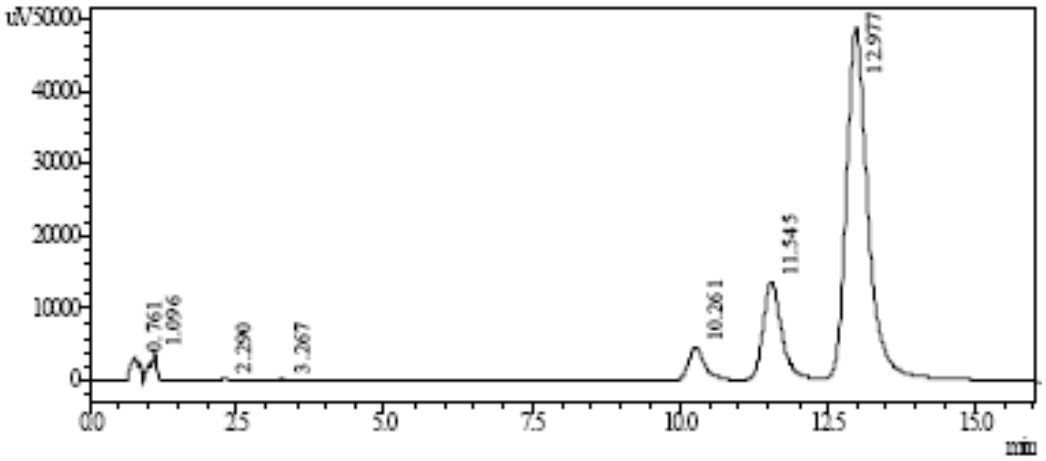
Figures 5C The chromatogram of turmeric extract powder raw material



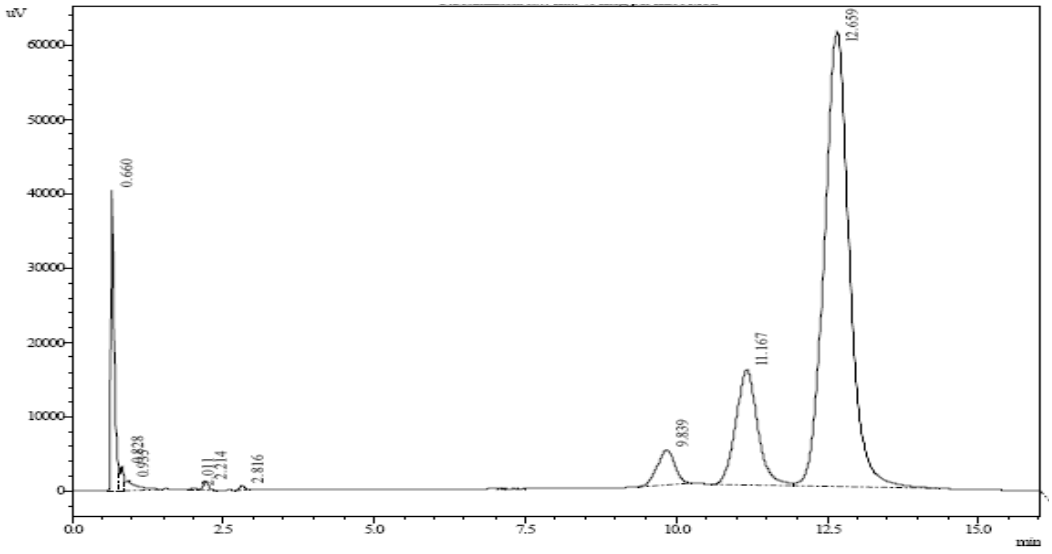
Figures 6C The chromatogram of chromatogram of initial shrimp feed



