CHARACTERIZATION OF EXTRACTED FISH OIL FROM EEL: EFFECTS OF PROCESS PARAMETERS ON EXTRACTION YIELD. PARAMETERS:

a) DRYING TEMPERATURE
b) SOLVENT DIFFERENT

AMIRAH BINTI IZAM

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL : CHARACTERIZATION OF EXTRACTED FISH OIL FROM EEL: EFFECTS OF PROCESS PARAMETERS ON EXTRACTION YIELD. PARAMETERS: a) DRYING TEMPERATURE, b) SOLVENT DIFFERENT

SESI PENGAJIAN: **2008/2009**

Saya **AMIRAH BINTI IZAM**
(HURUF BESAR)

mengaku membenarkan tesis Projek Sarjana Muda (PSM) ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut:

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. **Sila tandakan (✓)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan oleh

_____ (TANDATANGAN PENULIS)

Alamat Tetap: **14, JALAN PINGGIR 8/1L**
40000 SHAH ALAM
SELANGOR

Tarikh: 2 Mei 2009

_____ (TANDATANGAN PENYELIA)

SUREENA BT ABDULLAH
Nama Penyelia

Tarikh: 2 Mei 2009

CATATAN:
* Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.
◆ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertai bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM).

"I hereby declare that I have read this thesis and in
my opinion this dissertation is sufficient in terms of scope and
quality for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)"

Signature : .....

Supervisor : Sureena Bt Abdullah

Date : .....

CHARACTERIZATION OF EXTRACTED FISH OIL FROM EEL: EFFECTS OF PROCESS PARAMETERS ON EXTRACTION YIELD. PARAMETERS:

c) DRYING TEMPERATURE
d) SOLVENT DIFFERENT

AMIRAH BINTI IZAM

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

May 2009

DECLARATION

I declare that this thesis entitled "Characterization of Extracted Fish Oil from Eel: Effects of Process on Extraction Yield. Parameters: a) Drying Temperature, b) Solvent Different" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :……

Name : Amirah binti Izam

Date : 2 May 2009

DEDICATION

Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

ACKNOWLEDGEMENT

I would like to forward my appreciation to my thesis supervisor, Miss Sureena Bt Abdullah for her guidance and support in completion this research. I would also very thankful to my academic advisor, Mr. Rozaimi B Abu Samah, for his support and believe in me during my studies.

I'm very thankful to Universiti Malaysia Pahang (UMP) for providing good facilities in the campus. To all the staff in Faculty of Chemical & Natural Resources Engineering, a very big thanks you to all.

My sincere appreciation also extends to all my fellow colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Thank you for the time sacrificed to accompany me. And last but not least, I am grateful to all my family members.

ABSTRACT

Fish oil is recommended for a healthy diet because it contains the omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), that reduce inflammation throughout the body. The eel, scientifically known as *Monopterus albus* was used as the raw material. This study aimed at determining the best conditions to extract the fish oil in terms of drying temperature and solvents used for extraction process and to determine the compounds available in the extracted fish oil. The eel fillets were dried by using oven at five different temperatures ranging from 60 – 100 ^{o}C and then were grounded into powder form. The oil was extracted by using Soxhlet extractor for 6 hours and then was purified by using rotary evaporator to obtained concentrated oil. The fatty acid composition of the oil was analyzed using gas chromatography after being converted into methyl ester derivatives. Chemical quality of the oils which was acid value and free fatty acid composition were analyzed by using free fatty acid analyzer, 785 DMP Titrino. Results show that at temperature 70^{o}C with solvent ethanol were the best conditions to extract the fish oil. The acid values obtained are not beyond the accepted value thus, all the oils are in high quality. In the fatty acid analysis it was discovered that the major fatty acids in the oil were palmitic acid, stearic acid, oleic acid (omega-9) and linoleic acid (omega-6). As a conclusion, the best drying temperature and solvent to achieve high quantity of fish oil are 70^{o}C and ethanol respectively. The acid values reveal that the fish oils are in high quality. Four major fatty acid component detected in the fish oil are palmitic acid, stearic acid, oleic acid (omega-9) and linoleic acid (omega-6).

ABSTRAK

Minyak ikan telah disyorkan sebagai pemakanan yang sihat sebab ia mengandungi asid lemak omega-3, asid eikosopenaenoik (EPA), asid dokosahexaenoik (DHA), yang boleh mengurangkan keradangan. Belut, secara saintifiknya *Monopterus albus* (sejenis ikan air tawar) telah digunakan sebagai bahan ujikaji. Kajian ini bertujuan untuk menetukan keadaan yang paling baik untuk mengekstrak minyak ikan dari segi suhu pengeringan dan pelarut untuk proses pengekstrakkan dan juga menetukan komponen yang terdapat di dalam minyak belut. Isi belut dikeringkan menggunakan ketuhar pada 5 suhu yang berbeza dari suhu 60–100^{o}C dan kemudian dikisar menjadi serbuk. Minyak ikan diekstrak menggunakan pengekstrak Soxhlet selama 6 jam dan kemudian ditulenkan menggunakan penyejat berputar untuk mendapatkan minyak yang pekat. Komposisi asid lemak ditentukan menggunakan kromatografi gas setelah ditukarkan kepada terbitan metil ester. Kandungan asid dan kandungan asid lemak bebas ditentukan menggunakan penentu asid lemak bebas model 785 DMP Titrino. Keputusan menunjukkan pada suhu 70^{o}C dan pelarut etanol adalah keadaan yang paling baik untuk mengekstrak minyak ikan ini. Kandungan asid adalah tidak melebihi had yang diterima maka, semua minyak adalah berkualiti tinggi. Dalam analisis asid lemak, asid lemak utama di dalam minyak tersebut adalah asid palmitik, asid sterik, asid oleic (omega-9) dan asid linoleik (omega-9). Kesimpulannya, suhu pengeringan dan pelarut yang paling baik adalah 70^{o}C dan etanol. Minyak yang didapati adalah berkualiti tinggi. Empat komposisi asid lemak dalam minyak ikan ini ialah asid palmitik, asid sterik, asid oleik (omega-9) dan asid linoleik (omega-9).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

g	-	gram
h	-	hour
mL	-	mililiter
%	-	percentage
°C	-	degree Celsius
µL	-	microliter
rpm	-	rotation per minute

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Asthma is characterized by overly reactive bronchi (increased "twitchiness"). This increased responsiveness, doctors and researchers believe, is due to underlying bronchial inflammation. The walls of the bronchi contain muscles, and the interiors are lined with a membrane (mucous membrane) that secretes mucus, or phlegm. In people with asthma, the bronchi decrease in size when they come in contact with certain triggering factors (Boutin, 1995). Doctors agree that the best treatment for asthma entails identifying and then avoiding its triggers. In some instances these are obvious for example exposure to tobacco smoke and other noxious fumes, cold air, exercise, or an allergy to animal dander. Seasonal asthma is usually due to various pollens, molds and other environmental factors (Schwarcz and Berkoff, 2004). Wheezing, chest tightness, labored breathing and other asthma symptoms occur when the tiny muscles that control the airways to the lungs constrict, causing a bronchospasm. Normally, the airways narrow somewhat when exposed to smoke, pollutants, very cold air or substances that are harmful if inhaled (Schwarcz and Berkoff, 2004). Heredity may be a factor too. The reason some people have hyperactive airways is unknown; heredity, however, suspected of playing a role, because the disease runs in the families (Schwarcz and Berkoff, 2004).

