ULTRASONIC EXTRACTION OF FISH OIL FROM EEL

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A thesis submitted in partial fulfillment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2009

I declare that this thesis entitled "*Ultrasonic Extraction of Fish Oil from Eel*" is the result of my own research except cited in the references. The thesis has not been accepted for any degree and is not currently submitted in candidature of any other degree.

Signature	:	…….....
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"Especially to my beloved parents, brothers, sisters and friends …"

ACKNOWLEDGEMENT

First and foremost, thanks to God Almighty for the guidance and help in giving me the strength to complete this study successfully. I would also like to take this opportunity to express my utmost gratitude to my supervisor, Mrs. Siti Kholijah Bt Abdul Mudalip for his valuable guidance, encouragement, critics and advice throughout this study. Furthermore, to my second supervisor, Miss Zatul Iffah Bte Mohd Arshad, thank you for all your help in this project. Besides that, I have furthermore to thank to my undergraduate colleague for their support, valuable tips and ideas that helped me to complete this project within the timeline given.

Beside that, appreciation is also to all laboratory personnel who were willing to give help and good co-operation during the various laboratory tasks that helped me to complete this study within the timeline given.

Lastly, I am also forever indebted to my parents, Mr. Che Pi Bin Ibrahim and Mrs. Siti Mereyam Bt Zain, also to my siblings for their understanding and especially in giving strong moral supports during the preparation of this study.

ABSTRACT

The eel is rich in calcium and iron as well as vitamins B and D, also believed by many people to be the cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds. In this research, fish oil was extracted from Eel (*Monopterus albus*) by using ultrasonic extraction method. Effects of different ultrasonic power, solvent to solid ratio and sonication time on extraction yields were investigated. Then, the extracted fish oil was analyzed using 785 DMP Titrino and Gas Chromatography Mass Spectrometer. The ultrasonic power was used at 100, 200, 300 and 450W. The solvents used were 50, 100, 200 and 500ml of ethanol. In terms of sonication time it was set at 20, 30, 50, and 60 minutes. Results obtained show that the ultrasonic power of 200 W, solvent amount of 500 ml and 60 minutes of sonication time produce the higher yields of extracted fish oil 7.20 %. The FFA and acid value content of the extracted fish oil was higher at 100 W of ultrasonic power with content of 0.53 g/100 g and 0.30 MgKOH/1 g. Qualitative analysis Gas Chromatography Mass Spectrometer shows that the compound of Fatty Acid Methyl Ester (FAMEs) which is palmitic and stearic acid were detected. As a conclusion, the objectives in this study were achieved by extract the fish oil from eel using ultrasonic extraction method that effects of different ultrasonic power, solvent to solid ratio and sonication time on extraction yields.

ABSTRAK

Belut kaya dengan kalsium and zat besi seperti vitamin B dan D, ia juga dipercayai oleh orang ramai dapat memulihkan penyakit buah pinggang, lelah, serangan jantung dan berkemampuan dalam memulihkan luka selepas pembedahan dengan cepat. Di dalam kajian ini, minyak ikan telah diekstrak daripada belut (*Monoptherus Albus*) dengan menggunakan kaedah ekstrak gelombang ultrasonik. Kesan kepada kuasa ultrasonik, nisbah larutan kepada bahan dan perubahan masa kepada kadar ekstrak telah disiasat. Kemudian, minyak ikan yang diekstrak akan di analisis menggunakan 785 DMP Titrino dan Gas Kromatografi Berjisim Spekrometer. Kuasa ultrasonik tang telah digunakan pada 100, 200, 300, dan 450 W. Larutan yang telah digunakan adalah 50, 100, 200, dan 500 ml etanol. Dari segi perubahan masa ianya diselaraskan pada 20, 30, 50, dan 60 minit. Keputusan diperolehi menunjukkan bahawa kuasa tertinggi ultrasonik pada 200 W, jumlah larutan adalah 500 ml dan masa 60 minit menghasilkan kadar ekstrak minyak ikan yang tinggi adalah 7.20%. Kandungan asid lemak bebas dan nilai asid didalam minyak ikan yang diekstrak adalah tinggi pada 100 W kuasa ultrasonik dengan kandungan 0.53 g/100 g dan 0.30 MgKOH/1 g. Kualitatif analisis Gas Kromatografi Berjisim Spekrometer menunjukkan bahawa kandungan asid lemak metil ester (FAMEs) terdiri daripada palmitic dan stearic acid telah dikesan. Sebagai kesimpulannya, objektif didalam pembelajaran ini diperolehi dengan mengekstrak minyak ikan daripada belut dengan menggunakan kaedah ekstrak gelombang ultrasonik kesan kepada kuasa ultrasonik, nisbah larutan kepada bahan dan perubahan masa kepada kadar ekstrak.