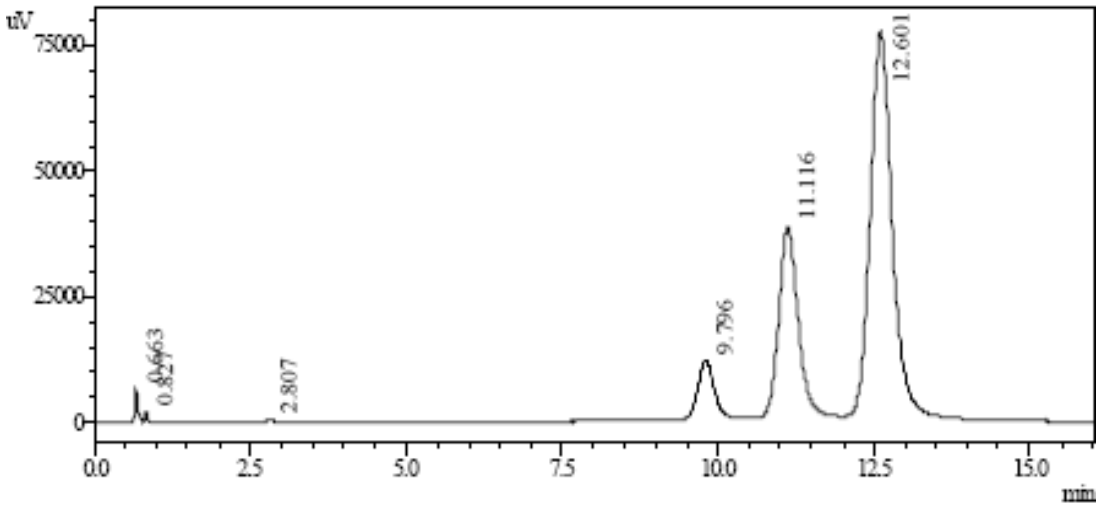
Figures 7C The chromatogram of CS



Figures 8C The chromatogram of CE



Figures 9C The chromatogram of ES



Figures 10C The chromatogram of EE

Figures 1C, 2C and 3C shown the chromatogram of references standard of bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin were eluted about 9, 11 and 12 min respectively. Figures 4C and 5C shown the references standard mixture of bisdesmethoxycurcumin, desmethoxycurcumin, curcumin and turmeric extract powder raw material respectively. Figures 6C, 7C and 8C shown the chromatogram of initial shrimp feed, CS and CE, while Figures 9C and 10C shown the chromatogram of ES and EE respectively. From the data obtained it indicated that the other ingredients did not interfered the peaks of drugs.

Accuracy

The closeness of the test results obtained by that method to the true value. The turmeric extract powder standard solution concentrations of 10, 20, 30, 40, 50 and 60 µg/ml were spiked in the placebo solution containing shrimp feed, deoiled enzyme-modified soy lecithin, ethyl alcohol, squid liver oil and BHT and injected. Accuracy was calculated as the percentage of recovery of each standard solution. The mean percentage of recovery of 95-105% with percent of coefficient of variation (%RSD) <2.00% indicates the high accuracy of the method.

Table 1C The percentage of recovery of curcuminoids

Analytical concentration µg/ml	**% Recovery of curcuminoids**			**Mean**	**RSD**
	1	**2**	**3**		
10	98.15	98.14	98.14	98.14	0.01
20	101.03	101.26	101.11	101.13	0.11
30	99.78	99.79	99.72	97.76	0.03
40	100.66	100.84	100.58	100.69	0.13
50	99.24	9945	99.36	99.35	0.11
60	100.11	100.10	100.17	100.13	0.03

Table 1C shows the percentage of analytical recovery of curcuminoids. The mean percentage of analytical recovery complied to the range of 95-105 % with low % RSD (<2.00 %) indicated the high accuracy of this method.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatly to multiple samplings of homogenous sample. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of drugs both within run and between run of less than 2.00 % which indicates that HPLC methods can be used to determine the amount of model drugs over period of time studied.

-Within run precision (Repeatability)

Five sets of six standard solution of turmeric powder were analyzed on the same day to determine within run precision. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of the drug from each concentration were determined.

-Between run precision (Reproducibility)

The between run precision was determined by comparing each concentration of turmeric powder standard solution prepared and analyzed on different days. The percentage of relative standard deviation (%RSD) values of concentration of turmeric powder from tree sets of the calibration curves was determined.

Table 2C shows data of within run precision of HPLC method that use to determine curcumin. Table 2C shows data of between run precision of HPLC method that use to determine curcumin. The percentage of coefficient of variation (%RSD) values of peak area both within run and between run were low (<2.00 %) which indicated that HPLC methods could be determine the amount of the drugs over period of time studied.

