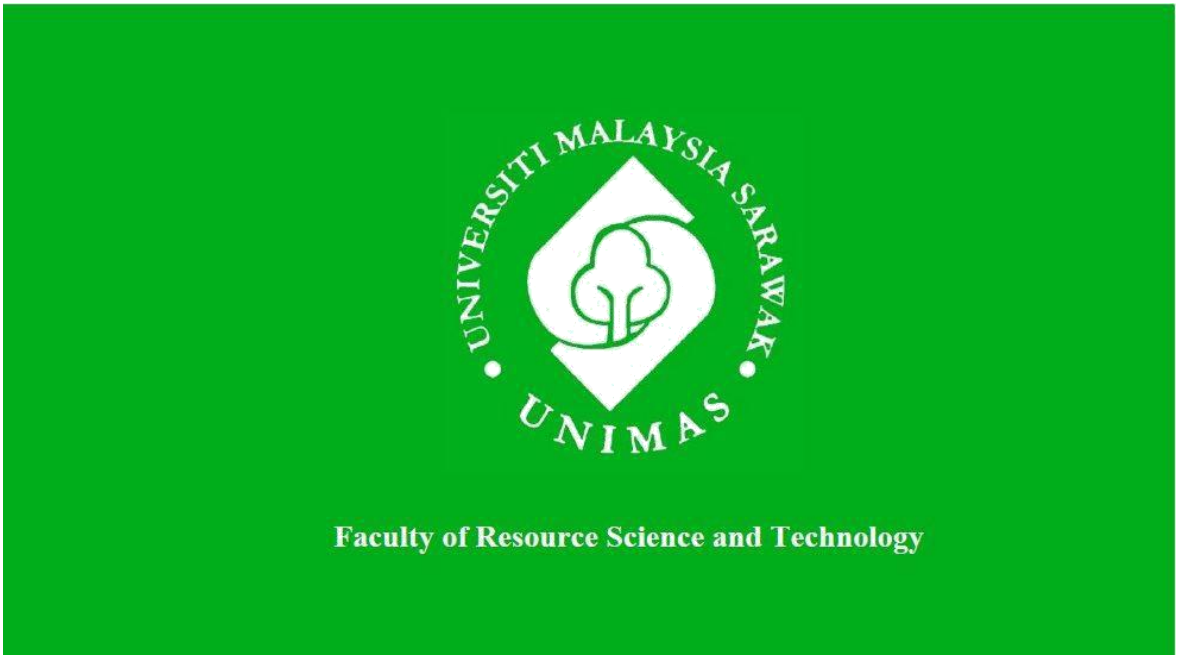

FATTY ACIDS COMPOSITION IN OILS FROM SEVERAL SPECIES OF *Tetraodontidae* (PUFFER FISH)

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Bachelor of Science with Honours
(Resource Chemistry)
2015

FATTY ACIDS COMPOSITION IN OILS FROM SEVERAL SPECIES OF *Tetraodontidae* (PUFFER FISH)

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A Final Year Project report is submitted in partial fulfilment of the requirements for the award of the degree of Bachelor in Resource Chemistry (Resource Chemistry)

Department of Chemistry

Faculty of Resource Science & Technology,

UNIVERSITI MALAYSIA SARAWAK

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LIST OF ABBREVIATIONS

AA	arachidonic acid
ALA	alpha-linoleic acid
CAD	coronary artery diseases
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FAMEs	fatty acid methyl esters
FAO	food and agriculture organization
FID	flame ionization detector
GC	gas chromatography
GC-FID	gas chromatography-flame ionization detector
GLA	γ-linoleic acid
HCA	hierarchical cluster analysis
IFMR	institute of fishery and marine research
LA	linoleic acid
LSD	least significant differences
MANS	malaysian adult nutrition survey
MUFA	monosaturated fatty acid
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
TFA	trans fatty acid

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FATTY ACIDS COMPOSITION IN OILS FROM SEVERAL SPECIES OF *Tetraodontidae*

(PUFFER FISH)