Most people with asthma cannot cure this disease but it can be controlled through preventive medicines and symptomatic treatments. Recently, fish and fish oil fatty acids are currently under intense scientific investigation because of the numerous

health benefits attributed to them (Rahman *et al*., 1995). Marine lipids are associated with high level of long chain omega-3 polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wu and Betchel, 2008). Many health experts suggest that two to three servings per week of seafood should be consumed in order to meet the recommended level of essential fatty acids for pregnant women, children and elderly people (Huhges, 1995; Olsen & Secher, 2002). Research studies done by expert found out that omega 3 fatty acids, particularly EPA, have a very positive effect on your inflammatory response. Through several mechanisms, they regulate your body's inflammation cycle, which prevents and relieves painful conditions like arthritis, prostatitis, cystitis and anything else ending in "itis" (Byrd, 2007)

In this study, swamp eel, *Monopterus albus* is used to extract the fish oil. Swamp eel naturally occurs in ponds, canals, ditches, rice fields, and swamps where it may be a dominant species (Smith, 1945). Asian swamp eels typically inhabit fresh-waters, but they may be found in brackish and saline waters (Nichols, 1943). It is no surprise that the eel's flesh is believed by many people to be the cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds (www.bernama.com). In traditional practitioner, the eel soup is believed to cure asthma disease (www.yuhuii.com). Although there are not many studies on the eel benefits, it is verbally proven by the consumers of the meat where it can cure asthma disease. The reason to extract the swamp eel oil is because of its feature that looks like snake makes the consumers feel disgusted and afraid to consume the meat. The fish oil can be an alternative medicine to prevent asthma. Furthermore, this study is a preliminary research and later it will be a pioneer to do more research on this fish oil.

Extraction process is the best method to separate the oil from the fish. In this production of swamp eel oil, organic solvents such as ethanol, isopropanol and n-hexane are used to extract oil from the eel. Selection of extraction conditions depends on the nature of the extraction process, the temperature, pH and residence time could have an effect on the yield and selectivity (www.cheresource.com). Extraction process is conducted by using Soxhlet extractor. A Soxhlet extractor is a type of laboratory

glassware invented in 1879 by Franz von Soxhlet. This equipment is designed for the extraction of lipid from solid material, but also can use whenever it is difficult to extract any compound from the solid. The Soxhlet extractor enables solids to be extracted with fresh warm solvent that does not contain the extract. This can dramatically increase the extraction rate, as the sample is contacting fresh warm solvent. At the end of extraction, the excess solvent may be removed by using rotary evaporator, leaving behind the extracted lipid.

1.2 Problem Statements

A lot of studies had been conducted by using other types of fish species such as salmon and mackerel to produce fish oil. But studies on swamp eels are less known yet. This is due to lack of exposure on the benefits and also no proving conducted scientifically by the experts. Furthermore, there also no study conducted on the effects of process parameters on extraction yield where the parameters are on drying temperature an solvent used for extraction process.

Verbally mention by the consumers of the swamp eel said that by eating the fish meat it can reduce the risk of asthma disease. As mention above, some believed that by drinking the eel soup can also cure asthma disease. One of the problems of this study is where the swamp eel has a feature that looks like a snake that disgusted the consumers and some are afraid of it. In addition, swamp eel is difficult to catch due to its slippery body texture. Thus, this study is conducted to extract the swamp eel oil to give more pleasant consuming to the consumers. There are no researches documented scientifically of the contents inside the eel that can heal asthma disease. Doctors only prescribe medicines to keep asthma in control but there is no medicine produce for curing asthma disease. Furthermore, this is a preliminary research as there is not much evidence to prove the benefits of swamp eel oil. This study is conducted to search for the specific compound that gives benefits towards the asthma patients.

1.3 Objectives

The purposes of this study are to determine the best condition to extract fish oil in terms of drying temperature and solvent use for extraction process and to determine the compounds available in the extracted swamp eel oil.

1.4 Scopes of Study

Raw material used in this study is swamp eel. Preparation of the raw material before the process of extraction is needed to increase extraction yield. By knowing the objective of this study, drying temperature of the eel are varied in order to determine the best drying temperature to yield a lot of fish oil quantity. Drying is a process of removing water moisture by evaporation from a solid or semi-solid material. In this study, the fish is dried by using oven. Drying temperature ranges from 60^{o}C to 100^{o}C is used to dry the fish. Drying temperature of 60^{o}C is the lowest temperature use to dry the fish because of to evaporate the moisture content inside the fish very quickly. Hundred degree Celcius is the highest temperature choose to dry the fish because of to avoid any damages or any lost of biological components inside the fish where it is important to be determine during chemical analysis.

In the extraction process by using Soxhlet extractor, there are three solvents used to extract the fish oil which are ethanol, isopropanol and n-hexane. These three solvents are choose because it is easy to get from any chemical supply companies in Malaysia.

The compounds inside the fish oil is identify by using Gas chromatography and also the acid value is quantified to measure the quality of the fish oil.

1.5 Rationale and Significance

The rationales of doing this study are:

1. To increase the extraction yield by monitoring parameters so that the pure oil can be used effectively.
2. To do preliminary research on the extracted oil using swamp eel. This study is a pioneer toward further research and studies.
3. To do clinical test on the swamp eel oil for determination of certain compounds that gives cure to asthma disease.
4. To open up opportunities to increase the demand on manufacturing the swamp eel oil in the form of capsules as proven by the researchers that fish oil have a lot of benefits.
5. To enhance the medication treatment for asthma illness and to research for alternative medicines that give benefits to the asthma patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Eel

Eel, scientifically known as *Monopterus albus*, naturally occurs in ponds, canals, ditches, rice fields, and swamps where it may be a dominant species (Smith, 1945). Asian swamp eels typically inhabit fresh-waters, but they may be found in brackish and saline waters (Nichols, 1943).

The body is more or less cylindrical; tail compressed tapering to a slender point much shorter than the trunk. Scales are absent. The snout is bluntly rounded, the jaws and palate have rows of viliform teeth. The upper lip is thick overlapping part of the lower lip. The eye is small, covered by a layer of skin (Nichols, 1943; Jayaram, 1981). Body color is slate brown above, white or light-brown below with small dark spots on sides and occasionally on the ventral surface (Inger and Kong, 1962). The lateral line is well developed and conspicuous (Jayaram, 1981). In adults, paired fins are lacking, and the dorsal, caudal and anal fins are reduced (Nichols, 1943; Sterba, 1983). Although specimens over 70 cm are relatively rare, this species may grow to a meter in length. Most grow to between 25 and 40 cm (Smith, 1945).

Figure 2.1: Eel (www.wikipedia.com)

This species occur in Asian country such as India, China, Japan, Malaysia and Indonesia. Probably also occurring in Bangladesh (www.fishbase.org). Based on the Annual Fisheries Statistic 2004 (Volume 1) by Department Of Fisheries, Malaysia, estimated fish production from public waterbodies (rivers) shows that only in Terengganu (0.2 tonnes) and Kelantan (2.80 tonnes) is the main producer of swamp eel. The estimated wholesale and retail value are as in Table 2.1

Table 2.1: Estimated wholesale and retail value of swamp eel in 2004, RM '000 (Annual Fisheries Statistic 2004, Department of Fisheries)

State	Wholesale value	Retail value
Terengganu	1.10	1.60
Kelantan	13.92	20.02

Even though many people are quite reluctant to eat the eel, it cannot be denied that this freshwater fish has many nutritional benefits. Its nutritional values are said to be on par with that of the 'tenggiri' (Spanish mackerel) and 'selar' (crevelle) which have 18.6 per cent protein and 15 per cent fat. The eel is also rich in calcium and iron as well as vitamins B and D. (www.bernama.com)

According to Razak *et al,* the eel, *Monopterus albus* contain a high level of docosahexanoic acid (DHA) which is 6.21 g/100 g lipid. Study by Rahman et al shows amount of eicosapentanoic acid (EPA) were found to be less than 3.5 % with freshwater eel being the highest (3.48 %) followed by eel (2.66 %). This would explain the usage of these two fish as a traditional medicine for muscles pain (Mohsin & Ambak, 1983).