Table 2C Within run precision data of curcumin

Concentration (µg/ml)	**Area under the curve**				
	10	**20**	**30**	**40**	**50**
1	640666	1392235	2095743	2843003	3519493
2	640589	1395486	2096015	2848127	3527246
3	640592	1393364	2094590	2840571	3524080
4	640652	1394681	2095972	2840427	3519964
5	640599	1395877	2095977	2846965	3522733
Average	640619.60	1394328.60	2095659.40	2843818.60	3522703.20
SD	36.49	1514.51	607.38	3576.91	3175.77
%RSD	0.01	0.11	0.03	0.13	0.09

Table 3C Between run precision data of cucumin reference standard day 1

Run	Area under the curve				
	Concentration (µg/ml)				
	10	**20**	**30**	**40**	**50**
1	640666	1392235	2095743	2843003	3519493
2	640589	1395486	2096015	2848127	3527246
3	640592	1393364	2094590	2840571	3524080
4	640652	1394681	2095972	2840427	3519964
5	640599	1395877	2095977	2846965	3522733
Average	640619.60	1394328.60	2095659.40	2843818.60	3522703.20
SD	36.49	1514.51	607.38	3576.91	3175.77
%RSD	0.01	0.11	0.03	0.13	0.09

Table 4C Between run precision data of cucumin reference standard day 2

Run	Area under the curve				
	Concentration (µg/ml)				
	10	**20**	**30**	**40**	**50**
1	645014	1402090	2194727	2941048	3584535
2	644320	1405445	2194484	2941799	3587424
3	644487	1403777	2195194	2941595	3586672
4	644785	1404129	2195595	2942876	3587560
5	644923	1405196	2195786	2942828	3587746
Average	644705.80	1404127.40	2195157.20	2942029.20	3586787.40
SD	293.75	1337.14	553.78	799.91	1323.60
%RSD	0.05	0.10	0.03	0.03	0.04

Table 5C Between run precision data of cucumin reference standard day 3

Run	Area under the curve				
	Concentration (µg/ml)				
	10	**20**	**30**	**40**	**50**
1	630571	1351170	2069922	2813311	3509862
2	630560	1351405	2069872	2808989	3509288
3	630522	1352198	2069991	2810285	3504969
4	630670	1355509	2075407	2810565	3519062
5	630618	1346521	2075985	2811046	351335
Average	630588.20	1351360.60	2072235.40	2810839.20	2878903.20
SD	57.12	3215.99	3165.97	1577.82	1412962.91
%RSD	0.01	0.24	0.15	0.06	49.08

Linearity

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the concentration of turmeric extract powder standard solution in samples within a given range. Five replicates of each concentration of standard solutions containing turmeric extract powder in various concentrations ranging from 10 to 60 µg/ml were prepared and analyzed. The linear regression analysis of the curve obtained by plotting the absorbance versus the concentrations was calculated.

Mobile Phase Prepare a filtrate

Standard preparation

Working standard was accurately weighed about 20 mg to 50 ml volumetric flask, then diluted with methanol to volume. This solution was used as the standard

stock solution. The 5 ml of stock solution were transfered to 50 ml volumetric flask, diluted with methanol or diluent to volume. The final concentration of standard solution was 40µg/ml. The HPLC method was used to determine curcuminoids content. The validation of HPLC methods used are presented as follows.