ABSTRACT

Polyunsaturated fatty acids (PUFAs) are well known with its various benefits to human health especially in reducing cardiovascular diseases, reducing the potential of diabetes and many more. This study was carried out to determine the fatty acids composition from several species of puffer fish such as *Lagocephalus lunaris, L. spadiceus, L. sceleratus, Xenopterus naritus, Ostracion nasus* and *Chelonodon patoca*. *C. patoca* has the highest value of ash and moisture content with 80.41% and 4.25%, respectively while *L. lunaris* has the highest total organic matter with 97.47 % from the proximate analysis. In general, liver contain the highest oil composition compared to muscle tissue as more fatty acids compound detected in the liver. This study also revealed that monounsaturated fatty acids with cis-10-heptadecenoic acid (C17:1) is the most compound formed. The chemometric analysis on fatty acids data revealed that *L. spadecius, X. naritus* and *O. nasus* species showed large similarity while *L. sceleratus* was very distinct to the other species in terms of fatty acid composition.

Keywords: puffer fish, polyunsaturated fatty acid (PUFAs), chemometric, proximate analysis, liver.

ABSTRAK

Rangkaian asid lemak tidak tepu (PUFA) terkenal dengan pelbagai manfaat untuk kesihatan manusia terutama dalam mengurangkan penyakit kardiovaskular, mengurangkan potensi kencing manis dan lain-lain. Oleh itu, kajian ini telah dijalankan untuk menentukan komposisi asid lemak dari beberapa spesies ikan buntal seperti *Lagocephalus lunaris, L. spadiceus, L. sceleratus, Xenopterus naritus, Ostracion nasus* dan *Chelonodon patoca. C. patoca* mempunyai kandungan abu dan kelembapan tertinggi dengan 80.41% dan 4.25%, masing-masing, manakala *L. lunaris* mempunyai jumlah bahan organik yang paling tinggi dengan 97.47% daripada analisis proksimat. Secara umumnya, hati mengandungi komposisi minyak tertinggi berbanding tisu otot di mana lebih komposisi asid lemak dikesan. Kajian ini juga menunjukkan bahawa asid lemak mono tidak tepu dengan cis-10-heptadecenoic asid (C17: 1) adalah sebatian yang paling banyak ditemui. Analisis kemometrik ke atas data asid lemak mendedahkan bahawa spesies *L. spadecius*, *X. naritus* and *O. nasus* menunjukkan persamaan yang besar manakala *L. sceleratus* menunjukkan perbezaan berbanding spesies lain dari segi komposisi asid lemak.

Kata kunci: ikan buntal, asid lemak poli tidak tepu (PUFA), kemometrik, analisis proksimat, hati.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Human always consumes fish in daily life. According to Norimah *et al.* (2008), the familiarity of consuming fishes among people in the remote and city area was rather great at 51% and 34%, based on the data obtained from the survey conducted by the Malaysian Adult Nutrition Survey (MANS) of 2008. The most predominant part consumes by human is the muscle tissue of the fish as it contains a lot of nutrients. In fish intake as a diet, the importance of nutrition related to its benefits of fatty acid profile (Sidhu, 2003). People often attracted to the outward perfection such as freshness, flavour, availability and other physical factors instead of the nutritional content and normally, little concerned about the fatty acid compositions in choosing the type of fishes for diet (Hearn *et al.,* 1987).

Generally, it is said that the composition of fatty acid might be different from one fish to the others (Muhamad and Mohamad, 2012). Several factors such as fishing season, place of origin, size, sex, and breeding season would influence the composition of total lipid and fatty acid in various fish (Luzia *et al*., 2003).Thus, it is important to be aware of the different nutrient contents in fishes, especially fatty acid content, which is related to various health problems effects (Abd Aziz *et al*., 2013). The omega-3 and omega-6 fatty acid which can be found in fish tissue, increasing attention because it would have positive effects on cardiovascular problems and certain types of cancer (Iwasaki *et al*., 2011).

A study conducted by Simopoulos (2002), reported that the person taking fish in moderate amounts, managed to avoid from primary cardiac arrest comparing to the person that did not consume fish. This fact is assigned in the lipid content in aquatic animals,

mainly fish which is rich in polyunsaturated fatty acid (PUFAs) such as omega-3 and omega-6 acids with the fact that PUFA cannot be made by the body but must be consumed daily (Wagner, 2012). The omega-3 fatty acid can be obtained through the intake of fish oil because the oils contain high composition of eicoesapentaenoic acid (EPA) and docoesahexaenoic acid (DHA) that can stimulate the production of good health (Guil-Guerrero *et al*., 2011). EPA and DHA that only can be found in fish and seafood are identified to be beneficial in the avoidance of human coronary heart disease (Leaf and Weber, 1988).

In this study, fish oil was obtained from the muscle tissue as well as the liver of *Tetraodontidae* (puffer fish) in order to determine the fatty acid composition and to show its benefits to the consumer. Puffer fish as food intake is rarely found among Malaysians and considered as trash fish but considered as a delicacy in certain places. *L. lunaris, L. sceleratus* and *L. spadiceus* are the most common species in Malaysia that always being consumed by some locals in the diet which lead to various poisoning cases (Kan et al., 1987) while *X. naritus* is considered a delicacy by the local community.

1.2 Problem Statement

Fatty acids originate from many aquatic animals, especially fishes, including freshwater and marine fishes. This study is focuses on the *Tetraodontidae* (puffer fish) to determine the fatty acid composition in oils. Numerous studies had been carried out to determine the fatty acid composition in freshwater fishes, for example *Channa striatus* (Haruan), *Pangasius hypothalamus* (Patin) and *Clarias macrocephalus* (Keli) and marine fishes such as *Tenualosa toli* (Terubok), *Rastrelliger kanagurta* (Kembong) and *Stolephorus baganensis* (Bilis) (Muhamad and Mohamad, 2012). The fatty acid

composition in puffer fish from the sea around Malaysia is scarcely done and puffer fish is abundant, considered as trash fish with no economic value. Therefore, the present study revealed the proximate composition and fatty acid level from several species *Tetraodontidae* (puffer fish) to determine its nutritional value in order to meet the demand of fish consumption and help consumers in choosing fish based on its nutritional value.

1.3 Objectives of the Project

The objectives of the study were:

a) To perform the proximate analysis, such as ash content, moisture content and total organic matter from liver and muscle of several *Tetraodontidae* species.

b) To isolate and analyze fatty acids in liver and muscle oils of several *Tetraodontidae* (puffer fish) species from the coast of Sarawak.

c) To quantify the polyunsaturated fatty acids (PUFAs) in the oil extracted from several species of *Tetraodontidae* (puffer fish).

d) To carry out chemometric analysis based on fatty acids in muscle and liver oils from several species of *Tetraodontidae*.

CHAPTER 2

LITERATURE REVIEW

2.1. Fatty Acids Content in Fishes

Fatty acid is a carboxylic acid with a long aliphatic chain, which is categorized as saturated or unsaturated fatty acid, depending on the number of hydrogen atoms and the double bond present. Fatty acids commonly derived from triglycerides and considered as free fatty acid when they are not attached to other molecules. According to the FAO of United Nations (2010), fatty acid can be classified into three groups based on their chemical structure as follows:

a) Saturated fatty acid (SFA, no double bond)

b) Monounsaturated fatty acid (MUFA, consist of one double bond)

c) Polyunsaturated fatty acid (PUFA, consist of 2 or more double bond)

Hames and Hooper (2001) reported that the properties of fatty acid are determined by the length of the chain and the number of double bond present. Melting temperature increases with decreasing the number of double bond and longer chain length. Melting temperature for shorter chain length fatty acid is lower than the longer chain while the saturated fatty acid has significantly higher melting temperature than unsaturated fatty acid with the same chain length. Therefore, PUFA have even lower melting temperature compared to the others.

Aquatic animals have been known as a basis of fatty acid composition, including freshwater and marine fish. The fatty acid content in fish has many benefits if being consumed in the appropriate amount which commercially used as food supplements. The research done by Muhamad and Mohamad (2012) was conducted on the fatty acid

composition of selected Malaysian marine fishes and freshwater fishes revolve about a comparative study between the individual species. Fatty acid profile data are important for food researcher and nutritionist in order to help them in formulation, processing and product advance as the total fatty acid compositions were determined simultaneously in the fish oil samples. Based on the result obtained, the fatty acid composition between both fishes showed similar patterns, but marine fishes contained more unsaturated fatty acids such as *EPA* and *DHA* (Muhamad and Mohamad, 2012). From the findings, n-3 PUFA would be appropriate for inclusion in the formulation of highly unsaturated low-fat diets that help to prevent various kinds of diseases.

Dejana *et al*. (2013) concluded that there are significant changes occur regarding the fatty acid composition of carp throughout the studying period that examine the change in growth. The research was done to investigate the changes in the proximate and fatty acid composition in the common carp. The presence of natural food on the carp farm affected the fatty acid composition with the exception of supplementary feeding during rearing. The extruded feed given as an additional feeding affected the amounts of MUFA and n-6 PUFA presence, and decline in the amounts of vital nutrition such as n-3 PUFA. Based on the result obtained, the quality of supplementary feed has to be upgraded to promote a better nutritional quality of the product.

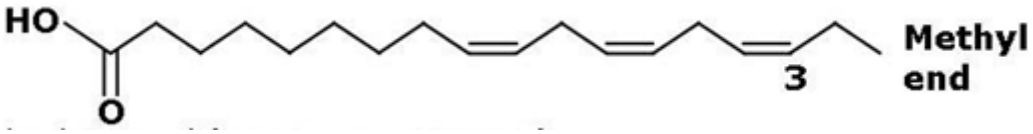
2.2. Introduction to Polyunsaturated Fatty Acids (PUFAs)

Fatty acids that mainly consist of omega-3 and omega-6 are known as PUFA which are important in human health. In terms of health, it is recommended to increase the consumption of food which is rich in n-3 PUFAs and to reduce the intake of SFAs and Trans fatty acid (TFAs) because TFAs affect cholesterol levels, increase the risk of

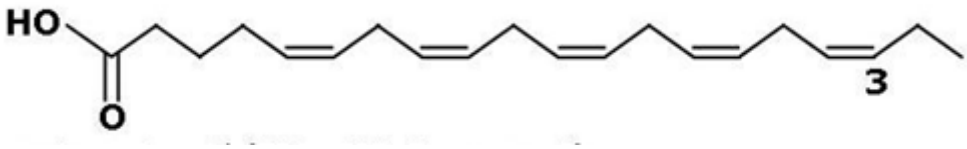
suffering coronary artery and heart diseases (Kummerow, 2009; Backholer and Peeters, 2012). Both n-3 and n-6 fatty acid are vital nutrients which cannot be released by the body but must be obtained in the diet. Throughout evolution, n-3 and n-6 fatty acid have been part of the human diet, whether through the consumption of fish oil or other natural sources of food that can provide a good alternative of PUFA as supplements.

Kapoor and Patil (2011) reported that the plant source offers alternative way in obtaining PUFA in the daily diet and can be established as supplements. The PUFA content such as EPA and DHA has been recommended in promoting human health and fish fecundity besides having a therapeutic effect on human physiology (Ackman, 1988; Saito *et al.*, 1997). Razak *et al.* (2001) have reported that the health agencies are recommending the tropical fishes which are rich in AA and DHA as infant food supplements. In his study, the fish oil from the body and head of *Monopterus albus* (a tropical freshwater fish) show that palmitic acid, oleic acid, AA and DHA were the dominant fatty acids.

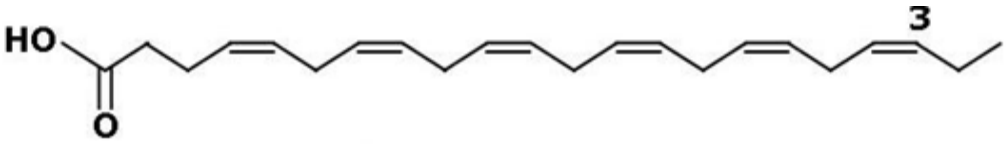
The position of the first double bond between the two groups of PUFA indicates the difference among them. Omega-3 has the first double bond occurs on the third carbon atom while omega-6 has the first double bond in the sixth carbon atom, from the methyl end of the chain. The main components of n-6 PUFA includes linoleic acid (LA), γ-linoleic acid (GLA) and arachidonic acid (AA) while n-3 PUFA mainly consist of alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Figure 2.1).



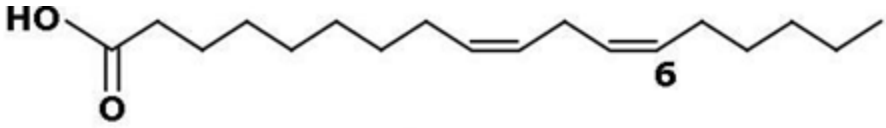
(ALA,C18:3, n-3)



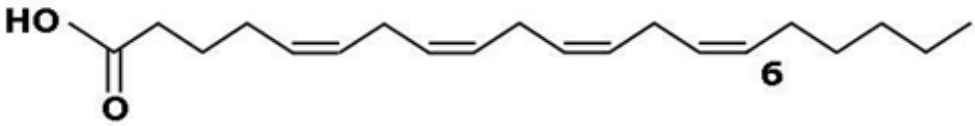
(EPA,C20:5,n-3)



(DHA,C22:6,n-3)



(LA,C18:2,n-6)



(AA,C20:4,n-6)

Figure 2.1: Chemical structures of several Omega-3 and Omega-6

2.3. Importance of Polyunsaturated Fatty Acids (PUFAs)

Numerous studies had been conducted in order to prove that the consumption of fish in daily diet can help in improving health. However, the result obtains are not consistent yet because of the limited evidence, especially for fatty acid composition, which

is associated to health related effects. Kar and Webel (2012) have presently providing an indication about the use of Omega-3 fatty acids in the treatment of Coronary Artery Diseases (CAD) and the potential benefits.

Several clinical tests have been done to evaluate the omega-3 fatty acids for CAD which is evaluated on 2,033 male patients and the patients were separated into 3 groups for the tests based on either fat consumption, fiber consumption or fatty fish consumption (Burr *et al*., 1989). Based on the data obtained, the group of individual who take fatty fish in their diet showed 29% decline in mortality in comparison to the other study groups. The incidence of cardiac death is due to lack of fatty fish intake. The study proved that the PUFA content in fishes can reduce the mortality rate with the assumption that the observed benefits of fatty fish intake have resulted in decreasing of sudden cardiac death.

Many studies have been conducted to prove that PUFA had largely beneficial values. Prato and Biandolino (2012) have successfully identified that the fish from Mediterranean Sea are important for coronary heart disease prevention based on the characterization from high composition of EFA such as EPA, DHA and n-3/n-6 ratios. A variety of data relate to the total PUFAs were found among the Mediterranean fish species where the highest amount of PUFAs was identified in the leanest fish and sea bass. Besides, the result obtained based on the epidemiological and experimental research agreed that the activity of the n-3 PUFAs encouraging in the prevention of cardiovascular diseases (Sidhu, 2003).

The high amount of lipid content in the local fishes might be commercialize as a fish oil capsule in order to meet the market demand. The findings from the previous study has been developed more to create a new market for fish oil as it can be used for food and dietary supplement consumption in daily life. Fish oil supplementation can help reduce muscle atrophy suggested to be the new evidence on the benefits of consuming the fish oil.

Smith *et al*. (2011) found that the stimulation of muscle protein synthesis in adults is due to the fish oil which contains omega-3 fatty acid supplementation. You *et al*. (2010) also successfully proved that the consumption of fish oils in the diet may alleviate muscle loss during immobilization. From these findings, it is recommended significant use of fish oil supplements in immobilized injured athletes.

In another point of view, study conducted by Abd Aziz *et al*. (2013) reported that most of the marine fish and shellfish have suitable ratio of fatty acids which higher than the level in the PUFA supplement recommendation, Menhaden oil. The results presented that marine fish and shellfish give numerous advantages in terms of health if taken frequently and may have potential in various nutraceutical purposes (Abd Aziz *et al.,* 2013).