2.2 Fish Oil

The fatty acid composition of marine lipids varies significantly, especially when compared with vegetable oils (Shahidi, 2005). Fatty acids can be divided into two which are saturated fatty acids or unsaturated fatty acids. Fish oils are rich in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and they are good sources of omega-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Shahidi, 2005). Omega-3 PUFA has benefits towards human health. These fatty acid decrease the stickiness of blood platelets, making it less likely that they will clump together to form clots. They also increase the flexibility of red blood cells, enabling them to pass more readily through tiny vessels, reduce inflammation of the artery walls and lower levels of triglycerides in the blood (Schwarcz and Berkoff, 2004). The human body uses omega-3 fatty acids to manufacture prostaglandins, chemicals that play a role in many processes, including inflammation and other functions of the immune system (Schwarcz and Berkoff, 2004). Not only the human need PUFAs to give them a lot of benefits, but fish also need it for their own good. Fish need PUFAs to provide tolerance to low water temperatures (Rahman *et al.,* 1994).

The fat content in fish varies according to seasons, species and geographical variation. Age variation and sex maturity in the same species also contribute to the significant differences in the total lipid contents (Piggott & Tucker, 1990; Tsuchiya, 1961; Rahman *et al.,* 1994). Researchers have found that freshwater fish contain lower proportions of omega-3 PUFA than do marine fish (Rahman *et al*., 1994). Decreases in PUFA concentrations in lipids would therefore be expected in warmer

waters (nearer the equator) like Malaysia. Wang et al. (1990) found that the ω-3: ω-6 ratio of freshwater fish was lower than marine fish. Freshwater fish normally consist of more omega-6 PUFA whereas the marine fish are rich in omega-3, especially DHA and EPA (Wang *et al.,* 1990). Figure below shows the chemical structure of DHA and EPA.

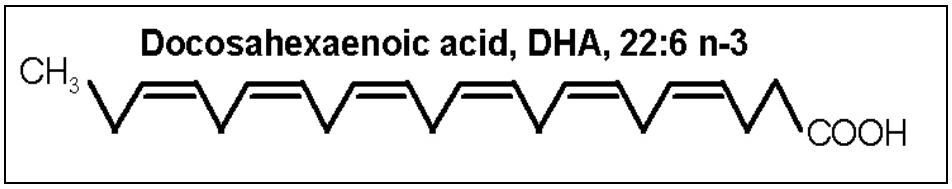


Figure 2.2: Chemical structure of docosahexaenoic acid (DHA)

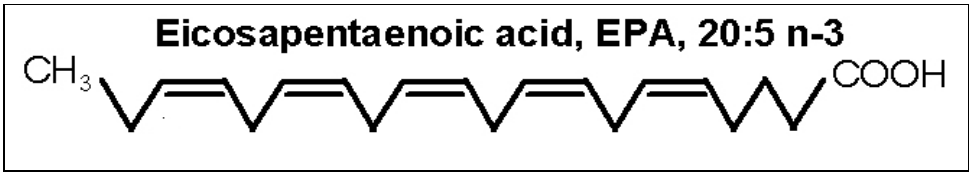


Figure 2.3: Chemical structure of eicosapentaenoic acid (EPA)
(www.omega-3-forum.com)

2.3 Asthma

Currently available therapy for asthma is highly effective and, if used appropriately, usually has no problems in terms of adverse effects. However, some patients (5–10% of asthmatic patients) remain poorly controlled, despite what appears to be optimal therapy (Barnes *et al*., 1998). In this study, the fish oil is used as an alternative medicine for curing or preventing asthma disease.

2.3.1 Asthma Disease

Asthma is characterized by overly reactive bronchi (increased "twitchiness"). This increased responsiveness, doctors and researchers believe, is due to underlying bronchial inflammation. The walls of the bronchi contain muscles, and the interiors are lined with a membrane (mucous membrane) that secretes mucus, or phlegm. In people with asthma, the bronchi decrease in size when they come in contact with certain triggering factors. The triggering factors can be due to exposure to smoke, pollutants, very cold air or substances that are harmful if inhaled (Schwarcz and Berkoff, 2004). However, the response in exaggerated and often triggered by otherwise harmless substances or activities, such as pollen and other allergens and exercise (Schwarcz and Berkoff, 2004).

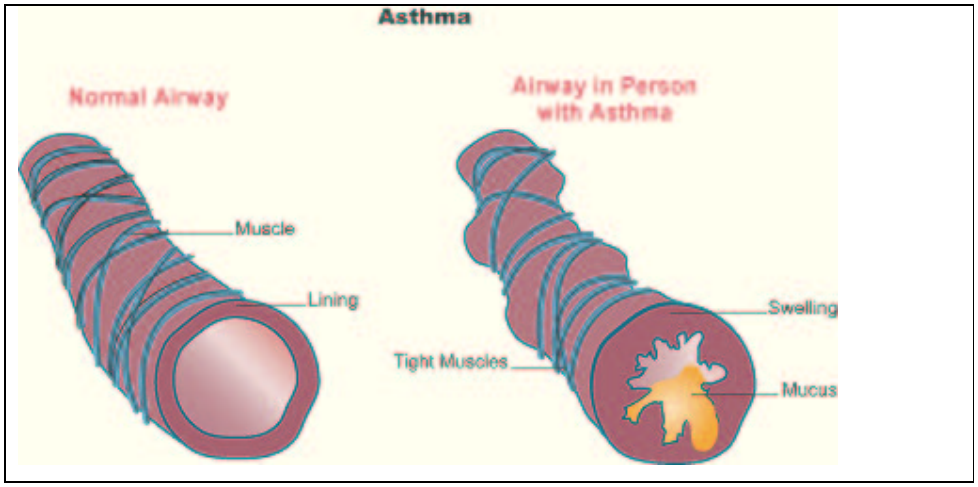


Figure 2.4: Lung airway in normal person and asthma patient (http://www.nhlbi.nih.gov)

The bronchi in young children, which are smaller, are more easily obstructed. During an asthma attack, the following changes take place in the bronchi and bronchioles:

1. The muscles encircling the bronchi contract, their interior diameter (lumen) narrows and air cannot reach the lungs as easily (in medical terms, this phenomenon is called bronchospasm).

2. The membrane lining the inside of the bronchi becomes inflamed and swollen, making it even more difficult for air to pass through (this is inflammation).

3. Excess secretions can lead to the formation of mucus plugs, which reduce the air passages even more (Boutin, 1995).

There are no specific foods that prevent asthma, but some may lessen its complications. Omega-3 fatty acids, found in salmon, mackerel, sardines and other cold-water fish, have an anti-inflammatory effect and may counter bronchial inflammation (Schwarcz and Berkoff, 2004).