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EPA	-	Eicosapentaenoic acid
DHA	-	Docosahexaenoic acid
GCMS	-	Gas Chromatography Mass Spectrometer
IgE	-	Immunoglobulin E
^{o} C	-	Degree Celcius
min	-	Minutes
SFE	-	Supercritical fluid extraction
TLC-FID	-	Thin layer chromatography-flame ionization detection
UE	-	Ultrasound extraction
MAE	-	Microwave assisted extraction
Hz/kHz	-	Hertz/ kilohertz
H	-	Hour
LKIM	-	Lembaga Kemajuan Ikan Malaysia
NaOH	-	Sodium Hydroxide
SPE	-	Solid Phase Extraction
PTFE	-	Polytetrafluoroethylene
Rpm	-	Red Hat Package Manager
mmHg	-	millimeter of mercury
V	-	Volume
g	-	gram
kg	-	kilogram
ml	-	millimeter
cm	-	centimeter
W	-	Watt
HCl	-	Hydrochloric acid
N	-	Normality

M	-	Mass
RM	-	Ringgit Malaysia

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malays used material that comes from plant, animal and natural resources as a traditional medicine. The fishery department in Malaysia is now encouraging and expanding the freshwater fish industry among the agriculturist and fisherman to increase their income. The fish framing technique are improving with the introduction of new techniques in order to have consistent supply of freshwater fish throughout the year. Fisheries Department reported that the landing of freshwater in Malaysia in 1995 and 1996 were 3, 9384.94 tones and 3,683.21 tones, respectively (Mokhtar *et al.*, 2005).

The eel, scientifically known as Monopterus Albus, is included in the fish genus. The body is long and its head is rounded, with the presence of gills. 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' (crevalle) which have 18.6 per cent protein and 15 per cent fat. The eel is rich in calcium and iron as well as vitamins B and D. Hence, 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. According to traditional medicine

practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (BERNAMA, August, 2007).

In traditionally medical, eel can be used as medical of various diseases such as for kidney diseased. The liver of eel is use for asthma prevention. Usually the liver will be smoked and then burnt to cinders and eats with onion and ginger. Beside that, eel soup can cure blood problem and prevent erectile dysfunction. Patient that just had operation done, it can cure the injured faster (http://www.cpintrass.com, 23 February 2009).

According to Leaf and Weber (1988), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which were found only in fish and others seafood's, possess extremely beneficial properties for the prevention of human coronary artery disease.

Many type of methods can be used to extract fish oil. The most popular methods are ultrasonic extraction. This method is very effective in terms of time taken for extraction process and can operate at room temperature. In many situations, ultrasonic treatment is a fast, inexpensive and efficient alternative to conventional leaching processes compare to another treatment (Rodrigo *et al.,* 2005). In other hand, the utilization of ultrasonic cavitation for extraction and food preservation is a new powerful processing technology that can not only be applied safely and environmentally friendly but also efficiently and economically. The homogenizing and preserving effect can be easily used for fruit juices and purees as well as for vegetable sauces and soups (http://www.hielsher.com, 18 August 2009). The sonication of liquids will generate sound waves that propagate into the liquid media resulting in alternating high-pressure and low pressure cycles. The high-pressure cycles of the ultrasonic waves support the diffusion of solvents, such as hexane into the cell structure. As ultrasound breaks the cell wall mechanically by the cavitations shear forces, it facilitates the transfer of lipids from the cell into the solvent. After the oil dissolved in the solvent, the pulp/tissue is filtered out. The solution is distilling to separate the oil from the solvent (Adeniyi *et al.*, 2002).

