NUTRITIONAL ANALYSIS AND HAZARD RISK ASSESSMENTS OF SELECTED AQUACULTURE SPECIES IN PENANG FOCUSING ON FISH OIL CHARACTERISTICS OF GOLDEN POMPANO (*TRACHINOTUS BLOCHII*)

NAUFAL BIN ARSHAD

UNIVERSITI SAINS MALAYSIA

2024

NUTRITIONAL ANALYSIS AND HAZARD RISK ASSESSMENTS OF SELECTED AQUACULTURE SPECIES IN PENANG FOCUSING ON FISH OIL CHARACTERISTICS OF GOLDEN POMPANO (*TRACHINOTUS BLOCHII*)

by

NAUFAL BIN ARSHAD

**Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy**

August 2024

ACKNOWLEDGEMENT

All praise to the Almighty God for giving me patience, health, and knowledge for me to complete this research. I would like to express my gratitude to my supervisor, Dr Lee Lai Kuan, for patiently providing me valuable guidance, motivation and willingly in stimulating the suggestion throughout my research study. She has always been the great mentor, who is willing to share the knowledge, and constantly gives the advice and moral support. It was my pleasure to have a dedicated and hardworking supervisor and thank you again for all your supervision.

I also like to acknowledge of appreciation to my co-supervisor, Professor Dato' Gs. Dr. Narimah Samat, for her guidance during this project. Without the kind helps from her, this work might face the difficulties and would not be done as planned. Furthermore, the express of gratitude will be dedicated to the Long-Term Research Grant Scheme, which helped to fund this research.

The gratitude of expression was also extended to the staff of School of Industrial Technology, for their cooperation in administration and technical support. The warmest appreciation was also extended to my labmates and friends for sharing their pearl of wisdom during the entire course time of research. Lastly, my profound gratitude was given to my lovely family, whose always give me encourage, motivation and support. Without them, the accomplishment of this research would not possibly been achieved.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF PLATES

Page

LIST OF SYMBOLS

%	Percentage
&	And
$<$	Less than
$>$	More than
±	Plus minus
^{o}C	Degree celcius
μ	Micro
=	Equal
β	Beta
ω	Omega
κ	Kappa

LIST OF ABBREVIATIONS

µg	microgram
µL	microlitre
As	Arsenic
B	Boron
B.C.	Before christ
cAMP	Cyclic adenosine monophosphate
C	Carbon
Ca	Calcium
Cd	Cadmium
Cu	Copper
Cr	Chromium
COVID-19	Coronavirus disease 2019
d	Day
DMEM	Dulbecco's Modified Eagle Medium
DoFM	Department of Fisheries Malaysia
Eq.	Equation
eV	electron volt
FAO	Food and Agricultural Organization of the United Nation
Fe	Iron
g	Gram
h	hour
H	hydrogen
ha	Hectare
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
INFOODs	International Network of Food Data Systems
khz	Kilohertz
kJ	Kilojoule
kV	Kilovolt

KOH	Potassium hydroxide
m/z	Mass to charge
min	Minute
N	Normality
nm	nanometer
O	Oxygen
ppb	Part per billion
ppm	Part per million
ppt	Part per trillion
RM	Ringgit Malaysia
rpm	Revolution per minute
sec	second
Sp.	Species
SPE	Solid Phase Extraction
SPSS	Statistical Package for the Social Science
UV-vis	Ultraviolet visibie
USD	United State dollar
WHO	World Health Organization

LIST OF APPENDICES

ANALISIS NUTRISI DAN PENAKSIRAN RISIKO BAHAYA BAGI SPESIES AKUAKULTUR TERPILIH DI PULAU PINANG MEMFOKUS KEPADA CIRI MINYAK IKAN BAWAL EMAS (*TRACHINOTUS BLOCHII*)

ABSTRAK

Akuakultur adalah sektor pengeluaran makanan yang berkembang pesat untuk mengurangkan kelaparan global, malnutrisi, keselamatan makanan, serta deprivasi nutrisi. Kajian ini bertujuan untuk meneroka spektrum komposisi pemakanan yang komprehensif menggunakan spesis akuakultur. Nilai komoditi akuakultur yang tinggi di Pulau Pinang, Malaysia telah disenaraikan, dan kuantifikasi pemakanan serta penilaian risiko kesihatan telah dinilai. Kajian juga mengekstrak minyak ikan daripada produk akuakultur dan menilai aktiviti biologi. Enam spesies akuakultur marin, iaitu ikan nyok-nyok, bawal emas, kerapu, merah, dan siakap, dikaji secara mendalam. Semua akualkutur spesies mengandungi kandungan protein dan lemak yang mencukupi, diantara 21.1 hingga 26.9%, dan 0.4 hingga 10.7%, masing-masing. Akuakultur spesis juga menunjukkan nisbah asid amino perlu (EAA) kepada jumlah asid amino (TAA) yang setanding dengan nilai rujukan FAO (50%), dengan kandungan leusina dan lisina menunjukkan EAA yang paling penting. Profil asid lemak menunjukkan bahawa siakap dan kerapu kaya dengan asid lemak tak tepu (PUFAs), terutamanya asid dokosaheksaenoik (DHA). Bawal emas dan ikan merah mengandungi nilai riboflavin dan piridoksina yang tertinggi. Indeks kualiti pemakanan menunjukkan bahawa spesies berada dalam nisbah yang dibenarkan (<1) untuk kedua-dua indeks aterogenik dan trombogenik. Semua spesies berpotensi menyumbang lebih daripada 10% protein, vitamin D_3, dan B_2, K, Mg, P, dan kurang daripada 1% vitamin A, E, B_6, Na, dan Ca untuk populasi dewasa dan kanak-kanak di Malaysia. Ampisilin,