2.3.2 Medication

Once asthma has been diagnosed, the goal of treatment is to minimize or eliminate respiratory symptoms and restore normal lung function by:

- Identifying the triggering factors and effectively correcting the environment.
- Using the minimum of drugs appropriate to the severity of the asthma;
- Preventing and treating flare-ups early and effectively (using a written action plan).

Medication varies according to the severity of asthma in each person. That person must know and understand:

- How to use asthma drugs and what effects they have
- What drugs you must take

- How to change medication if asthma symptoms worsen;
- When to consult a doctor.

Today, people take asthma medication mainly by inhalation. An inhaled drug can be taken at a lower dose and thus causes fewer side-effects than if it was taken in the form of pills or syrup (Boutin, 1995). In this study, the fish oil is extracted from flying fish as an alternative medicine to prevent asthma disease. This alternative medicine is basically free from chemicals. Chemicals base medicines are not very good in long term because it might cause harm to human body if it is not suitable to them.

2.4 Factors effecting extraction process

There are many factors that can affect extraction process. In this study focuses more on drying temperature and solvent different.

2.4.1 Drying

Drying generally means removal of small amount of water from material. In drying, the water is usually removed by as vapor in the air (Geankoplis, 2003). The moisture content of the final dried product varies for different material to dry. Dried salt contains about 0.5% water, coal about 4% and many foods products about 5 % (Geankoplis, 2003). Several different ways and methods can be classified for drying processes. Drying processes can be done in batch, where the material is inserted in the drying equipment and drying process proceeds for given period of time. Drying also can be done in continuous where the material is continuously added to the dryer and dried material is continuously removed. The drying temperature range of 60^{o}C to 100^{o}C is used to dry the filleted fish. Predrying facilitates the grinding of the samples, enhances extraction and may break fat-water emulsions to make fat dissolve easily in the organic solvent and helps to free tissue lipids (Akoh and Min, 2002). Drying the

samples at elevated temperatures is undesirable because lipids become bound to proteins and carbohydrates and such bound lipids are not easily extracted with organic solvents (Pomeranz and Meloan, 1994).

Figure 2.5: Oven

2.4.2 Solvent

The solvents mostly used for isolation of lipids are alcohols (methanol, ethanol, isopropanol, n-butanol), acetone, acetonitril, ethers (diethyl ether, isopropyl ether, dioxane, tetrahydrofuran), halocarbons (chloroforms, dichloromethane), hydrocarbons (hexane, benzene, isooctane) or their mixtures (Akoh and Min, 2002). Three solvents which are ethanol, isopropanol and n-hexane are used in the extraction process. Ethanol and isopropanol are alcohol solvent and n-hexane is hydrocarbon solvent. The ideal solvent for lipid extraction would completely extract all lipid components from a food. The efficiency of solvent extraction depends on the polarity of the lipids present compared to the polarity of the solvent. Lipids containing no distinguishable polar groups are highly soluble in hydrocarbons solvents such as hexane, benzene, or cyclohexane (Akoh and Min, 2002). Neutral lipids are hydrophobically bound and can

be extracted from tissues by nonpolar solvents, whereas polar lipids, which are bound predominantly by electrostatic forces and hydrogen bonding, require polar solvents capable of breaking such bonds (Akoh and Min, 2002). In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point (so that it can easily be removed by evaporation), be non-toxic and be nonflammable (for safety reasons) (http://www-unix.oit.umass.edu/~mcclemen/581Lipids.html)

Table 2.2 below shows methods for the routine extraction of lipids in foods for the purpose of using the extract for the subsequent analysis of individual lipid components.

Table 2.2: Methods for routine extraction of lipids in foods, for the purpose of using the extract for the subsequent analysis of individual lipid components. (Ötleş, 2005)

Methods for the Routine Extraction of Lipids in Foods, for the Purpose of Using the Extract for the Subsequent Analysis of Individual Lipid Components

Method	Solvents	Types of Lipid Extracted	Ref.
Soxhlet-related methods	Hexane or petroleum ether	Nonpolar lipids	Table 5.2
Supercritical fluid extraction	CO_2, sometimes with ethyl alcohol	Nonpolar lipids	5
Folch	Chloroform, methanol, water	Nonpolar and polar lipids	10, 11
Bligh and Dyer	Chloroform, methanol, water	Nonpolar and polar lipids	12
Hara and Radin	Hexane, isopropanol, water	Nonpolar and polar lipids	15

As for this study, the three solvents choose as extraction solvent are because of it is easy to get in any chemicals supply companies and also the solvent did not react with the fish oil to form other unwanted by-product.

2.5 Soxhlet Extractor

To measure "total fat", various methods have been approved by the regulatory agencies of most major countries. Most older methods involved extraction and gravimetric mass measurement of the lipid residue (Ötleş, 2005). In gravimetric methods, lipids of the sample are extracted with a suitable solvent continuously, semi-continuously or discontinuously. In semi-continuous solvent extraction (e.g., Soxhlet, Soxtec), the solvent accumulates in the extraction chamber (sample is held in a filter paper thimble) for 5-10 minutes and then siphons back to the boiling flask. This method requires a longer time than the continuous method, provides a soaking effect for the sample and does not result in channeling. After a certain period of time the solvent layer is recovered and the dissolved fat is isolated by evaporating the organic solvent (Akoh and Min, 2002).

The most outstanding advantages of conventional Soxhlet extractor are as follows:

1. The sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium.
2. The temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent.
3. No filtration is required after the leaching step.
4. Sample throughput can be increased by simultaneous extraction in parallel since the basic equipment is inexpensive.
5. It is very simple methodology which needs little specialized training.
6. Has the possibility to extract more sample mass than most of the latest methods and is non-matrix dependent. (Garcia-Ayuso *et al*, 1998)

Figure 2.6: Soxhlet extractor

CHAPTER 3

METHODOLOGY

3.1 Materials and Solvents

The fish species used in this study is swamp eel, *Monopterus albus* which was bought from Giant supermarket for about six kilogram. The solvents used are ethanol (R&M Chemicals), isopropanol (Systerm) and n-hexane (Fisher Scientific).

3.2 Apparatus

For drying process an oven is used to remove moisture content by evaporation of water content from the fish (swamp eel). Another purpose of drying is to saturate the oil content in the fish. In the extraction process, Soxhlet extractor is used to extract the oil from the fish. Purification of oil need to be done in order to obtained pure fish oil. Rotary evaporator is the equipment responsible to remove the fish oil from solvent. For analysis purpose, free fatty acid analyzer model 785 DMP Titrino is used. This model also can determine the acid value inside the fish oil. Another equipment for analysis purpose is Gas Chromatography, Agilent 5975C MSD is used to detect what compounds exist in the fish oil.

3.3 Sample Preparation

Prior to analysis, the internal organs were removed and the fish was washed with cold water to removed residual blood. Then, it was evisterated and filleted by cutting the fish lengthwise along the backbone to obtained maximum amount of flesh without including the backbone. The fillet was cut into small pieces to attain large surface area for dying reason. The fillets were then dried overnight inside the oven at temperature of 60, 70, 80, 90 and 100^{o}C. The drying will stop until reaching constant weight. After that, grind the fillets into powder form by using Waring Blender and sieve the powder into homogenized form using plastic sieve. The powder form fillet was put into clean seal plastic bags and labeled.