Calibration curve of bisdesmethoxycurcumin reference standard

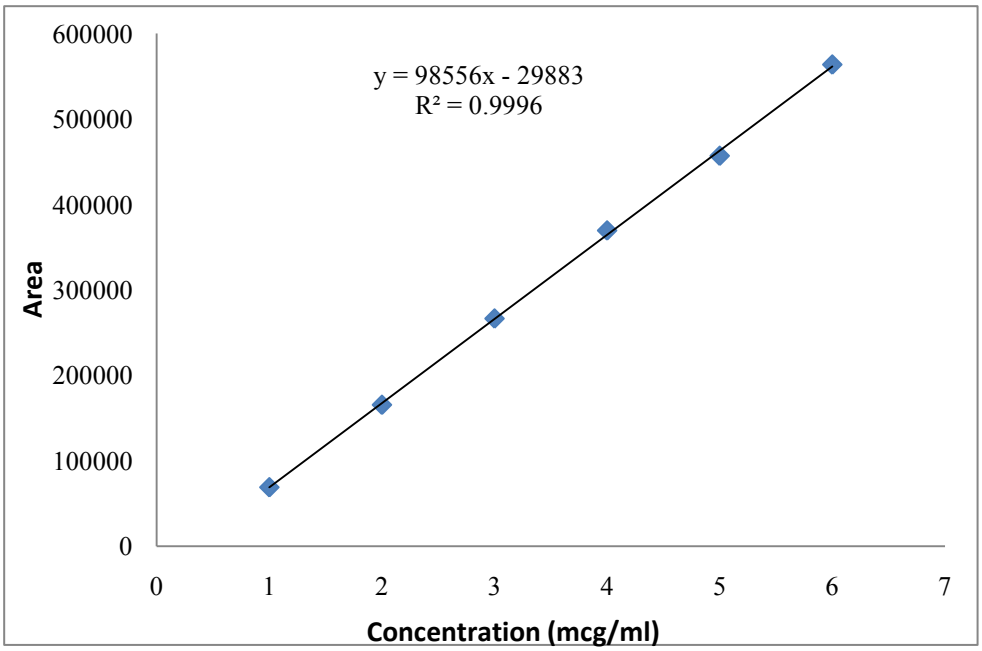


Figure 11C Calibration curve showing linearity between area under the curve and bisdesmethoxycurcumin reference standard concentrations analyzed by HPLC method

Calibration curve of desmethoxycurcumin reference standard

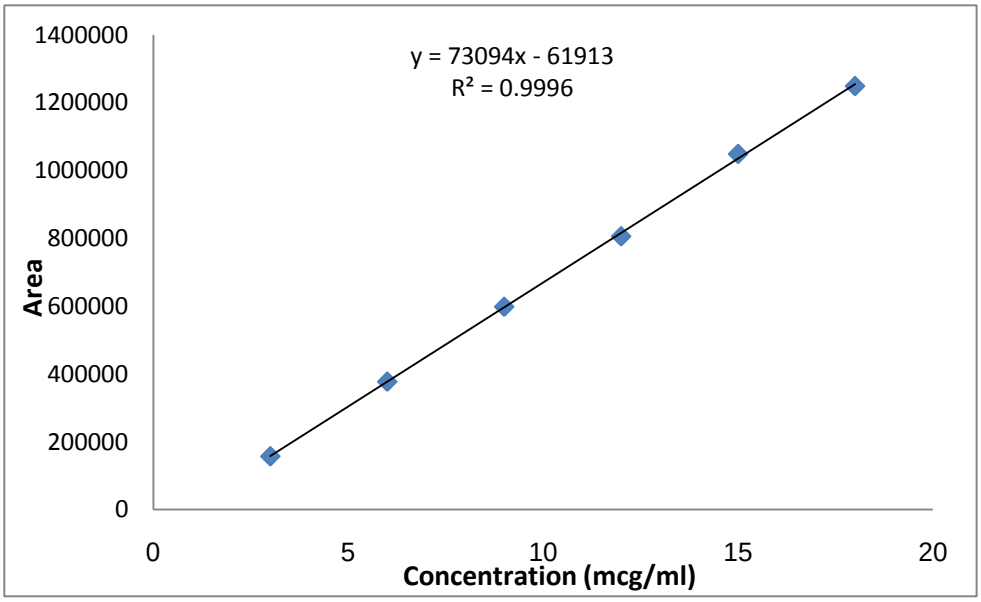


Figure 12C Calibration curve showing linearity between area and desmethoxycurcumin reference standard concentrations analyzed by HPLC method