streptomisin, dan tetrasiklina tidak dikesan (<1 μg kg^{-1}) dalam spesies yang dikaji. Pengambilan harian yang dianggarkan bagi mikro-mineral dan logam berat bagi populasi Malaysia berada dalam had harian yang selamat (PTDI). Penilaian indeks bahaya (HI) menunjukkan bahawa semua spesis berpotensi untuk menunjukkan risiko bukan karsinogenik bagi dewasa dan kanak-kanak dalam pengambilan yang berpanjangan. Semua spesis yang dikaji menunjukkan kebarangkalian yang tinggi terhadap risiko kanser tahap serdehana disebabkan oleh pendedahan kepada kepekatan kadmium (Cd) jikalau pengambilan secara harian lebih dari tempoh 30 tahun. Minyak bawal emas (GPO) diekstrak melalui tiga kaedah pengekstrakan yang berbeza, iaitu pengekstrakan pelarut (SE), pengekstrakan tekanan hidrolik (HPE), dan kaedah hidrolisis enzimatik (EHE). HPE menghasilkan GPO yang berkualiti tinggi dengan jumlah PUFAs tertinggi serta dipilih untuk menjalani proses penulenan. GPO yang ditulen (RGPO) menujukkan peningkatkan nilai peroksida dan *p*-anisidin tetapi penurunan terhadapan kandungan kelembapan dan logam berat. RGPO juga mengandungi jumlah fenolik sebanyak sebanyak 123 mg asid galik kg^{-1}, kandungan karotenoid (4 mg 100 g^{-1}), vitamin A (95 μg 100g^{-1}), dan vitamin E (3300 μg 100g^{-1}). RGPO menunjukkan nilai IC_{50} sebanyak 143.2 mg mL^{-1} untuk aktiviti menangkap DPPH dan 61.37 mg mL^{-1} untuk aktiviti penghalang α-amylase. Kesan antiproliferasi terhadap $HePG_2$ ditunjukkan pada kepekatan 50 μg mL^{-1}, yang menghasilkan peratusan keboleh hayatan sel sebanyak 61.1. Kesimpulannya, kajian ini mempunyai kepentingan dalam memberikan pandangan kepada pengguna mengenai komposisi pemakanan dan risiko kesihatan yang berkaitan dengan spesis akuakultur. RGPO juga dikenalpasti sebagai minyak kesihatan yang berpotensi bagi penggunaan manusia.

NUTRITIONAL ANALYSIS AND HAZARD RISK ASSESSMENTS OF SELECTED AQUACULTURE SPECIES IN PENANG FOCUSING ON FISH OIL CHARACTERISTICS OF GOLDEN POMPANO (*TRACHINOTUS BLOCHII*)

ABSTRACT

Aquaculture is a rapidly growing food production sector to alleviate global hunger, malnutrition, food security and nutrition deprivation. This study is aimed to explore a comprehensive spectrum of nutritional composition using aquaculture species. High commodity values aquaculture species in Penang, Malaysia were selected, and their nutritional quantification and health risk estimation were assessed. The study also extracted fish oil from the aquaculture products and evaluating their biological activities. Six marine aquaculture species, namely bigeye trevally, golden pompano, golden snapper, grouper, red snapper, and seabass were studied extensively. All cultured species contained adequate protein and lipid content, ranging from 21.1 to 26.9%, and 0.4 to 10.7%, respectively. Essential amino acid (EAA) to the total amino acid ratio (TAA) of culture species was comparable to the FAO reference values (50%), with leucine and lysine demonstrated the most important EAA. Fatty acid profiling showed that seabass and grouper were rich in polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA). Golden pompano and red snapper contained the greatest values of riboflavin and pyridoxine. The nutritional quality indices indicated that the cultured species were within the permissible ratios (<1) for both atherogenic and thrombogenic indices. All cultured species potentially contributed more than 10% of protein, vitamin D_3, and B_2, K, Mg, P, and less than 1% of vitamin A, E, B_6, Na, and Ca for both the adult and children populations in Malaysia.

Ampicillin, streptomycin, and tetracycline were undetected (<1 μg kg^{-1}) in the cultured species. The estimated daily intake of microminerals and heavy metals for the Malaysian population was within the tolerable daily limit (PTDI). Hazard index (HI) assessment indicated that all cultured species were potent to demonstrate non-carcinogenic risk for both adult and children in prolonged consumption. All species studied predicted to pose a moderate carcinogenic risk due to exposure to cadmium (Cd) if daily consumption over a 30-year period. Golden pompano oil (GPO) was extracted via three different extraction modes, namely the solvent extraction (SE), hydraulic pressure extraction (HPE), and enzymatic hydrolysis extraction (EHE). HPE produced high quality GPO with the highest amount of PUFAs and subjected to the refinement process. The refined GPO (RGPO) elevated peroxide and p-anisidine values, but reduced moisture and heavy metals content. RGPO contained an adequate amount of total phenolic content (123 mg Gallic acid kg^{-1}), total carotenoid content (4 mg 100 g^{-1}), vitamin A (95 μg 100g^{-1}), and vitamin E (3300 μg 100g^{-1}). RGPO exhibited IC_{50} values of 143.2 mg mL^{-1} for DPPH scavenging activities and 61.37 mg mL^{-1} for α-amylase inhibitory activity. The anti-proliferative effect of on $HePG_2$ was demonstrated at a concentration of 50 μg mL^{-1}, resulting in a cell viability percentage of 61.1. In conclusion, this study holds significance by providing valuable insights to the consumers with regards to the nutritional composition and health risks associated with aquaculture species. RGPO could serve as a potential functional oil for human consumption.

CHAPTER 1

INTRODUCTION

1.1 Background

The median projection predicts the global population will grow to over 9.7 billion by 2050, and food demand is expected to rise even faster due to the emergence of a more significant proportion of high-income societies who typically consume more animal protein than low-income societies (Kharas, 2010). The annual global consumption of seafood products per capita has doubled over the last 50 years, and more than 3.1 billion people consumed at least 20% of their animal protein via seafood (FAO, 2016). Seafood protein is an essential nutritional component in many countries, particularly low-income ones (Guillen et al., 2019). In parallel, food security has become of utmost importance to third-world countries, where a large percentage of their population is low in income and a high share of total household expenditure is devoted to food (Jennings et al., 2016). As such, securing a sustainable food supply chain is the critical determinant for the balance of food demand and food consumption (Azra et al., 2021).

Aquaculture is one of the essential sectors that contribute to fishery products as the primary source of protein for human consumption (FAO, 2023). This sector has been the fastest-growing food production sector in the past three decades, with an annual growth rate of 6.9%. With the rapid increase in mass production, this sector is now close to overtaking the capture fisheries as the leading source of aquatic foods for human consumption and a sustainable option for attaining food security (FAO, 2022). Indeed, aquaculture is frequently promoted as an economic enhancement, substantial expansion of world trade, raising household income, and elevating urbanization

(Troell et al., 2021). At the current state, Asia is by far the largest aquaculture producer, accounting for 92% of global live-weight production in 2020, with China alone contributing 57% of total aquaculture volume and 59% of global value, respectively (FAO, 2022). Driven by a significant expansion in Asia, global aquaculture development grew broadly, including Malaysia.

In line with the Malaysia national food security plan, aquaculture has evolved substantially in the last 15 years towards cultivation techniques, species, and contribution to national income (Roslina, 2018; Samah & Kamaruddin, 2015). Numerous cultivation methods are currently being adopted, and the growth of marine cultivation is highly correlated with the domestic and international demands for these commodities (Roslina, 2018). The aquaculture industry in Malaysia engages approximately forty thousand culturists actively, and brackish water culture alone has contributed an estimated USD 0.75 billion to the industry (DoFM, 2023). Penang state, located in the northwest region of Peninsular Malaysia, is one of the biggest marine aquaculture producers, accounting for 64% of Malaysia's total brackish cage-cultured production (DoFM, 2023). The main cultivation species in Penang include seabass (*Lates calcarifer*), snapper (*Lutjanus* sp.), golden pompano (*Trachinotus blochi*), grouper (*Epinephelus* sp.), and bigeye trevally (*Caranx sexfaciatus*).

Knowledge of aquaculture nutritional composition is invaluable to unravel the links between successful cultivation, access, and nutrient intake to ensure a sustainable food supply chain. Aquatic animals are highly nutritious food that play significant roles in human health. The importance of marine animals to provide at least 20% protein and rich sources of fish oils, especially ω-3 polyunsaturated fatty acids (PUFAs), to the global population has increased the worldwide consumption of seafood products (Golden et al., 2016). In addition to its rich sources of protein and essential fatty acids, aquatic animals are densed with micronutrients, hence play a pivotal role in eradicating micronutrient deficiencies, notably in developing countries (Roos et al., 2003). Given the positive nutritional and health attributes of the aquatic species, their nutritional composition varies depending on the species, geographical origin, sources, and feeding behaviors (Taylor & Savage, 2006). Moreover, several studies have identified variations in nutrient composition among fish species and their potential contribution to deficiencies in vulnerable groups in different regions, such as India (Mohanty et al., 2016), Bangladesh (Bogard et al., 2015), and Nigeria (Egun & Oboh, 2022).

Despite its high nutritional values, aquatic products remain vulnerable to a range of safety risks attributed to their cultivation practices and environmental hazards (Hussan & Gon, 2016). Epidemiological studies define risk as a probability (without reference to the potential size of the adverse health impact), while hazard is interpreted as the presence of material or conditions that have the potential to cause harm or loss (Rout & Sikdar, 2017). There are 3 major broad categories of hazards that interact and impact the seafood supply chain from aquaculture subsectors: (1) chemical hazards derived from either natural or anthropogenic sources that may affect the survival rate of animals for human consumption; (2) human pathogen hazards that interact with aquatic products; and (3) animal pathogen hazards that may affect the quality of life of the products for use as seafood. Aquatic animals have a particularly intricate relationship with their environment, that prone them to easy exposure and bioaccumulating to the diverse chemical and pathogen hazards present in water, sediment, and their feeds (Stentiford et al., 2022).

This possibility risk of the aquaculture organism could be evaluated through the quantitative risk assessment based on the consumption pattern (Cohen et al., 2005).

The European Food Safety Authority outlines four steps for the implementation of risk assessment: (1) the identification of hazard compounds; (2) the characterization and identification of hazard compounds related to the reversibility, severity, and single-dose relationships; (3) the probability and exposure assessment of each identified compound for hazard compounds in a population group; and (4) the risk assessment. Globally, there are three approaches to evaluating the risk in aquaculture practice, namely, the hazard quotient, hazard index and carcinogenic evaluation (EFSA, 2010). This approach has successfully been implemented in the mariculture farm in China (Liang et al., 2016).

Aligned with the nutritional benefits and hazard risks associated with aquaculture species, fish oils represent another important component that can be derived in this fisheries sector. Fish oils are considered functional foods due to their superior content of ω-3 and ω-6 PUFAs. Mounting evidence in recent years demonstrating that these functional components are positively correlated with human health benefits, particularly in the prevention of cardiovascular diseases, cancer, and diabetes (Zhang et al., 2019). The emphasis on the benefits of ω-3 long-chain PUFAs has led to the widespread availability of purified fish oil supplements accessible in health food stores. Fatty fish such as salmon and cod are commonly used in extracting the fish oil due to the adequate amount of PUFAs (NIH, 2019a). The extraction method are crucial factors in the production of fish oils as they can influence both yields and quality. Various methods, such as wet rendering, enzymatic hydrolysis, and solvent extraction, are employed to extract the oil component. Extracted oils may contain impurities, necessitating a purification process to meet quality standards for human consumption. This purification process aims to eliminate undesirable impurities while retaining essential compounds like ω-3 and other PUFAs. Thus, the refining process is designed to achieve specific characteristics, minimizing oil losses, and maximizing the availability of beneficial constituents. The refined fish oil may possess natural compounds that not only contribute to the oxidative stability of the oil but also offer health benefits. Furthermore, oil extracted from farmed seabream and seabass exhibited cellular antioxidant activity and the highest cytotoxic effect against breast cancer cells (Fuente et al., 2022a). These findings indicate that aquaculture species could be exploited for oil production with nutritional and bioactive properties, in conjunction with circular bioeconomy concepts.

1.2 Problem statement

Globally, there is insufficient information on the nutrient composition of fish, with only 25.7% of fish species documented in the Food and Agriculture Organization's INFOODS database (FAO, 2016). Furthermore, policymakers have primarily focused on the production of commercial marine fish, often overlooking at their nutritional values. Due to the scarcity of nutritional data and limited policy attention, fish is commonly treated as a homogeneous food group in dietary planning, despite the notable nutrient content of many species that could potentially address specific nutrient deficiencies if promoted for consumption in sufficient quantities (Rifat et al., 2023). A parallel situation is evident in Malaysia, where only 8 published articles have documented the nutritional composition of marine fish in the country, and none of the studies focus on aquaculture species (Airina & Jamaluddin 2012; Babatunde et al., 2020; Irwandi & Farida, 2009; Nurnadia et al., 2013; Siong et al., 1989.; Rozaina et al., 2018; Tramice et al., 2021). Moreover, the parameters within these studies were limited to proximate analysis, mineral content, vitamin and fatty acid composition.

Despite the nutritional quality of aquaculture species, the bioaccumulation of contaminants in maricultural farm raises safety concerns as it can occur at any point in the value chain. Adverse health effects of contaminant exposure on human become practical following long-term dietary ingestion (Ling & Liao, 2007). These contaminants may stem from ecological pollution, socio-cultural habits prevailing in various regions, or malpractice in the cultivation process. Simultaneously, inappropriate cultural behavior and practices can lead to eutrophication and environmental degradation (Hussan & Gon, 2016). The concern regarding the aggressive use of antibiotics in aquaculture farms could be validated by the presence of antimicrobial drug residues in the cultivated species (Gibson et al., 2020). These antibiotics were used to prevent diseases caused by microorganisms such as bacteria, viruses, fungi, and parasites in cultured species, thereby increasing the mortality of the species. Moreover, half of production losses in aquaculture are attributed to the diseases with more pronounced impacts in developing countries (Chowdhury et al., 2022). Many antibiotics, including ampicillin, tetracycline, streptomycin, amoxicillin, and sulfadiazine-trimethoprim, were used in aquaculture to treat fish diseases (Alderman & Hastings, 1998). However, such antimicrobial residues may be transferred to human, potentially leading to the emergence of multi-drug resistance (Okocha et al., 2018). To date, only two published reports on antimicrobial residues have been documented in Malaysia (Henderson et al., 1997; Thiang et al., 2021).

Moreover, the presence of heavy metals in aquaculture products is another concerning scenario among consumers. As, Cd, Pb and Ni are the most common heavy metals detected in the aquaculture products (Biswas et al., 2023; Hossain et al., 2022; Kazemi et al., 2022). These metal contaminants primarily derive from the industrialization and urbanization surrounding the cultured area (Mahat et al., 2018).

Hence, risk assessment was introduced to evaluate the harmful effects of consuming fish within a certain period. The assessments were conducted based on specific farmed species, with detailed investigations into the health impacts of different consumption scenarios and exposure to targeted sub-groups. Analysis of the risk assessment in fish samples has been initiated in Malaysia (Anuar et al., 2023; Azmi et al., 2019), but there is limited data to reveal the existence of trace metals, particularly in aquaculture. It is essential to provide knowledge about aquaculture products in several aspects, notably on health and safety (Carrassón et al., 2021).

Fish oils are acknowledged for their benefits to human, and the demand for this component has increased over the years. Traditionally, fish oils are extracted from wild-caught fatty fish. However, due to the depletion of wild-caught stocks, it is suggested that fatty fish from aquaculture can serve as an excellent source for producing fish oils to meet consumer demand. Limited research evidence has been devoted to seek the potential of fish oil production from farmed fish (Aitta et al., 2021; Rahman et al., 2018; Ghaly, 2013). Therefore, the fatty fish from the selected aquaculture species were chosen as precursors for extracting fish oil. Wet pressing, solvent extraction, and enzymatic hydrolysis are among the most common approaches to extract fish oil due to their simplicity and efficiency (Dennis, 2019; Routray et al., 2018; Silva & Soares, 2013; Zhang et al., 2021). It should be noted that the fish species and extraction process are likely to influence the physicochemical properties, rheological properties, composition of fatty acids, and oil quality (Sathivel et al., 2008; Yin & Sathivel, 2010). To date, no study has been conducted to explore the correlation between the extraction process and the physicochemical properties of marine-cultured fish oil. Such study is crucial for industrial applications of fish oil production.

Moreover, refined fish oil might possess natural biological activity components such as antioxidant, anti-cancer and anti-bacterial properties (Fuente et al., 2022a).

1.3 Objective

The objectives of the study are outlined as below:

General:

To explore the nutritional value, hazard risks, and produce fish oil derived from high commodity values aquaculture species in Penang.

Specific objectives :

1. To examine the full nutritional spectrum of the selected high commodity values of marine aquaculture species in Penang.
2. To quantify the hazards compound and predict hazard risk in selected aquaculture species.
3. To extract, characterize and refine the fish oil derived from selected fatty cultured species.
4. To assess the biological activities of the refined fish oil from selected fatty cultured species.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture in Malaysia

Geographically, Malaysia is a country that consists of two regions divided by the South China Sea: Peninsular Malaysia (West Malaysia) and East Malaysia. This multiracial land has a long coastline of approximately 4800 km, with Peninsular Malaysia around 2031 km long, while East Malaysia has a 2778 km long coastline. The rich natural sources such as ponds, rivers, lakes, and sea have made Malaysia a perfect country for developing aquaculture. Aquaculture has been developed in Malaysia since the 1920s, beginning with Chinese carp, followed by shrimp and blood cockle culture in the 1930s and 1940s, respectively. In the early 1970s, the cage cultivation of marine fish was first introduced to this industry (Samah & Kamaruddin, 2015). Malaysia has ranked in the top 20 of global aquaculture production, with the highest production of seaweed and brackish species.

The trends of the fisheries sector in Malaysia are shown in Figure 2.1. Marine capture fisheries contributed 1.31 million tonnes, whereas 0.57 million tonnes accounted for aquaculture production in 2022. For marine capture fisheries sectors, the trends were evident until 2016, with production amounts of 1.59 million tonnes, and it began to decline drastically to the lowest production of about 1.31 million tonnes in 2022. Such trend indicated that the exploitation of fish from the oceans had reached its maximum level and has limited its contribution to the fisheries sectors (DoFM, 2023). In contrast, the aquaculture industry in Malaysia has become relatively unstable over the last few years. The production has been facing a declining effect since 2012, decreasing from 0.63 million tonnes in 2012 to 0.53 million tonnes in the following year, and eventually reaching the lowest production of about 0.39 million tonnes in 2018.

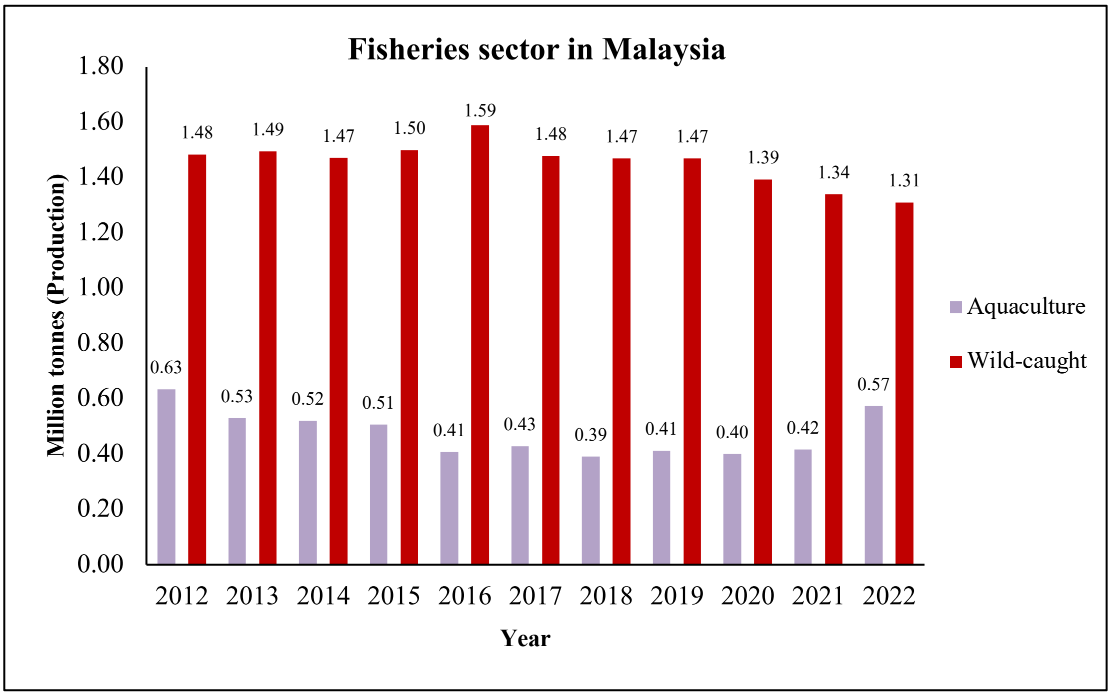

Figure 2.1 Trends of the fisheries sector in Malaysia (2012-2022) (DoFM, 2023)

The decrease might be attributed to the challenges (such as increased production cost of labor, disease, safety, and quality of aquaculture practice) faced by fish farmers in managing the farms. This sector grew in 2019 with production of 0.41 million tonnes, reflecting a 5.2% rise in production volume over 2018. Nonetheless, the production experienced a slight decrease in 2020, with production of 0.4 million tonnes due to the COVID-19 pandemic outbreaks. In 2022, the sector reported its output to 0.57 million tonnes, showing the highest percentage rise (15.2%) since 2012. The current trend has shown the total fisheries production of Malaysia and dealing with security issues to limited stocks of marine fisheries and fishing beyond the sustainable reproductive capacity (Roslina, 2009).

According to DoFM (2023), approximately 38 759 fish farmers and culturist are involved in the aquaculture industry, In 2022, brackish water aquaculture contributed up to 150 349.12 tonnes of fish, valuing more than RM 3.51 billion (approximately USD 0.75 billion). Pulau Pinang has recorded the highest production for brackish water systems, amounting to 41 387.32 tonnes, followed by Perak (34 660.33 tonnes) and Kedah (23 498.82 tonnes) (Table 2.1).

Table 2.1 Brackishwater culture production by states in 2022 (DoFM,2023)

State	Brackishwater culture production (tonnes)
Pulau Pinang	41378.32
Perak	34660.33
Kedah	23498.82
Johor	20365.69
Sabah	10001.58
Selangor	6164.81
Sarawak	3880.63
Pahang	3547.87
Terengganu	2977.46
Kelantan	1898.61
Negeri Sembilan	1217.95
Melaka	582.31
Perlis	171.32
W.P. Kuala Lumpur	1.90
W.P. Labuan	1.52
Total	**150349.12**

Note: W.P. = Wilayah persekutuan

2.1.1 Aquaculture practice in Penang

Penang is located at the northwestern coast of Peninsular Malaysia and consists of two parts: Penang Island and the mainland of Seberang Perai. This state has five administrative districts, namely the northeast district (daerah Timur Laut) and the southwest district (daerah Barat Daya) located at Penang Island. The Northern Seberang Perai district (daerah Seberang Perai Utara), Central Seberang Perai district (daerah Seberang Perai Tengah), and Southern Seberang Perai district (daerah

Seberang Perai Selatan) are located at the mainland of Seberang Perai (known as Province Wellesley).

The fisheries sector is one of the important sectors in Penang, and it has recorded the highest production of marine culture in Malaysia, accounting for more than a quarter of Malaysia's aquaculture revenues (DoFM, 2023). This industry sector thus has excellent potential for producing fish and seafood products for both domestic and international consumption, and become the major driving force in the state's economic development (Vaghefi, 2017). Brackish water cultivation is the major cultivation, highly distributed in the Northeast district of Penang Island and South Seberang Perai. The main cultivation site in South Seberang Perai is Kampung Sungai Udang, which has been operated for at least 60 years. The place has been famous for the fishing activities in the sea, and prawns breeding along the coastal area. Pulau Jerejak, located in North East District of Penang Island, was a highly distributed aquaculture site with a total of 55 marine cultivation farms (Zain, 2020).

2.1.1(a) Brackish water species in Penang

Brackish water is defined as water with salinity between freshwater and seawater, and it is often discovered in transition zones where the two waters mix. According to Rich and Maier (2015), a well-known example of salty water is the estuary, the meeting point between river and the sea. Brackish water species have gained lots of attention due to their high export demand and ability to generate significant foreign exchange for the country. There are three main categories of brackish water species cultivated in Penang: finfish, crustaceans, and bivalves, as shown in Table 2.2. Finfish cultivation is normally produced in open sea cages or fish ponds. The main species cultivated in Penang are seabass (*Lates calcifer*), snapper

(*Lutjanus sp.*), golden pompano (*Trachinotus blochi*), grouper (*Epinephelus sp.*), and bigeye trevally (*Caranx sexfaciatus*).

Table 2.2 Brackishwater culture species in Penang in 2022 (DoFM, 2023)

Categories	Cultures species	Production (Tonnes)	Values (in thousands)
Finfish	Seabass	29 770.59	366 196.95
	Red snapper	2 009.04	62 594.75
	Golden snapper	2 089.79	51 890.09
	Golden pompano	1 637.41	31 857.14
	Grouper	803.60	31 644.70
	Bigeye trevally	1 596.94	30 682.87
	Red tilapia	947.66	9 476.61
	Threadfin	19.45	544.72
	Grunt	0.07	1.66
	Milkfish	2.30	16.87
Crustacean	White-leg shrimp	1 233.24	32 084.35
	Tiger prawn	726.94	18 765.23
Bivalve	Cockles	541.38	5 631.09
	Oyster	0.06	1.42
	Mussels	0.26	1.28
	Total	**41 378.72**	**610 798.86**

(i) Seabass

Seabass (*Lates calcifer*), also known as giant sea perch or barramundi and in Malaysia as *siakap* (Plate 2.1) is a euryhaline and demersal fish in high demand (Irmawati et al., 2020). Seabass culture in Malaysia was started in the mid-1970s, with a cage system using wild and imported fry. In 1982, the larval rearing of the seabass was developed in Penang, and the first successful spawning and breading was achieved in 1985 at Terrenganu (Lovatelli, 1991). At present, the cultivated species is sustained by locally produced or imported fry (Thailand).

Plate 2.1 Seabass (*Lates calcifer*)

In sea cage cultivation, the fingerling was stocked with sizes of 8-10 cm, and approximately 8 g each of fish, and the growth up rate for the species to reach its marketable size (about 600 to 700 g) is 7-8 months (Aguru, 1985). Efficient breeding programs for improving this farm have been developed by the Fisheries Research Institute (FRI) since 2016 with the aim of improving economically important traits of Asian seabass (Idris et al., 2022). In 2022, seabass contributed approximately 29.8 thousand tonnes, valued at RM 366 million, accounting for the highest production of brackish species in Penang (DoFM, 2023). In fact, seabass has been recognized as a high-commodity value fish species in Entry Point Projects (EPPs) under the agriculture sector of the National Key Economic Area (NKEA) in Malaysia (Idris et al., 2022).

(ii) Snapper

Red snapper and golden snapper are the most commercially available snapper species in Malaysia, which ranked second among the most productive species in Penang. Snappers are grown in Malaysia in both small- and large-scale operations, with culture systems ranging from cage cultures to brackish water ponds. Red snapper, scientifically known as *Lutjanus argentimaculatus*, and in Malaysia as Ikan Merah

(Plate 2.2), is an euryhaline and demersal fish live in water with high salinity containing 35 ppt of salt content. This species was first introduced in Malaysia in 1988 and has recorded the production of 188 tonnes in similar years (DoFM, 2023). The fingerling was commonly collected from local water or imported from Taiwan and Indonesia. In 2022, the production of snapper had risen to 5 833 tonnes, increasing up to 96% from their initial year (DoFM, 2023). The significant increase has shown the demands and favour of the species from worldwide consumers due to its favourable quality.

Plate 2.2 Red snapper (*Lutjanus argentimaculatus*)

Meanwhile, the golden snapper (*Lutjanus johnii*), locally known as Jenahak (Plate 2.3), is widely cultivated in Asian countries such as China, Pakistan, Hong Kong, Indonesia, India, the Philippines, Singapore, and Malaysia. This demersal species has a high survivability rate, and it can grow to its marketable sizes (600-800 g) in 7-8 months. The fingerling production was achieved by induced spawning (Philipose et al., 2017). Currently, the production of golden snapper in Malaysia has increased tremendously, from 1339 tonnes in 1994 to 4617 tonnes in 2022 (DoFM, 2023).

Plate 2.3 Golden snapper (*Lutjanus johnii*)

(iii) Golden pompano

Golden pompano (*Trachinotus blochii* or Bawal Emas in Malay) (Plate 2.4) is a marine fish species belonging to the family of Carangidae, mainly distributed in the tropical and subtropical waters of the Indian Ocean and Pacific Ocean (McMaster & Gopakumar, 2016). This species is pelagic, active, and able to acclimate at a wide range of temperatures and salinity (0-65 ppt). The cultivation of the species can yield a good harvest after 240 days, with an average size of fish at a weight of 450-500 g and a survivability rate of 94% (McMaster & Gopakumar, 2016). Owing to its high survivability, fast growth rate, and good meat quality, golden pompano has become one of the high-market demand species in Asia region (FAO, 2008). This species was first introduced to Malaysia by Taiwanese investors to produce the fry for both local and overseas demands (Ransangan et al., 2011) At present, this species in Penang was ranked third in Penang among the most productive species, amounting to 1637 tonnes (DoFM, 2023).

Plate 2.4 Golden pompano (*Trachinotus blochii*)

(iv) Grouper

Grouper is classified in the subfamily of *Epinephelinae*, and several species have been cultivated in Malaysia, including *E. coioides, E. tauvina, E. fiscoguttatus*, *E. lanceolatus* and *E. suillus* (Robert et al., 2002). This aquatic animal was also a pioneer species in sea cage cultivation in Malaysia in 1973, and the species was cultivated using the wild-caught fry. The seeds of the groupers are mainly sourced from the local water or imported from Taiwan (Lovatelli, 1991). In sea cage cultivation, the grouper fingerlings with an approximate weight of 2 to 5 g are stocked in net cages, and the species are fed twice a day with either trash fish or pelleted feed. The culture period of the species to attain a marketable size of about 600-800 g is estimated at 6 to 8 months (Lovatelli, 1991). In 2022, the production of grouper in Penang was estimated at 803.6 tonnes, and *E. coiodes* (also known as orange-spotted grouper or Kerapu in Malay) (Plate 2.5) is the most abundant cultivated species. The interest in *E. coiodes* was started in 1989, with the research of hatchery production by induced egg spawning and growth proficiency in the juvenile stage (Ali, 1996; Azmi et al., 2021).

Plate 2.5 Grouper (*Epinephelus coioides*)

(v) Bigeye trevally

Bigeye trevally, scientifically known as *Caranx sexfaciatus* (Nyok-nyok in Malay) (Plate 2.6), is an economically important pelagic fish widely distributed in the Indian and Pacific oceans. According to Sumaila et al. (2007), this species is considered a high-price species, and commonly categorized within the pompanos family. This species was first cultured in cages in Sabah during the mid-1990s. Fingerlings were customarily collected from the wild, and the growth period of culture to reach its marketable size (600-800 g) ranged from 6-8 months (Rengarajan et al., 2017). The production of the species is ranked fourth as the most producing species in Penang, amounting to 1597 tonnes in 2022.

Plate 2.6 Bigeye trevally (*Caranx sexfaciatus*)

2.1.1(b) Marine cultivation and feeding technique in Penang

(i) Cultivation Technique

Cage culture was first introduced in Malaysia in the 1970s, and grouper (*Epinephelus tauvina*) was the first species being cultivated in this system at the Straits of Penang. Since then, it has become a major industry, particularly in marine fish production, due to the system having proved to be technically feasible and commercially viable (Chua & Teng, 1977). The cultivation cages in Malaysia are generally rectangular and comprised of net cages and frame support (Figure 2.2). Plastic drums or styrofoam blocks coated with fibreglass are normally used to keep the frame afloat (Hamid, 1993). The concrete blocks or wooden pegs are usually implemented on the system as an anchor to hold the floating system. Each net-cage system can be built from 1 unit (4 cages) to a maximum unit of 15 (60 cages). The latter is confined majorly to the East coast of Peninsular Malaysia due to the suitability of location that is limited by rough seas, particularly during monsoon seasons (Hamid, 1993). Currently, the main species cultivated in the marine cage were seabass (8 514 tonnes), red snapper (4 765 tonnes), golden snapper (4 500 tonnes), grouper (3 527 tonnes), golden pompano (2 641 tonnes), and bigeye trevally (1 897 tonnes) (DoFM, 2023). Pulau Pinang is the state that has the highest use of land for the brackish cage, amounting to 837,628 ha, with a total of 249 culturists (DoFM, 2023).

(ii) Feeding Technique

Food pellets are normally used by farmers in aquaculture practices and it comprised of two main components: protein (18-50%) and lipids (10-25%), which are important in promoting the growth of cultivated species (Craig & Helfrich, 2002). The nutritional content of the feed is dependent on the type and the maturity stage of the species. Protein levels of feed are formulated according to species: Shrimps (30-35%), catfish (28-32%), tilapia (35-40%), marine finfish (40-45%) (Craig & Helfrich, 2002). Moreover, the level of proteins dependent on the maturity of the fish and feeding behaviour. For instance, early-life stage fish require more protein compared to matured fish, and carnivorous fish will be feeding higher amounts of protein than herbivorous and omnivorous fish. The protein in the feed is commonly derived from fish meals or plant-based protein such as soybean meal (Malaysia and Thailand), groundnut (Nigeria), and cottonseed cake (Egypt) (Manaf, 2017; FAO, 1979). Lipids (fat) are also important nutrients required in fish feed. These essential nutrients serve as transporters for fat-soluble vitamins in fish metabolism. Generally, the fish feed consists of about 7-15% lipid content, derived from marine fish oil, algae, oil plant-based oil such as rice bran oil, maize oil, and cassava oil (Manaf, 2017; FAO, 1979).

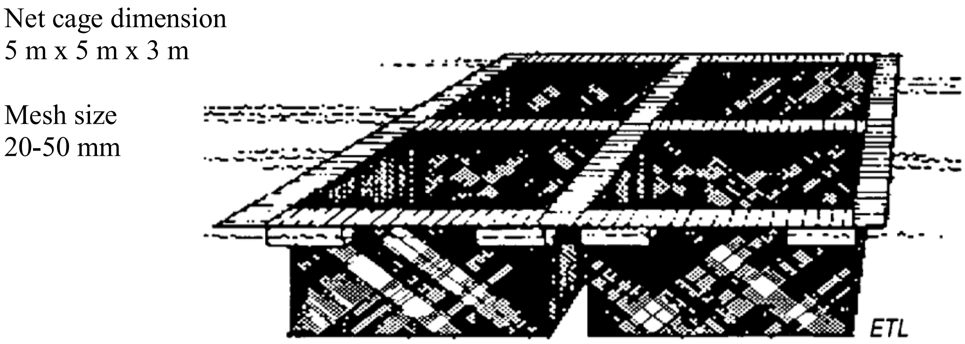

Figure 2.2 Malaysia cultivation sea cage system (SEAFDEC, 1994)

In mariculture, trash fish is generally being implemented as the feed due to its lower cost compared to pelleted food. Trash fish is defined as the low economic values of small-sized fish species that are easily perishable with relatively poor edible quality and often not being used for direct human consumption, which are normally caught in commercial inshore fisheries (Hasan et al., 2007). Mackerel, scads, and nemipterid are some examples of common trash fish in the aquaculture feeding (Chun, 2007). According to Giri et al. (2004), 85% of mariculturists in Malaysia rely on trash fish as the sole feed in their cultivation system, especially the carnivorous species such as groupers and snappers. In accordance to the feeding technique, the cultured species have normally been fed with trash fish twice a day with 8-10% of the biomass, up to 100 g, and 3-5%, up to 600 g (Araujo et al., 2022).

2.2 Nutritional value of marine species

Nutrient deficiency is one of the global concerns throughout the third world (Tacon & Metian, 2013). Compared to terrestrial meat products (bovine meat, poultry meat, pig meat), aquatic animals offer many benefits to human health. It is well documented that marine animals, particularly fish, contributing high levels of ω-3 fatty acids as dietary lipids sources, providing low calories but high-quality protein (Witten

& Cancela, 2018). The roles of the aquaculture sector in producing aquatic foods are quintessential not only to secure the global food supply but also to provide an adequate amount of nutritional benefits, especially to underdeveloped countries that are susceptible to malnutrition (Bogard et al., 2017)

Pioneer epidemiological evidence from Japan's fishing village has shown that consuming aquatic animal products could effectively reduce the risk of cardiovascular diseases (CVD) (Ebihara et al., 1982). Subsequently, Oomen et al. (2000) suggested that individuals with frequent fatty fish intake could reduce the risk of getting CVD by 34%. Consumption of fish up to 35 g d^{-1} would decrease CVD mortality. Recently, Zhang et al. (2020) also reported that the risk of CVD decreased by 4% through 20 g fish intake daily. This nutritional richness positions fish as a source of energy for maintaining a healthy lifestyle, often referred to as ‘rich food for poor people' (Balami et al., 2019; Bezbaruah & Deka, 2021). The nutritional values of aquaculture products are highly dependent on the variability of species, habitat, location and cultivation characteristics, as illustrated in Table 2.3.

Table 2.3 Nutritional composition of global aquaculture products

Location	Category	Proximate analysis				Amino acid composition		Vitamin Composition		Fatty acid composition			Mineral Detection			References
		Moisture	Protein	Fat	Ash	EAA	NEAA	Fat Soluble	Water soluble	SFA	MUFA	PUFA	Macro	Micro	Heavy metals	
China	Marine fish	69-72	21-22	0.5-8	1.1-1.4	45	55	-	-	33-34	24-37	30-43	Ca, K, Mg, Na	Cu, Fe, Mn, Zn	As, Hg	(Ali et al., 2023)
Bangladesh	Freshwater fish	-	-	-	-	-	-	-	-	-	-	-	-	Zn	As, Cd, Cr, Pb, Hg	(Biswas et al. 2023)
China	Freshwater fish	75-79	19-21	2-3	1.1-1.5	39	61	-	-	23-30	30-44	33-39	Ca, K, Mg, Na	Cu, Fe, Mn, Zn	As, Cd, Cr, Pb, Hg	(Wang et al., 2022)
Poland	Freshwater fish	74-78	17-18	1.2-2	-	54	46	-	-	29-30	51-53	11-13	-	-	-	(Sobczak et al., 2021)
Egypt	Freshwater fish	72	19	8	1.1	30	70	-	-	30	45	26	Ca, Mg, K	Fe, Mn, Zn	-	(Abdel-Mobdy et al., 2021)
China	Crustacean	69-85	13-18	0.7-1	-	39-42	58-61	-	-	18-21	22-42	22-50	-	-	-	(Wang et al., 2021)
Norway	Marine fish	65-66	14-15	13-17	12	45-46	54-55	-	-	21-25	45-56	29-33	-	-	-	(Esaiassen et al., 2022)
Italy	Bivalve	80-84	6-10	1-2	3-5	-	-	-	-	41-50	18-27	26-38	Ca, K, Mg, Na	Cu, Fe, Zn	-	(Biandolino et al., 2019)
Malaysia	Freshwater fish	68-72	15-19	4-16	1.1-1.2	44-46	54-56			26-33	34-49	8-9	-	-	-	(Noordin et al., 2019)
Thailand	Crustacean	77-80	17-18	1.2-1.3	1-1.5	55	45	-	-	32-35	20-24	44-45	Ca,Mg	Cu, Fe, Mn, Zn	Cd,Co, Ni,	(Sriket et al., 2007)

Note: Value were expressed in percentage. EAA = Essential amino acids; NEAA = Non-essential amino acids; SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids

2.2.1 Protein and amino acid

2.2.1(a) Protein

Protein is one of the essential macromolecule required by living organisms to serve the functionality of the biological process, and accounts for 18% of the human protein supply. This versatile macromolecule is an initial substance for the enzyme, which functions as the catalyst for the hemoglobin and contributes to most of the body functionalities (i.e., immune system, growth and movement) (Telser, 2002). Proteins, also often called as polypeptides, are made from amino acids, AAs, linked by peptide bonds. A peptide bond occurs by the condensation of AAs with the elimination of water (Mai et al., 2022). Each protein unit consists of either one or more polypeptides, and the number and order of the amino acid residues in the polypeptide chains can define its characteristics, nature and structure. Examples of proteins classified based on structures are either globular proteins polypeptide chains folded into globular or spherical shapes such as albumin or fibrous proteins polypeptide chains arranged in long strands such as collagen (Mai et al., 2022).

Fish is a good source of animal protein that has greater satisfaction effects in terms of affordability and variation than land animal sources like pork, beef and chicken. Additionally, protein content in fish is ranged between 14 and 21 g per 100g, with its higher biological values compared to plant-based protein (Mendivil, 2021). Protein derived from fish can be categorized into three groups, namely the myofibrillar (60-75%), sarcoplasmic protein (20-35%), and musculoskeletal protein (2-5%) (Fernando et al., 2023). Myofibrillar proteins (MPs) are composed of myosin, actin, and regulatory make up to 66 to 77% of total proteins in flesh muscles, and contribute to several important functional properties (Manzano et al., 2017). In contrast, fish sarcoplasmic protein (SP) is a large family of protein that include myoglobin,