Figure 3.1: Sample preparation

3.4 Extraction by Soxhlet Extractor

Ten grams of powder fillet at different temperatures was weighted and put inside a thimble while 400 ml of different solvents was measured. The total time spent for the extraction process was 6 hours for each sample. The extracted oil was then collected in a flask.

Figure 3.2: Soxhlet extractor

3.5 Rotary evaporator

When the extraction process was finished, the solvent will be removed by using Heidolph Laborata 4000 rotary evaporator, yielding the extracted compound. The temperature of the rotary evaporator must be set above the boiling point of the solvent used to extract the fish oil so that the solvents can evaporate and leave only the pure oil. Rotation speed was set at 50 rpm. The solvent collected was recycled back for the next extraction process. Volume of fish oil obtained was measured and put inside a 25 ml of universal bottle.

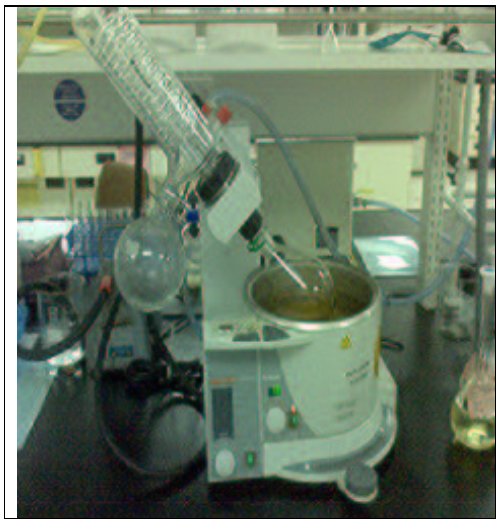


Figure 3.3: Rotary evaporator

3.6 Chemical Analysis

This research will focus on three different chemical analyses which are:

1. Analysis of chemical compositions
2. Analysis of free fatty acid content
3. Acid value

3.6.1 Analysis of Chemical Compositions

Lipid samples were converted to their constituent fatty acid methyl esters (FAME). Weigh 10 mg sample in a 20 ml test tube (with screw cap) or reaction vial. Dissolve the sample in 10 ml hexane. Add 100 µL 2 N potassium hydroxide in methanol (11.2 g in 100 ml). Close the tube or vial and vortex for 30 s. Centrifuge at 4000 rpm for 10 min. Transfer the clear supernatant into 2 ml autosampler vial and was taken for GC-MS analyses.

Routine analysis of methyl esters was performed by Gas Chromatography – Mass Spectrometry (GC-MS Agilent 5975C MSD) on a polyethylene glycols DB-Wax column (30 m length x 0.25 mm internal diameter). Carrier gas used was helium. Split injection with split ratio of 50:1 was used. Injection volume was 1 µL. Operating conditions were 250^{o}C injection port; 280^{o}C flame ionization detector and oven temperature was 50^{o}C. Compounds were tentatively identified by comparison with retention times of Razak *et al* GC Chromatogram of the FAME derived from the body oil of eel.

3.6.2 Analysis of free fatty acid content and acid value

Free fatty acids (FFA) values were used as the quality indicator of oil. It can be determined by using free fatty analyzer model 785 DMP Titrino. This equipment can also determine acid value of the oil without using conventional method which is titration method. The acid value (AV), which is defined as the number of milligrams of KOH required to neutralize the free fatty acids in 1 g of sample, is a measure of FFA content or a measure of the amount of *free acids* present in a given amount of fat. Five milliliter of fish oil was diluted with 50 ml of ethanol and the free fatty acid content and acid value was detected by this equipment.

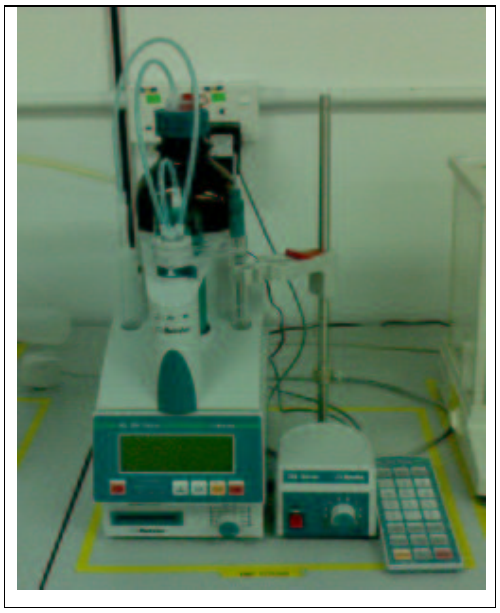


Figure 3.4: 785 DMP Titrino

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

For this chapter, there are four aspects being considered. The aspects are the drying temperature, the solvent for extraction process, acid value content and the components available in the eel oil.

4.2 Observations

After the purification steps, observations were made to see the differences between the eel oils obtained. Table 4.1 shows the observation on the eel oils based on its color and the presence of white fat.

Table 4.1: Table of observations on the eel oil obtained.

Drying temperature, ^{o}C	Observations					
	Ethanol		Isopropanol		n-Hexane	
	Presence of white fat	Colour of eel oil	Presence of white fat	Colour of eel oil	Presence of white fat	Colour of eel oil
60	Yes		Yes		No	
70	Yes		Yes		No	
80	Yes		Yes		No	
90	Yes		Yes		No	
100	Yes		Yes		No	

From the observations, the color of eel oil for extracted using ethanol as solvent was darker followed by isopropanol and n-hexane. The color of the oils was different because of the polarity of the solvent used to extract the fish oil. Table 4.2 shows the polarity index of the solvents. Polarity index, P' is a relative measure of the degree of interaction of the solvents with various polar test solutes. High polarity index means that the solvent have high interaction with other solutes. Ethanol have high polarity index followed by isopropanol and lastly is n-hexane.

Table 4.2: Table of polarity index of solvents used to extract eel oil

Solvent	**Polarity index, P'**
Ethanol	4.3
Isopropanol	3.9
n-Hexane	0.1

Polarity is referring to the dipole-dipole intermolecular forces between the slightly positively charged ends of one molecule to the negative end of another or the same molecule. Molecular polarity is dependent on the difference in electronegativity between atoms in a compound and the asymmetry of the compound's structure. Atoms with high electronegativities such as fluorine, oxygen, and nitrogen exert a greater pull on electrons than atoms with lower electronegativities. In a bonding situation, this can

lead to unequal sharing of electrons between atoms as electrons will spend more time closer to the atom with the higher electronegativity. A non-polar compound occurs when there is an equal sharing of electrons between two different atoms. Examples of household non-polar compounds include fats, oil and petrol or gasoline. In this case, eel oil is a non-polar molecule. Therefore, most non-polar molecules are water insoluble (hydrophobic) at room temperature but very soluble in solvent (www.wikipedia.com). Table 4.3 shows on how to predict the molecules polarity.