1.2 Identification of Problem

The eel or known as *Monopterus Albus* is rich in calcium and iron as well as vitamins B and D. Hence, 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. According to traditional medicine practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (Nurul, BERNAMA 6 August 2007).

There are many type of methods used for extraction of fish oil; the most popular method is ultrasonic extraction due the advantages of this method. Ultrasonic enhancement on mass transfer has been realized in such unit operations as leaching, extraction, adsorption and desorption (Carcel *et al.,* 2005). In addition, in the fields of heat transfer, chemical reaction, fine particles preparation, waste water treatment and the concerning processes also benefit from ultrasonic irradiation. The ultrasonic power supply transforms line voltage to high frequency 20 kHz electrical energy. This electrical energy is transmitted to the probe or water bath where it is converted to mechanical energy. The vibrations from the probe are coupled to and intensified by the titanium tip. The probe vibrates in a longitudinal direction and transmits this motion to the titanium tip immersed in the solution. Cavitation results, in which microscopic vapor bubbles are formed momentarily and implode, causing powerful infinitesimal shock waves to radiate throughout the solution in proximity to the radiating face of the tip (Carcel *et al.,* 2005).

Beside that, in comparison with conventional Soxhlet extraction, less extraction time and solvent consumption were required by ultrasonic extraction. The efficiency of the ultrasonic extraction was similar or even higher that the routine Soxhlet method. Additionally, the new extractor is very simple and easy to use and can accelerate the extraction procedures of organic components from various solid samples (Lee *et al.,* 2000). Therefore, due to the advantages of the ultrasonic extraction method, there are used this method in these studies to investigated the

effects of different ultrasonic power, solvent to solid ratio and sonication time on extraction yields.

1.3 Statement of Objective

The main objective of this study is to extract fish oil from eel by using ultrasonic extraction method. Beside that, works also done to study the effect of ultrasonic power, different solvent to solid ratio, and sonication time on extraction yields.

1.4 Research Scope

The scope of research is included as below:

i. To study the effects of ultrasonic power at 100W, 200W, 300W and 450W with frequency of 25 kHz and 300 ml of ethanol on extraction yields.

ii. To examine the effects of solvents amount at 50ml, 100 ml, 200ml, and 500ml with constant intensity obtained from 1.4 (i) on extraction yields. The solvent used was ethanol.

iii. To investigate the effects of different sonication time at 20, 30, 50 and 60 minutes with 200W constant ultrasonic power and constant solvent volume obtained from 1.4 (ii) on extraction yields.

iv. To analyze the extraction yields using Gas Chromatography Mass Spectrometer (GCMS).

1.5 Rational and Significance of Study

The eel was need to extract in order to obtained the concentrated oil from the eel that believes have some benefit in their nutrition content of eel to human life as mention before. The eel also regular to consume that can help to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (Nurul, BERNAMA 6 August 2007). The extraction process of eel also investigated to obtain the effect of different ultrasonic power, solvent to solid ratio and sonication on extraction yields.

There used the ultrasonic extraction method to extract the fish oil from eel due to the cavitation phenomena applied in the ultrasonic extraction method. Ultrasound is an efficient non-thermal alternative. Ultrasonic cavitation creates shear forces that break cell walls mechanically and improve material transfer. This effect is being used in the extraction of liquid compounds from solid cells (solid liquid extraction). In this case, the compound to be dissolved into a solvent is enclosed in an insoluble structure. In order to extract it, the cell membrane must be destructed. For this, ultrasound is faster and more complete than maceration or stirring. The particle size reduction by the ultrasonic cavitation increases the surface area in contact between the solid and the liquid phase significantly (Singh *et al.*, 2002).