Calibration curve of curcumin reference standard

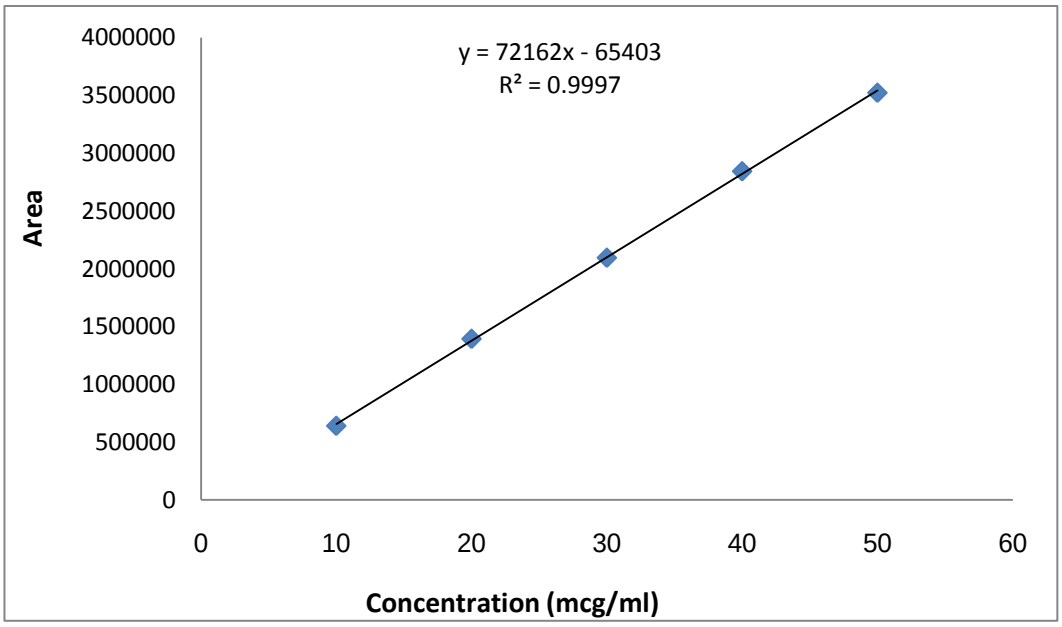


Figure 13C Calibration curve showing linearity between area and curcumin reference standard concentrations analyzed by HPLC method.

APPENDIX D

Evaluation

Dissolution study

Table 1D Percentage amount of curcuminoids release from shrimp feed in water

Product	Time (min)	% Leaching						Mean (%)	SD
		n1	n2	n3	n4	n5	n6		
CS	10	40.68	40.99	41.03	41.11	44.79	48.80	42.90	3.28
	20	33.04	34.22	33.64	33.93	34.29	34.75	33.98	0.59
	30	31.81	31.11	31.60	31.31	31.33	31.65	31.47	0.26
	40	30.41	30.99	30.27	30.48	30.97	30.56	30.61	0.30
	50	30.05	30.35	29.90	29.97	29.65	28.74	29.78	0.56
	60	29.97	30.41	29.82	30.02	29.19	28.03	29.57	0.85
CE	10	52.12	50.13	51.92	51.66	54.85	52.23	52.15	1.53
	20	35.16	35.93	35.85	36.16	34.15	35.31	35.43	0.73
	30	31.78	30.45	31.42	30.79	30.45	30.60	30.92	0.56
	40	29.98	30.36	29.90	30.17	29.34	28.37	29.69	0.73
	50	27.65	27.79	27.50	27.64	30.84	33.67	29.18	2.54
	60	26.87	27.22	29.98	27.65	26.87	26.71	27.55	1.24
ES	10	62.30	65.52	62.01	64.25	65.52	68.84	64.74	2.52
	20	59.82	63.40	62.34	63.62	63.40	64.09	62.78	1.56
	30	58.41	57.72	56.98	55.58	53.12	57.56	56.56	1.94
	40	41.02	41.14	41.14	41.14	41.67	41.92	41.34	0.37
	50	36.42	36.15	36.37	36.80	27.22	36.24	34.87	3.75
	60	31.62	32.07	31.97	32.07	32.13	31.71	31.93	0.21
EE	10	75.03	75.62	75.38	75.44	75.62	75.03	75.35	0.27
	20	68.16	70.69	69.51	69.99	69.11	68.78	69.37	0.90
	30	68.78	66.01	68.05	66.77	66.01	65.99	66.94	1.21
	40	60.11	61.07	59.85	60.18	62.66	60.99	60.81	1.03
	50	54.25	53.66	53.12	53.74	54.21	70.69	56.61	6.91
	60	40.19	40.60	40.13	40.94	40.85	40.02	40.46	0.39

Table 2D Reduced variable and observed responses in experimental design

Run	x_1	x_2	x_3	Y1	Y2
1	-1	-1	-1	9.37	63.01
2	-1	-1	+1	13.02	61.39
3	-1	+1	-1	8.08	69.42
4	-1	+1	+1	17.63	48.36
5	+1	-1	-1	5.43	91.74
6	+1	-1	+1	7.04	74.63
7	+1	+1	-1	6.72	86.07
8	+1	+1	+1	7.2	77.25

Table 3D The percentage remaining of curcuminoids after storage in ambient condition for 6 months

Storage period (month)	% Remaining of curcumin			Mean	SD
	1	2	3		
CS					
1 month	87.78	87.67	87.46	87.64	0.16
2 month	80.16	80.63	80.38	80.39	0.24
4 month	72.94	72.78	72.55	72.76	0.20
6 month	67.59	67.94	67.76	67.76	0.18
CE					
1 month	89.85	89.77	89.55	89.72	0.16
2 month	83.26	83.43	83.17	83.29	0.13
4 month	76.17	76.32	76.08	76.19	0.12
6 month	71.46	71.46	71.46	71.46	0.00
ES					
1 month	87.67	88.02	87.89	87.86	0.18
2 month	83.16	82.63	82.38	82.72	0.40
4 month	78.12	78.12	78.12	78.12	0.00
6 month	70.75	70.54	70.38	70.56	0.19
EE					
1 month	91.03	91.11	91.36	91.17	0.17
2 month	87.57	87.49	87.64	87.57	0.08
4 month	85.63	83.43	83.17	84.08	1.35
6 month	79.68	79.74	80.11	79.84	0.23

Table 4D The equation and correlation coefficient (R^2) of the relation between the storage periods versus the percentage remaining amount of the total curcuminoids active constituents for testing the kinetic order

Product	R^2	
	Zero order kinetics	First order kinetics
CS	$y = -6.6052x + 108.33$ $R^2 = 0.9786$	$y = 108.88e^{-0.069x}$ $R^2 = 0.9849$
CE	$y = -6.1024x + 107.19$ $R^2 = 0.9885$	$y = 110.38e^{-0.076x}$ $R^2 = 0.9745$
ES	$y = -6.0226x + 106$ $R^2 = 0.9935$	$y = 107.67e^{-0.069x}$ $R^2 = 0.9875$
EE	$y = -3.9084x + 103.99$ $R^2 = 0.9989$	$y = 104.62e^{-0.042x}$ $R^2 = 0.9969$

Table 5D Physical properties of processed shrimp feed [mean (SD)]

Physical properties	**CS**	**CE**	**ES**	**EE**
Moisture content (%LOD)	10.45(0.01)	10.87(0.01)	8.12(0.03)	9.96(0.01)
Bulk density (g/ml)	0.47(0.00)	0.44(0.01)	0.33(0.01)	0.29(0.02)
Tapped density (g/ml)	0.48(0.00)	0.46(0.02)	0.37(0.01)	0.33(0.01)
%Compressibility	2.08(0.00)	5.05(1.04)	10.91(0.17)	15.69(1.70)
Flow rate (g/sec)	19.18(0.03)	12.74(0.06)	10.06(0.09)	8.61(0.12)
Angle of repose (deg)	23.21(0.02)	37.62(0.09)	18.70(0.01)	41.62(0.03)
Diameter (mm)	1.3(0.01)	1.29(0.01)	0.89(0.01)	0.92(0.01)
Hardness(kp)	6.26(0.08)	5.98(0.17)	2.83(0.89)	-
Watability(sec)	38(1.00)	40.33(2.08)	56.67(1.53)	45.33(1.53)

APPENDIX E
Statistical analysis

Table 1E One-way ANOVA analysis of curcuminoids content (%) in shrimp feed each method freshly prepared

Assay of curcuminoids content

ANOVA

Percent

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	85.728	3	28.576	58.348	.000
Within Groups	15.672	32	.490		
Total	101.400	35			

Multiple Comparisons

Dependent Variable: Percent

	(I) Percent Curcuminoids	(J) Percent Curcuminoids	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	AIC	Em C	-2.38556*	.32990	.000	-3.3588	-1.4123
		AIEx	-3.28667*	.32990	.000	-4.2599	-2.3134
		EmEx	-4.12889*	.32990	.000	-5.1021	-3.1556
	Em C	AIC	2.38556*	.32990	.000	1.4123	3.3588
		AIEx	-.90111	.32990	.078	-1.8744	.0721
		EmEx	-1.74333*	.32990	.000	-2.7166	-.7701
	AIEx	AIC	3.28667*	.32990	.000	2.3134	4.2599
		Em C	.90111	.32990	.078	-.0721	1.8744
		EmEx	-.84222	.32990	.111	-1.8155	.1310
	EmEx	AIC	4.12889*	.32990	.000	3.1556	5.1021
		Em C	1.74333*	.32990	.000	.7701	2.7166
		AIEx	.84222	.32990	.111	-.1310	1.8155

*. The mean difference is significant at the .05 level.

Table 2E One-way ANOVA analysis of leaching profile in term of the percent remaining the curcuminoids content in shrimp feed after processed each preparing method sampling every 10 in 1 hour total time

At 60 minutes

ANOVA

Percent Remain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1773.994	3	591.331	1179.059	.000
Within Groups	34.104	68	.502		
Total	1808.098	71			

Multiple Comparisons

Dependent Variable: Percent Remain

	(I) Percent Remain at time	(J) Percent Remain at time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	AIC	Em C	3.14222*	.23606	.000	2.4655	3.8190
		AIEx	-1.15611*	.23606	.000	-1.8329	-.4794
		EmEx	-10.21056*	.23606	.000	-10.8873	-9.5338
	Em C	AIC	-3.14222*	.23606	.000	-3.8190	-2.4655
		AIEx	-4.29833*	.23606	.000	-4.9751	-3.6216
		EmEx	-13.35278*	.23606	.000	-14.0295	-12.6760
	AIEx	AIC	1.15611*	.23606	.000	.4794	1.8329
		Em C	4.29833*	.23606	.000	3.6216	4.9751
		EmEx	-9.05444*	.23606	.000	-9.7312	-8.3777
	EmEx	AIC	10.21056*	.23606	.000	9.5338	10.8873
		Em C	13.35278*	.23606	.000	12.6760	14.0295
		AIEx	9.05444*	.23606	.000	8.3777	9.7312

*. The mean difference is significant at the .05 level.

Table 3E One-way ANOVA analysis of percent curcuminoids content in shrimp feed each method after storage for 6 month

At 6 month

ANOVA

Percent

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	243.550	3	81.183	2721.986	.000
Within Groups	.239	8	.030		
Total	243.788	11			

Multiple Comparisons

Dependent Variable: Percent

	(I) Percent Curcuminoids	(J) Percent Curcuminoids	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	AIC	Em C	-3.69667*	.14101	.000	-4.1892	-3.2042
		AIEx	-2.79333*	.14101	.000	-3.2858	-2.3008
		EmEx	-12.08000*	.14101	.000	-12.5725	-11.5875
	Em C	AIC	3.69667*	.14101	.000	3.2042	4.1892
		AIEx	.90333*	.14101	.002	.4108	1.3958
		EmEx	-8.38333*	.14101	.000	-8.8758	-7.8908
	AIEx	AIC	2.79333*	.14101	.000	2.3008	3.2858
		Em C	-.90333*	.14101	.002	-1.3958	-.4108
		EmEx	-9.28667*	.14101	.000	-9.7792	-8.7942
	EmEx	AIC	12.08000*	.14101	.000	11.5875	12.5725
		Em C	8.38333*	.14101	.000	7.8908	8.8758
		AIEx	9.28667*	.14101	.000	8.7942	9.7792

*. The mean difference is significant at the .05 level.

VITA

Miss Jirisuda Suthiprapa was born on June 18, 1980. She received her Bachelor of science in Pharmacy degree on March 2004 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. After graduation, she started working as an Instructor in the Thai Traditional Medicine Program at Kanchanabhishek Institute of Medical and Public Health Technology, Praboromarajchanok Institute, Ministry of Public Health for two years before entering the Master's Program in Manufacturing Pharmacy at Chulalongkorn University in 2006.