Table 4.3: Table of predicting molecule polarity (www.wikipedia.com)

	Formula	Description	Example
Polar	AB	Linear Molecules	CO
	HA_x	Molecules with a single H	HCl
	A_xOH	Molecules with an OH at one end	C_2H_5OH
	O_xA_y	Molecules with an O at one end	H_2O
	N_xA_y	Molecules with an N at one end	NH_3
Non-polar	A_2	Diatomic molecules of the same element	O_2
	C_xA_y	Most carbon compounds	CO_2

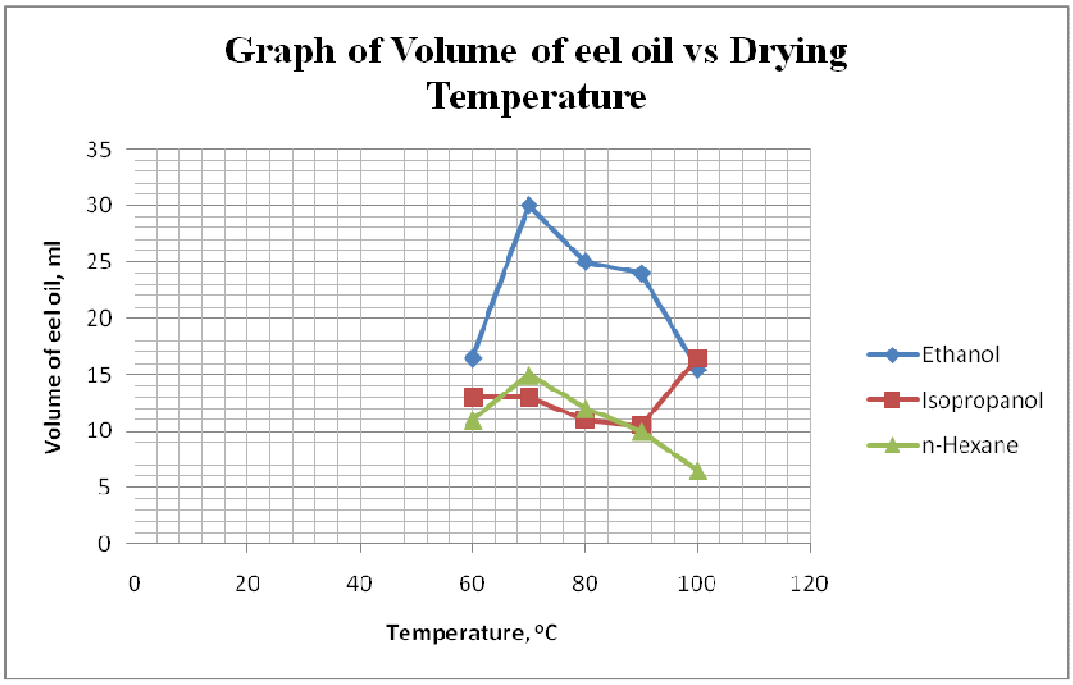
Since ethanol and isopropanol is in alcohol (-OH) group which possess oxygen in their chemical formula, therefore these solvents are polar molecules based on Table 4.3 while n-hexane is a non polar molecule. Because ethanol and isopropanol possess oxygen atom that have high electronegatives, thus, they exerts a greater pull on atom that have low electronegatives such as the oil atoms. For that reason these two solvents can extract more amount of fish oil than n-hexane. Using n-hexane as solvent was not good enough to extract the fish oil because it has low electronegativity that weakens the pulling effects on atoms. Ethanol is a primary alcohol while isopropanol is a secondary alcohol.

The primary alcohol is not a stable molecule thus, it has high tendency to exert greater pull with other atom to bind with it. For that reason the polarity index of ethanol is higher than isopropanol. This also explained why the fish oil extract with

ethanol had darker color than extracted with isopropanol. This also explains on the presence of white fats. These white fats were also known as fatty acids.

4.3 Volume of Eel Oil Obtained

The volume of eel oil can be measured after the purification step by using rotary evaporator. Figure 4.1 illustrate the graph of volume of eel oil versus drying temperature based on different solvent.



.

Figure 4.1: Graph volume of eel oil versus drying temperature based on different solvent

Generally, Figure 4.1 shows that as the temperature increased, the volume of eel oil decreased. This was because as the drying temperature increased, the more water content was removed from sample, therefore, the more efficient the extraction process will be. High drying temperature was not necessary because lipid became bound to proteins and carbohydrates where it was not easily extracted with organic solvents. The drying temperature should be high enough to evaporate moisture from

the foods, but not high enough to cook the foods. From the graph also, the optimum temperature to obtain high amount of eel oil was the temperature of 70^{o}C. This can be considered the best drying temperature because all of the water content inside the fish has been removed and therefore extraction process can be done efficiently. From here it was known that the best drying temperature was 70^{o}C and the best solvent to achieved high yield of eel oil was ethanol.

4.4 Acid Value and Free Fatty Acid Content

Acid value (or "neutralization number" or "acid number" or "acidity") is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize the free acids in one gram of sample, is a measure of free fatty acids content. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds (www.wikipedia.com). Generally, from Figure 4.2 shows that by using ethanol as solvent, the acid value increased as the drying temperature increased. While by using the other two solvents, generally would decreased in acid value as the drying temperature increased. Increased in acid value is generally associated with lipase activity originating from microorganisms or biological tissue (Boran *et al*). Hydrolysis of ester bonds in lipids by enzymatic action or heating in the presence of water liberates free fatty acids (FFA) (Pastoriza, 2005). Bimbo reported that the acceptable limit for acid value was to be in between 7-8 mg KOH. The result shows that the acid values of the eel oil were not beyond the acceptable limit. It was important to note that the samples were not sterilized nor studied them under aseptic conditions. Thus, it was possible that some enzyme or microorganism contamination might occur during sample removal. De Koning (1999) observed that hydrolysis of fish was greatly reduced upon sterilization.