CHAPTER 2

LITERATURE REVIEW

2.1 Eel

The eel, scientifically known as Monopterus Albus, is included in the fish genus. The body is long and its head is rounded, with the presence of gills. 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' (crevalle) 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. Hence, 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. According to traditional medicine practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (Nurul, BERNAMA 6 August 2007). There are the figure of eel breeding in a pond is illustrated in Figure 2.1 below:

Figure 2.1: The eel breeding in pond

Although its impacts are unknown, Monopterus albus predaceous and generalized feeding habit poses a potential threat to native fish, frogs and aquatic invertebrates. In Florida and Georgia, introduction is likely due to an aquarium release or a fish farm escape or release. It is believed that Monopterus albus was originally brought to Hawaii by Asian immigrants as a food fish. Description Aguirre and Poss (1999) state that the body of Monopterus albus is more or less cylindrical; the tail is compressed, tapering to a slender point much shorter than the trunk. Scales are absent. The snout is bluntly rounded, and 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. Body colour is slate brown above and white to light-brown below with small, dark spots on its sides and occasionally on the ventral surface. Monopterus albus may grow to a metre in length, but most grow to between 25cm and 40cm. The habitat for Monopterus albus lives in muddy ponds, swamps, canals, and rice fields, where it burrows in moist earth in the dry season, surviving for long periods without water.

According to Bricking (2002), the impacts of Monopterus albus are uncertain, however, they are likely to affect the population size of their prey, as well as the availability of food sources for larger fish, turtles, frogs and wading birds.

Monopterus albus consumes crayfish, tadpoles, small fish, and worms. They can eat some larger prey as well, by grabbing them with their mouths, and spinning until they are torn in half. Monopterus albus may also play a role in altering the habitat beneath ponds and marshy regions where they burrow nests to wait out dry seasons. Declines in native centrarchids from some areas of the United States have been attributed to this species (Nico, 1999).

2.1.1 Benefit of Eel

According to Rosli, who has a restaurant at Pantai Seri Tujuh in Tumpat, said that initial not many knew about the eel dishes. He comes out with his rather unique eel delicacies for example eel laksa and paprik eel. One of the customer said the eel dishes were appetizing and eel's flesh was soft and tasted good, just like that of the catfish. Another customer picked the eel soup for lunch and he suggest eel based dishes should be promoted to the public because more people should know the deliciousness and nutritional benefits that an eel dish can offer.

Despite there is no commercial breeding of eels, those who rear this fish can reap handsome returns due to its high market demand. According to Rosli, his restaurant used some 15 to 20 kg of eels a day and he never had problems obtaining the supply. Eels caught in the wild are more expensive than those reared. This is due to the fact that the former has more nutritional values due to feeding in their own natural habitat. During the normal days, the wild eels are priced at between RM8.00 and RM11.00 a kg. However during the monsoon season, the price can shoot up to RM14.00 due to the difficulty in catching them. For eels bred in captivity, their price is between RM6.00 and RM7.00 a kg. In Kelantan during the yesteryears, those who ate eels were considered as "poor" as they were thought to be unable to afford the marine fish. Nowadays, the eel dishes, particularly the soup, are more expensive than the other fish dishes. As more people are now aware of its

nutritional benefits, it is no surprise that the price of this freshwater fish is getting more expensive (Nurul, 2007).

2.2 Fish Oil

Fish oil is oil that is taken from the tissues of fatty fish. Fish oil analysis indicates that there are a number of fish that are rich in Omega 3 fatty acid. These fatty acids are good fats that body needs in order to function properly. These fish have Omega 3 fatty acids are include salmon, mackerel, tilefish, shark and swordfish. The fish oil analysis also indicates that fish oil can help maintain cardiovascular health, aid diabetes and arthritis, and prevent certain types of cancer (Omega, 2006). There are some description about Omega 3 and their benefits.

2.2.1 Benefits of Omega 3 Fatty Acids

Omega 3 essential fatty acids were discovered in the 1970's, and have been studied in thousands of clinical trials. There is overwhelming evidence that is important for good health and protecting against disease. Omega 3 fatty acids are a form of polyunsaturated fat which is one of four types of fat that bodies derive from food. The other three are cholesterol, saturated fat, and monounsaturated fat). There are three main types of Omega 3 fatty acids. The first two, EPA (eicosapentaenoic acid) and DHA (docohexanoic acid), are both found in cold-water fish, such as salmon, tuna and mackerel. Fresh seaweed is the only plant food that contains much EPA or DHA. The third type of Omega 3 called ALA (alpha-linolenic acid) is found in flaxseeds, its may in fact not actually be getting these valuable substances (http://www.aboutomega3.com, 21 August 2008). The chemical structure of EPA and DHA are illustrated as Figure 2.2 below: