



Faculty of Resource Science and Technology

PROXIMATE, FATTY ACIDS AND MINERAL COMPOSITION
IN FISH OIL FOR SELECTED SPECIES OF
SERRANIDAE FROM SARAWAK

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(35077)

Bachelor of Science with Honours
(Resource Chemistry)
2015

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This Final Year Project Report is submitted in partial fulfillment of the requirements the
Degree of Bachelor of Science with Honours in Resource Chemistry

Program of Resource Chemistry
Department of Chemistry
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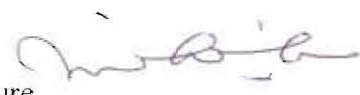
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LIST OF ABBREVIATIONS

SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
ALA	α -linoleic acid
AA	Arachidonic acid
LA	Linoleic acid
FAME	Fatty acid methyl ester
GC-FID	Gas chromatography flame ionization detector
GC	Gas chromatography
IUCN	International Union for Conservation of Nature
ICP-OES	Inductively coupled plasma-optical emission spectrometer

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PROXIMATE, FATTY ACIDS AND MINERAL COMPOSITION IN FISH OIL FOR SELECTED
SERRANIDAE SPECIES OF SARAWAK

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ABSTRACT

A comparative study on the proximate, fatty acids and mineral composition of *Epinephelus coioides*, *E. areolatus*, *Cephalopholis sonnerati* and *C. miniata* were carried out. The results revealed the presence of moisture content ranging from 76.33 to 82.60 %, ash 4.84 to 5.70 % and total organic matter 5.64 to 6.62 %. The composition of the extracted oil had been analysed using the GC-FID. The composition fatty acids showed that total monounsaturated fatty acid (MUFA; 50.65-57.84 %) was the highest followed by saturated fatty acid (SFA; 22.33-29.19 %) and polyunsaturated fatty acid (PUFA; 19.14-24.87 %) in both muscle and liver. Stearic acid and palmitic acid for SFA, cis-10-heptadecenoic acid and elaidic acid for MUFA and alpha-linolenic acid and docosahexaenoic acid (DHA) for PUFA have been identified as predominant fatty acid. The mineral analysis was done by using ICP-OES. Minerals included K (2391.5-2895.83 mg/kg), Na (507.17-1005.5 mg/kg), Mg (201.83-241.15 mg/kg) and Ca (177.30-354.10 mg/kg) while Zn, Pb, Mn, Cu and Cd were present in trace amounts.

Keywords: Proximate, Minerals, Fatty acids, MUFA, PUFA

ABSTRAK

Kajian perbandingan proksimat, asam lemak dan kandungan mineral empat jenis ikan Epinephelus coioides, Epinephelus areolatus, Cephalopholis sonnerati and Cephalopholis miniata telah dijalankan. Keputusan menunjukkan kehadiran kandungan kelembapan diantara 76.33-82.60 %, abu 4.84-5.70 % dan jumlah bahan organik 5.64-6.62 %. Komposisi-komposisi pati ikan telah ini telah di analisis menggunakan GC-FID. Komposisi asam lemak menunjukkan bahawa jumlah asam lemak mono tidak tepu (MUFA; 50.65-57.84 %) yang paling tinggi diikuti oleh asam lemak tepu (SFA; 22.33-29.19 %) dan asam lemak poli tidak tepu (PUFA; 19.14-24.87 %) di dalam kedua-dua otot dan hati. Asid stearik dan asid palmitik untuk SFA, asid cis - 10 - heptadekanoik dan asid elaidik untuk MUFA dan asid alfa - linolenik dan asid dokosahexaenoik (DHA) untuk PUFA telah dikenalpasti sebagai asam lemak paling utama. Analisis mineral telah dikenalpasti menggunakan ICP-OES Mineral termasuk K (2391.5-2895.83 mg/kg), Na (507.17-1005.5 mg/kg), Mg (201.83-241.15 mg/kg) dan Na (177.30-354.10 mg/kg) manakala Zn, Pb, Mn, Cu dan Cd dikenalpasti dalam jumlah surih.

Kata kunci: *Proksimat, Mineral, Lemak Asid, MUFA, PUFA*

CHAPTER 1

INTRODUCTION

1.1. General Introduction

Fish is consumed as food all over the world. In emerging countries, fish is the important sources of lipid and nutrients in diet of human. According to Minamy *et al.* (2014) reported that fish is the one of essential nutrient source for process of human survival. Fish communities change from one area to another because of the several factors such as water temperature, water velocity and clarity, alkalinity and available habitat (Adeniyi *et al.*, 2012). The lipid content and fatty acid composition in fish are affected by various factors such as their environment, size of fish, species, tissues and diet (Hwang *et al.*, 2004). Study by Waterman (1980) showed that the principal components of fish are water, protein, lipid and carbohydrate while Dana *et al.* (1985) study stated that the following minerals are commonly found in fish include sodium, potassium, calcium, magnesium, phosphorus, sulphur, iron, chlorine, silicon, manganese, zinc, copper, arsenic and iodine.

Nutritional composition of fish are varies greatly from one species and individual to another depending on age, feed intake, sex and sexual changes connected with spawning, the environment and season (Silva and Chamul, 2000). The nutritional important of fish consumption is associated largely with the fatty acids. Fatty acids are compose of omega-3 polyunsaturated fatty acids (PUFAs) that highly important for human health (Pourshamsian *et al.*, 2012). Diets in which unsaturated fatty acids replace the saturated ones are associated with low incidence of coronary disease. Sargent (1996) reported that by increased consumption of fish and fish products which are rich in PUFAs of the omega-3

but poor in PUFAs of omega-6 family can reduce the risk of cardiovascular diseases. According to Kandemir and Polat (2007) reported that muscle and liver are two organs involved in the lipids storage inside fish. Humans can obtain main parts of these fatty acids through consuming aquatic products.

In this research, the *Serranidae* species was studied. The common name of *Serranidae* species identify as grouper. Groupers species are extremely vulnerable to overfishing and many species are threatened worldwide (Hackradt *et al.*, 2014). Several marine species are seriously threatened by array anthropogenic actions (Lotze *et al.*, 2006). Fishing is the main human activity that effecting fish population abundance and size structure toward causing marine biodiversity loss (Hackradt *et al.*, 2014).

1.2. Problem Statements

The fatty acid and mineral composition in fish can classify into different types. This variation of the fatty acid and mineral composition are differing depending on the types of fish that been studied. The habitat, types and diet of fish was determine the amount of fatty acid and mineral composition present in that particular fish. This study was focus only on the *Serranidae* species. In Malaysia the information regarding the *Serranidae* species is still rather scanty and inadequate. Few studies and less publications deal with them because the fish is expensive. According to IUCN Red List criteria, *Serranidae* species have an elevated risk of extinction due to overfishing.

1.3. Objectives of the Study

The objectives of this study are:

- a) To carry out the proximate and mineral content analyses in liver and muscle from several species of *Serranidae* (Grouper).
- b) To determine the chemical composition of fatty acids in liver and muscle from several species of grouper.
- c) To identify the SFA, MUFAs and PUFAs in liver and muscle from several species of grouper.
- d) To perform chemometric analysis based on fatty acids content in fish oil of grouper family.

CHAPTER 2

LITERATURE REVIEW

2.1. *Serranidae*

The grouper was found in several different genera in the subfamily *Epinephelinae* in the family *Serranidae*. The definition of a grouper from the Portuguese 'garropa'. Groupers is a subfamily of *Epinephelinae* which any member of the genera *Aethaloperca*, *Alphestes*, *Anyperodon*, *Cephalopholis*, *Cromileptes*, *Dermatolepis*, *Epinephelus*, *Gonioplectrus*, *Gracila*, *Hyorthodus*, *Myteroperca*, *Paranthias*, *Plectropomus*, *Saloptia*, *Triso* and *Variola*. Currently, the subfamily of *Epinephelinae* contains 163 species (Craig *et al.*, 2011).

The taxonomy of grouper is in a state of fluctuation. According to Craig and Hastings (2007) presented a hypothesis of relationships among grouper species and proposed a reorganization of genera within the groupers. Groupers usually been treated as a subfamily of the family *Serranidae*. As turn of the 21st century, molecular techniques become well-establish methods for discerning relationship among animal groups. Early studies using these techniques highlighted novel relationship among groupers and seabasses and suggested some genera were in fact distantly related percomorph lineages and moreover *Serranidae* not a monophyletic lineage (Craig *et al.*, 2011). Groupers and remaining genera of the traditional *Serranidae* is thought that they are not allied groups. Those genera known as the subfamily *Epinephelinae*.

2.1.1. *Epinephelus coioides* (Orange-spotted grouper)

E. coioides is a well-known and common species. However, the population trend of *E. coioides* had shown decreasing (Craig *et al.*, 2011). Overfishing is the major threat to this species as it is taken not only as adults but also in large numbers as juveniles for the grow-out mariculture industry. Instead, the brackish water, increase in coastal development and habitat destruction was also threaten *E. coioides*. According to Craig *et al.* (2011) reported that *E. coioides* occupies a wide range of habitats including shallow reefs and lagoons with brackish and turbid waters in depths to at least 30 m. Juveniles are commonly found in shallow waters of estuaries over sand, mud and gravel and common mangroves. According to Matthews and Samuel (1991) stated that *E. coioides* can reaches a maximum age of 22 years. According to Craig *et al.* (2011) reported that *E. coioides* often mistaken from *E. malabarius* and *E. tauvina*. The colour patterns of all 3 species are similar but consists different of spots in their body. According to IUCN red list status (2004) *E. coioides* are considered near threatened.

2.1.2. *Epinephelus areolatus* (Areolate grouper)

E. areolatus is wide-spread and reasonably common throughout its range. Yet, the population trend of *E. areolatus* is unknown (Craig *et al.*, 2011). Fishing pressure is the major threat to this species which is taken both as adults and as juveniles also for grow-out mariculture. Habitat and ecology of *E. areolatus* is found in seagrass beds or on silty sand bottoms around isolated small rock outcrops, dead coral or soft coral in depths of 2-200 m. Juveniles are common at water depths to 80 m (Kailola *et al.*, 1993). The maximum reported age for *E. areolatus* is 15 years (Craig *et al.*, 2011). According to IUCN red list status (2008) the *E. areolatus* is least concern.

2.1.3. *Cephalopholis sonnerati* (Tomato hind)

C. sonnerati is considered to be a common species throughout its range. The population trend of *C. sonnerati* is stable. Overfishing is the major threat in *C. sonnerati*. According to Craig *et al.* (2011) reported that the habitat of *C. sonnerati* usually in deep lagoon reefs and steep outer reef slopes in depths of up to 100 m and the juveniles are often found near sponges or coral heads. According to IUCN red list status (2008) the *C. sonnerati* is least concern.

2.1.4. *Cephalopholis miniata* (Coral hind)

C. miniata is common in most coral-rich areas within its range. According to Craig *et al.* (2011) reported that the population trend of *C. miniata* was decreasing due to overfishing. The habitat of *C. miniata* in clear water of coral reefs and more often found in exposed rather than protected reef areas (Fischer *et al.*, 1990). *C. miniata* is easily confused with *C. hemistiktos* and *C. sexmaculata*. These species differ in colour pattern. According to IUCN red list status (2008) the *C. miniata* is least concern.

2.2. Fatty Acids in Fish

Human have utilized fishes as food material since several centuries ago due of several nutrients such as protein, fat, vitamin and minerals (Minamy *et al.*, 2014). The constituent of fatty acid namely saturated fatty acid (SFA) and unsaturated fatty acid contained monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) known as omega-3, omega-6 and omega-9 (Simopoulos, 2002; Kolanowski, 2005). PUFAs such as omega-3 was the fat contained in fish that can strength the brain (Simopoulos, 2002; Steffens, 2006; Fawole *et al.*, 2007).

Fish oil generally contained omega-3 such as eicopenthanoic acid and docohexanoic acid, omega-6 such as linoleic acid and arachodoneic acid and omega-9 such as oleic acid which believed have a healthy beneficial (Calder, 1996; Massaro *et al.*, 1999; Cleland *et al.*, 2003; Ruxton *et al.*, 2004). According to Lin *et al.* (2007) reported that fatty acid content of fish in liver and muscle generally reflects the fatty acid composition of the diet. The fatty acid composition in grouper muscle and liver were strongly influenced by the composition in dietary fatty acids (Wu *et al.*, 2002).

Relative liver weights did not differ among dietary groups indicated this variable was not affected by the dietary lipid composition (Wu *et al.*, 2002). Dietary lipid sources did not affect whole body composition and lipid deposition in either liver or muscle among grouper fed with various diet (Martino *et al.*, 2002). In *Epinephelus coioides*, the optimal dietary protein and lipid levels in juvenile fish were 48% (Luo *et al.*, 2004) and 10% (Luo *et al.*, 2005).

According Louly *et al.* (2011) reported on study of muscle lipid and fatty acids of three largely consumed seawater species of *Serranidae* which were *Epinephelus aeneus*, *Cephalopholis taeniops* and *Serranus scriba*. From the research reported that the lipid contents was relatively poor, ranging from 0.8% to 2.3% showing a significant seasonal dependency. Amongst the 35 fatty acid was identified, 35-51% were SFA, 21-33% MUFAs and 18-34% PUFAs. Palmitic acid was found to be the main SFA and seasonal dependent with a mean value of 30.5% for *E. aeneus*, 27.9% for *C. taeniops* and 20.9% for *S. scriba*. Amongst MUFA, oleic acid with 11-16% was the main acid in all three species. The omega-6 PUFA level was low in particular for *C. taeniops* (1.3-1.6%) and a little higher for *S. scriba* (3.6-4.2%). The three species was characterised by high amounts of omega-3 PUFA.

2.3. Mineral composition in fish

According to Shahidi *et al.* (1999) stated that the mineral composition which comprise the major element such as calcium, magnesium, sodium, potassium and trace element such zinc and iron was determined inside fish muscle. Potassium was observed to dominate other minerals in all fish samples (Adeniyi *et al.*, 2012). The variations recorded in the concentration of the different nutritional components in the fish examined could have been as result of the rate in which these components are available in the water body (Yeannes and Almandos, 2003). The ability of the fish to absorb and convert the essential nutrients from the diet and the water bodies where they live affect the nutritional components in fish (Adeniyi, *et al.* 2012).

Most of micro element were equally important in trace amounts but they tend become harmful when their concentration in the tissues exceed the metabolic demands (Ako and Salihu, 2004). Calcium was good for growth and maintenance of bones, teeth, and muscles (Turan *et al.*, 2003). Normal extra cellular calcium concentrations were necessary for blood coagulation, integrity and intracellular cement substances (Okaka and Okaka, 2001). Sodium was an activator of transport ATP-ases in animals and possibly also in plants (Adeyeye, 2005). There were also direct relationships of sodium intake with hypertension on human (Dahl, 1972). Iron was important constituent of haemoglobin (Onwordi *et al.*, 2009). The presence of zinc in the fishes could mean that the fishes can play valuable roles in the management of diabetes which result from the insulin malfunction (Okaka and Okaka, 2001).

The composition of a particular fish species often appears to vary from one fishing ground to another, and from season to season, but the basic causes of change in composition were usually variation in the amount and quality of food that the fish eats and

the amount of movement it makes. For example, fish usually stop feeding before they spawn and draw on their reserves of fat and protein. When fish were overcrowded there may not enough food to go round as well as intake will be low and composition will change accordingly.

2.4. Analytical Method of GC for Analysis of Fatty Acids

GC analysis of fatty acids was first reported by James and Martin (1952). This technique has been extensively used for the compositional determination of fatty acids (Manku, 1983; Sandra and Vanreolenbosch, 1981). Some lipids such as short chain free fatty acids can analysed by gas chromatography without derivatization. However, most of them must be derivatives before analysis because of their low volatility or poor thermal stability (Coltro *et al.*, 2005). Standard methodology for the derivatization of fatty acids is detailed by the International Union of Pure and Applied Chemistry (IUPAC) (Lee *et al.*, 1998). According to Coltro *et al.* (2005) reported that the methodology involves the alkaline saponification of extracted fat to break down their glycerides and for the subsequent esterification of the liberated fatty acids in the presence of methanol and boron trifluoride. The formed fatty acid methyl esters (FAMES) are then extracted with organic solvents and analysed by GC with flame ionization detector (FID).

CHAPTER 3

MATERIALS AND METHODS

3.1. Sample Collection & Preparation

Fresh sample of *Serranidae* species was collected from the coastal area of Sarawak. The sample was kept in a cold iced box and transported to the laboratory. On arrival at the laboratory, the fresh fish was washed immediately. Fresh fish was then placed in separated plastics bag, sealed, labelled and kept in freezer at -20°C before they been analysed. Figures 3.1 to 3.4 show the sample of *Serranidae* species that had been studied.

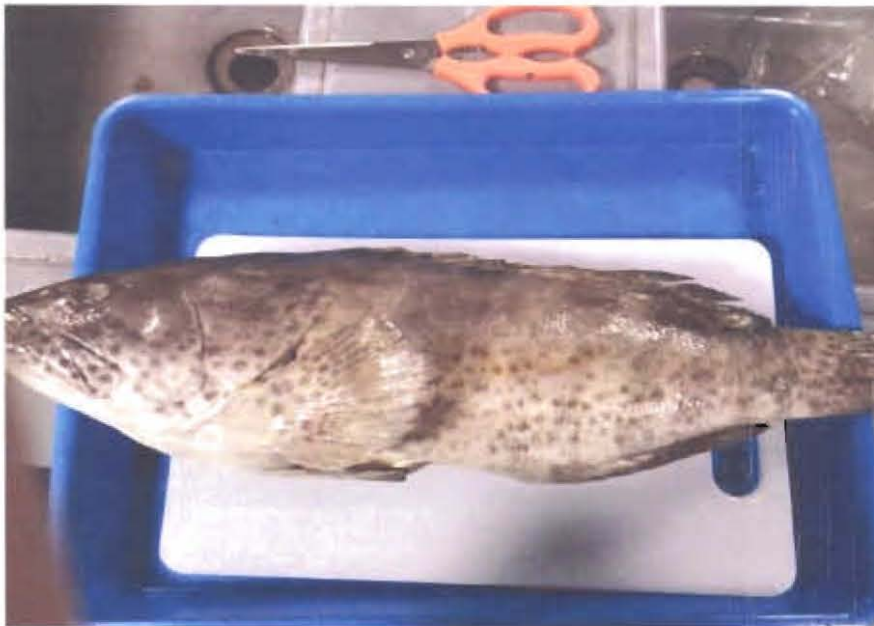


Figure 3.1 : *E. coioides* species



Figure 3.2: *E. areolatus* species



Figure 3.3: *C. Sonnerati* species