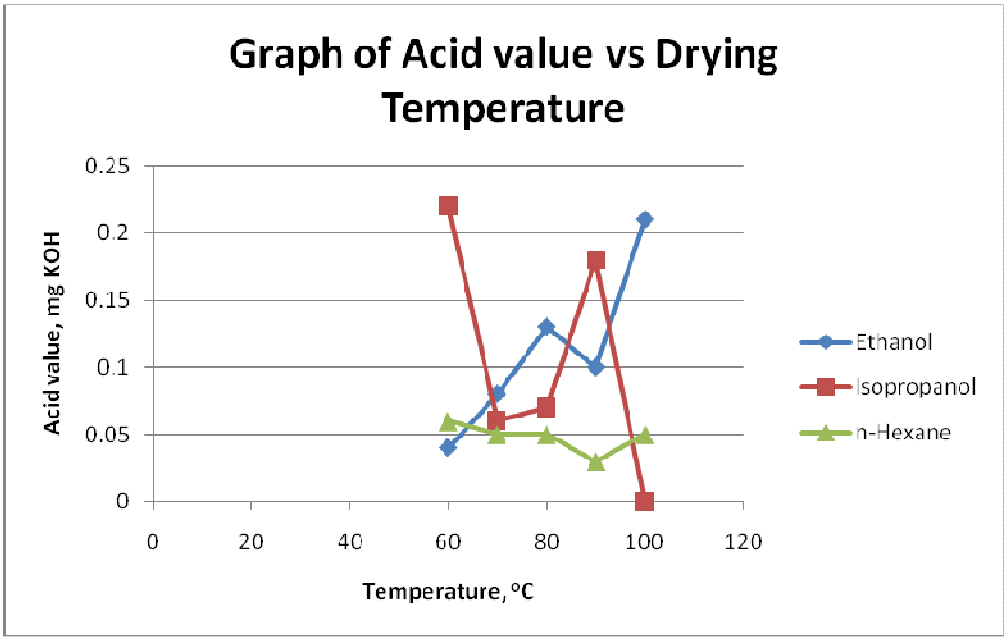


Figure 4.2: Graph of acid value versus drying temperature

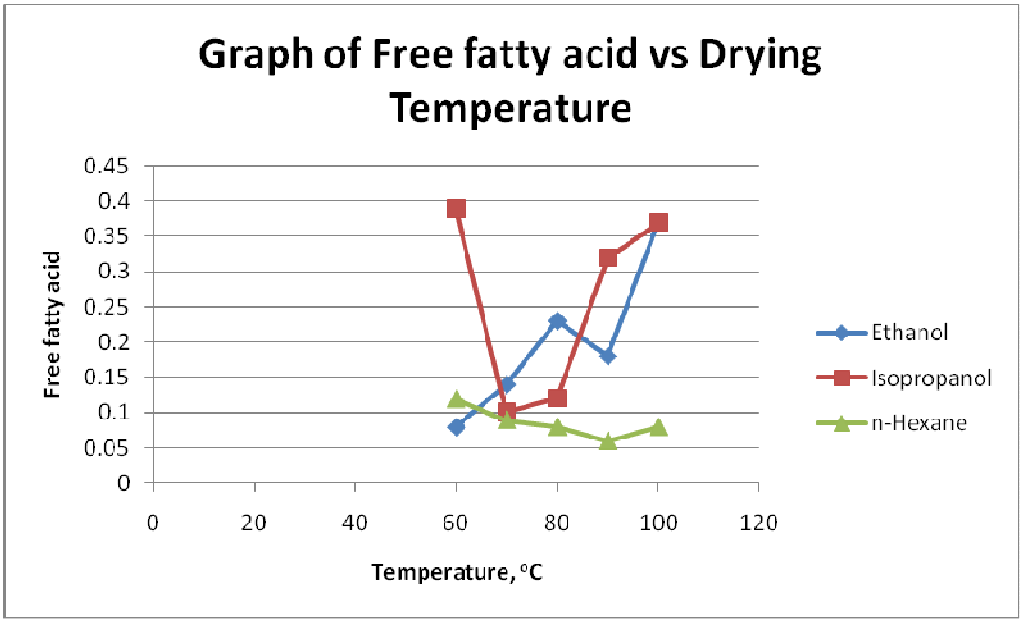


Figure 4.3: Graph of free fatty acid versus drying temperature

Free fatty acid content was used as quality indicator of the oil. From Figure 4.3, illustrate the graph of free fatty acid content versus drying temperature. High content of free fatty acid shows that the low quality of the oil due to the lipolytic enzymes from microorganisms (De Koning, 1999). In some cases, the quantification of free fatty acid serves to establish the limits by which the food is not organoleptically acceptable (Pastoriza, 2005). From the graph, even though the free fatty acid content of eel oil extracted by ethanol increased as the drying temperature increased, the eel oils were still in high quality due to the acceptable limit of acid value. These reason also the same by using isopropanol as solvent.

4.5 Gas Chromatography Results

The analysis of FAMEs was used for the characterization of the lipid fraction in foods, and was one of the important analyses for food. The results obtained from Gas chromatography reveals the main components that contained in the eel oil. All of the chromatogram results shows that there were abundance of palmitic (C16:0) and stearic (C18:0) acid. Table 4.7 shows the main fatty acid components that contain in

the fish oil according to different drying temperature. Oleic acid (omega-9) and linoleic acid (omega-6) are detected also in the fish oil.

Table 4.4: Table of fatty acid components in eel oil

Drying temperature, ^{o}C	**Fatty acids**
60	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
70	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
80	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
90	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
100	- Lauric acid - Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)

4.6 Conclusion

From the results obtained, it can be concluded that the best drying temperature was 70^{o}C and the best solvent used in extraction process was ethanol. The acid value analysis shows that the eel oils were in the acceptable limit, thus, the oils were in high quality. The results from gas chromatography reveals four fatty acids that were found abundantly which were palmitic acid, stearic acid, oleic acid (omega-9) and linoleic acid (omega-6).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Fish oil has been recognized as good sources of polyunsaturated fatty acids (PUFA) which are widely used for pharmaceutical purposes and as food supplements. It has been reported that tropical fishes including eel are rich in arachiodic acid (AA) and docosahexaenoic acid (DHA). These fatty acids have been recommended as infant food supplements by health agencies (Razak *et al,* 2001).

Overall, this study gives results in terms of best drying temperature, solvent, acid value and also the components inside the eel oil. For best drying temperature it was 70^{o}C while ethanol was the best solvent used to extract the fish oil. These two conditions results in the highest volume of fish oil extracted. Acid value analysis shows that the eel oils were less exposed to the lipase activity originating from microorganisms or biological tissues. This had made that the eel oils were in good quality. The fatty acid composition of the eel oil that was found abundantly is palmitic acid and stearic acid. The World Health Organization claim there is convincing evidence that dietary intake of palmitic acid increases risk of developing cardiovascular diseases (www.wikipedia.com).

There are no researches that scientifically proven of the active compounds that can heal asthma disease. Further studies on this fish could reveal the important compounds that can cure asthma disease. For now it is important to collect as much data as possible on what components that available in the eel. This preliminary study can be a start towards more studies in future.

5.2 Recommendations

To achieve a very good result for future study, there are several recommendations that need to be considered. To obtain larger amount of eel oil, increase the amount of the fish powder that will be used in the extraction process. In addition, to increase extraction yield, the extraction process need to extend longer so that all the oil has been extracted from the sample. A series of repetition of the experimental process should be conducted in order to produce good results.

Further recommendations are also made for future studies on this research. A further research on oleic acid (omega-9) and linoleic acid (omega-6) should be conducted whether it can be the components that heal asthma disease. All the information that has been obtained for this study must gather as a reference in future research. Lastly, put in document of this research as to prove scientifically on the findings by the researchers.

REFERENCES

Akoh C. C. and Min D. B. (Eds.) (2002). *Food Lipids. Chemistry, Nutrition, and Biotechnology*. Madison Avenue, N. Y.: Marcel Dekker

Annual Fisheries Statistic (Volume 1), (2004). Department of Fisheries Malaysia

Barnes P. J., Rodger I. W. and Thomson N. C. (1998). *Asthma: Basic Mechanisms and Clinical Management* (3rd Ed.). London: Academic Press

Bimbo, A.P. (1998). *Guidelines for Characterizing Food-Grade Fish Oils*. Inform vol. 9, No. 5, Heerfordshire: UK.

Boran G., Karaçam H., Boran M., (2005). Changes in the Quality of Fish Oils Due to Storage Temperature and Time. *Food Chemistry*. 98, 693-698.

Boutin, Helene (1995). *Understand and Control Your Asthma.* Montreal, QC, CAN: McGill-Queen's University Press

Byrd, Michael (2007). http://ezinearticles.com/?7-Fish-Oil-Benefits-Proven-by-Research&id=415032. 7 Fish Oil Benefits Proven by Research.

De Koning, A.J. (1999). The free fatty acid content of fish oil, part V. the effects of microbial contamination on the increase in fatty acid content of fish oils during storage at 25 ^{o}C. Fett/Lipid, 101, 184 - 186.