2.4. Toxicity of Puffer Fish

Toxin that contain in the puffer fish is known as tetradotoxin. The toxin is heat stable and does not decompose through freezing, drying and cooking (Silva et al., 2010). Therefore, puffer fish poisoning due to consumption of toxic species is familiar. Several actions such as setting restricted rules has been taken by the several countries to ban the ingestion of any products related to puffer fishes but consumers are still exposed to the threat of this species due to the illegal consumption. Information and knowledge about toxic and non-toxic puffer fish species are still limited in Malaysia as many cases of puffer fish poisoning in human were reported.

Study conducted by Monaliza and Mohamad (2011) reported *L. lunaris* and *L. sceleratus* as toxic puffer fish. Man *et al.* (2010) also reported that *L. spadiceus* as a non-toxic among the *Lagocephalus* species which is safe for human consumption. According to Man *et al.* (2010), *L. lunaris* is considered notorious species among the three groups of

Lagochephalus as it contains potent neurotoxin in their muscle. However, it is problems rises when it comes to differentiate between all this three species as they have similar external morphology. Due to the misidentification which of the puffer fish considered safe, many puffer fish poisoning occurred mainly in Asian region as it has been a delicacy among human (Lin *et al*., 2002).

The first case was documented in Sabah due to the consumption of puffer fish which include 4 intoxications and 9 fatal cases (Lyn, 1985; Kan *et al.*, 1987). Several cases of puffer fish poisoning occurred in Bangladesh due to low cost and low availability of food which always been eaten especially by the poor people. 37 people from eight poor families were admitted which eight of them died after several days cause by the consumption of puffer fish (Rahman, 2005). This incident occurred due to lack of knowledge especially for rural communities. From the study conducted by Taguchi (1982), puffer fish poisoning also reported in Japan even it seldom found in temperate waters due to misidentified with non-toxic puffer, *L. wheeleri*, due to the similarity in external morphology. Nowadays, the habit of eating puffer fish especially in Sarawak is slowly spread among people and some species can simply be seen in the local market. Further analysis must be done on puffer fish species and described their morphological characteristics including their diet composition to provide basic knowledge in order to differentiate a toxic puffer fish from non-toxic.

A study carried out by Mohd *et al.* (2014) on 14 species of puffer fish has classified 11 species belong to the *Tetraodontidae* family; while 2 species belong to the *Diodontidae* family and only one species categorized in *Ostraciidae* family. *Tetraodontidae* family are toxic while other species of *Diodontidae* family and *Ostraciidae* family are weakly toxic. The study also reported that the tetradotoxin amount is the highest in liver and muscle and the lowest in the skin. Nunez-Vazquez *et al*. (2000) proposed that the ingestion or

preparation as food required special guidelines to prevent poisoning. In Malaysia, puffer fish is classified as trash fish with no market value and were not consumed by local people at certain places as the information on the safety is still inadequate.

2.5 Family of *Tetraodontidae* (puffer fish)

The family of *Tetraodontidae* consist of many familiar species which has large external spines on its body and relatively strong teeth in each jaw. Thus, *Tetraodontidae* name is come from (tetras= four; odontos= tooth). The puffer fish get its name because when it feels threatened it puffs up doubles its actual size by swallowing water or air (Rita, 2012). There are many different types and species of puffer fish and usually found in coral reefs in the Indian, Pacific and Atlantic Oceans (National Geographic, 1996). Puffer fish can be found in marine and freshwater with the habit of consuming invertebrates and algae as their sources of food. However, the puffer fish have a toxin inside of their body that causes other fish that eat them to die or become ill. According to Oliveira *et al*. (2006), there are 185 species and 28 genera of puffer fish in the family of *Tetraodontidae*. In Malaysia, there are at least 16 species of puffer fishes have been recorded (Cantor, 1849; Scott, 1959).

2.5.1 *Lagochephalus sceleratus*

L. sceleratus also known as silver cheeked toadfish which populates the tropical Indian and Pacific Oceans. Sometimes, it is also called green spotted puffer fish as it has finely distributed spots at the dark brown colour body. The first recognised species of *L. sceleratus* from *Tetrodontidae* family was found by Johan Gmelin, the German scientist in 1789. According to Yaglioglu *et al.* (2011), *L. sceleratus* species can be found at muddy