Garcia-Ayuso L. E. and Luque de Castro M. D. (1998). Soxhlet extraction of solids material: an outdated technique with a promising innovative future. *Analytica Chimica Acta*, 1-10

Geankoplis C. J. (2003). *Transport Processes and Separation Process Principles*. Upper Saddle River, N. J.:Prentice Hall

Huhges, D. A. (1995). Fish oil and the immune system. *Nutrition & Food Science,* 95(2), 12 - 16

Huah T. S., Suriah Abd. Rahman, Osman Hassan and Nik Mat Daud (1995). Fatty Acid Composition of Some Malaysian Freshwater Fish. Universiti Kebangsaan Malaysia. 45 – 49

Inger, R. F., and C. P. Kong. (1962). The Freshwater Fishes Of North Borneo. Fieldiana: Zoology 45:1-268.

Jayaram, K. C. (1981). The Freshwater Fishes Of India, Pakistan, Bangladesh, Burma, and Sri Lanka- A Handbook. Zoological Survey Of India, Calcutta. 475 Pp. + 13 Plates.

Mohsin, A. K. M. & Ambak, M. A. (1983). Freshwater fish in Peninsular Malaysia. Universiti Pertanian Malaysia

Nichols, J. T. (1943). The freshwater fishes of China. Natural history of Central Asia volume IX. *The American Museum of Natural History*, New York, NY.

Nurul Halawati Azhari (2007, August 06). Eel Goods the Palate, Health. *Bernama*. Retrieved February 20, 2009, from http://www.bernama.com

Olsen, S. F., & Secher, N. J. (2002). Low consumption of seafood in early pregnancy as a risk factor for preterm delivery:Prospective cohort study. *British Medical Journal.* 324(23), 1-5

Ötleş S. (Ed.) (2005). *Methods of Analysis of Food Components and Additives*. Boca Raton, F. L.:Taylor & Francis Group

Pastoriza, L., Bernadez, M., Sampedro, G., Herrera, J.J.R. and Cabo, M.L. (2005). Modified Method for the Analysis of Free Fatty Acids in Fish. Journal Argiculture Food Chemical. 1903 - 1906

Pomeranz Y. and Meloan C. L. (1994). *Food Analysis; Theory and Practice*, (4th Ed.) AVI, Westport, CT

Piggott, G. M. & Tucker, B. W. (1990). *Eflects of Technology on Nutrition.* New York, USA.:Marcel Dekker, Inc.

Razak, Z. K. A., Basri, M., Dzulkefly, K., Razak, C. N. A., and Salleh, A. B..(2001). Extraction and Characterization of Fish Oil from *Monopterus albus*, *Malaysian Journal of Analytical Sciences*, The Malaysian Analytical Sciences Society. 7(1), 217 - 220

Schwarcz J. and Berkoff F. (2004). *Foods That Harms, Foods That Heals*. Pleasantville, N. Y.:The Readers Digest Association Shahidi F. (Ed.) (2005). *Bailey's Industrial Oil & Fats Products* (6th Ed. Vol 3.).Hoboken, N. J.:John Wiley & Sons

Smith, H. M. (1945). The Fresh-water Fishes Of Siam, Or Thailand. *Bulletin Of The U.S. National Museum* 188:1-622.

Smith, M. W. & Miller, N. G. A. (1980). *In Animals and Environmental Fitness,* ed. R. Giles. Oxford,U.K.:Pergamon Press.

Sterba, G. (1983). The Aquarium Fish Encyclopedia. *The MIT Press.* Cambrige, Massachusetts. 605 pp

Tsuchiya, T. (1961). Biochemistry of fish oil. In *Fish as Food,* ed. G. Borgstrom. Academic Press, New York, USA, pp.21 l-58.

Wang, Y. J., Miller, L. A., Perren, M. & Addis, P. B. (1990). Omega-3 fatty acids in Lake Superior fish. *Journal of Food Science,* 55(l), 71-73.

Wu, T. H.and Betchel P. J. (2008). Salmon by-product storage and oil extraction. *Food Chemistry*. 868 – 871.

http://www.bernama.com Eels Good For The Palate, Health

http://www.cheresource.com Optimizes liquid-liquid extraction.

http://www.yuhuii.com Belut

http://www.fishbase.org *Monopterus albus*

http://www.hmgind.com Laboratory oven

http://www.nhlbi.nih.gov What is Asthma?

http://www.omega-3-forum.com Fatty acid structure

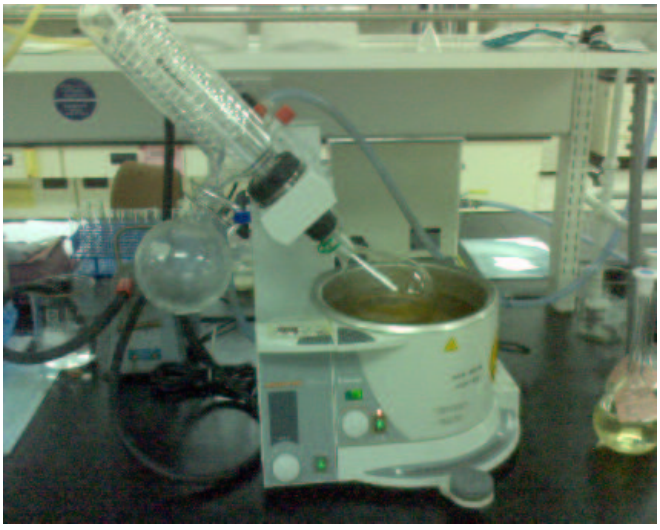
http://www-unix.oit.umass.edu/~mcclemen/581Lipids.html Analysis of Lipids

http://www.wikipedia.com Polarity

http://www.wikepedia.com Soxhlet extractor

APPENDIX A

Appendix A.1: Soxhlet extractor



Appendix A.2: Rotary evaporator (Heidolph Laborata 4000)

Appendix A.3: Oven

Appendix A.4: Waring Commercial blender

Appendix A.5: Fatty acid analyzer (785 DMP Titrino)

APPENDIX B

Appendix B.1: Table of observations on the eel oil obtained

Drying temperature, ^{o}C	Observations					
	Ethanol		Isopropanol		n-Hexane	
	Presence of white fat	Colour of eel oil	Presence of white fat	Colour of eel oil	Presence of white fat	Colour of eel oil
60	Yes		Yes		No	
70	Yes		Yes		No	
80	Yes		Yes		No	
90	Yes		Yes		No	
100	Yes		Yes		No	

Appendix B.2: Table of volume of eel oil obtained

Drying temperature, ^{o}C	**Volume of eel oil based on different solvents, ml**		
	Ethanol	**Isopropanol**	**n-Hexane**
60	16.5	13	11
70	30	13	15
80	25	11	12
90	24	10.5	10
100	15.5	16.5	6.5

Appendix B.3: Table of acid value according to different drying temperature and solvents

Drying temperature, ^{o}C	Acid value, mg KOH		
	Ethanol	**Isopropanol**	**n-Hexane**
60	0.04	0.22	0.06
70	0.08	0.06	0.05
80	0.13	0.07	0.05
90	0.10	0.18	0.03
100	0.21	0.21	0.05

Appendix B.4: Table of free fatty acid according to different drying temperature and solvents.

Drying temperature, ^{o}C	Free fatty acid		
	Ethanol	**Isopropanol**	**n-Hexane**
60	0.08	0.39	0.12
70	0.14	0.10	0.09
80	0.23	0.12	0.08
90	0.18	0.32	0.06
100	0.37	0.37	0.08

Appendix B.5: Table of fatty acid components in eel oil

Drying temperature, ^{o}C	Fatty acids
60	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
70	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
80	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
90	- Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)
100	- Lauric acid - Palmitic acid - Stearic acid - Oleic acid (omega-9) - Linoleic acid (omega-6)