CHARACTERISTICS OF FISH PROTEIN POWDER PREPARED FROM SNAKEHEAD (CHANNA STRIATA) RASIDE ON SPECIALISED LIPID DIET

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols/ Abbreviation	Description
a*	Redness/ Greenness
AA	Arachidonic acid
AABA	L-α-amino-n-butyric acid
ANOVA	One-way analysis of variance
b*	Yellowness/ Blueness
C	Control
Ca	Calcium
DB	Dry basis
DF	Directly frozen
DHA	Docosahexaenoic acid
DSC	Differential Scanning Calorimetry
EAA	Essential amino acid
EAI	Emulsion activity index
ESI	Emulsion stability index
EPA	Eicosapentaenoic acid
EU	European Union
EWP	Egg White Powder
FAME	Fatty-Acid Methyl Ester
FAO	Food and Agriculture Organisation
FC	Foaming capacity
FDA	U.S. Food and Drug Administration
Fe	Iron
FPC	Fish Protein Concentrate
FPH	Fish Protein Hydrolysate

FPI	Fish Protein Isolate
FPP	Fish Protein Powder
FS	Foaming stability
FW	Frozen with distilled water
g	Grams
x*g*	Gravity
HCl	Hydrochloric acid
HPP	Snakehead protein powder (from commercial fish)
K	Potassium
L*	Lightness
Mg	Magnesium
mg	Milligram
mL	Milliliter
MRL	Maximum Residue Limit
Na	Sodium
NaCl	Sodium chloride
ND	Not detected
NEAA	Non-essential amino acid
nm	Nanometer
Pb	Lead
pH	Power of hydryogen
PUFA	Poly-unsaturated fatty acid
R	Rotation
rpm	Rotatations per minute
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SFA	Saturated fatty acid

SFPP	Snakehead protein powder (from specially-fed fish)
t	Time
UFA	Unsaturated fatty acid
WB	Wet basis
WFP	Whole fillet powder (from commercial fish)
WHO	World Health Organisation
WI	Whiteness Index
w/v	Weight/Volume
Zn	Zinc
°C	Degree celcius
ΔH	Denaturation enthalphy
J/g	Joule/gram
$\mathbf{T_{max}}$	Peak maximum temperature
µL	Microlitre
%	Percentage
>	More than
<	Less than
≥	Equal or more than

CIRI-CIRI SERBUK PROTEIN IKAN YANG DISEDIAKAN DARIPADA IKAN HARUAN (CHANNA STRIATA) YANG DITERNAK DENGAN DIET LIPID KHAS

ABSTRAK

Penternakan ikan haruan (*Channa striata*) dengan diet kaya dengan lipid yang diperoleh daripada sumber ikan dan kacang soya dapat meningkatkan kecekapan reproduksinya. Objektif kajian ini adalah untuk menyedia dan menilai serbuk protein yang disediakan daripada haruan yang diternak dengan diet lipid khas (SFPP). Untuk mencapai objektif ini, langkah-langkah penyediaan SFPP perlu disediakan. Justeru itu, kajian ini dibahagi kepada dua bahagian. Dalam kajian fasa I, hanya ikan haruan komersial digunakan kerana sumber ikan yang diternak dengan diet lipid khas adalah terhad. Tujuan utama kajian awal adalah untuk menentukan cara penyediaan dua jenis produk iaitu serbuk filet haruan (WFP) dan serbuk protein haruan (HPP). Akibat penggunakan rawatan pembekuan ke atas hasil pengutipan fillet juga dikaji, iaitu pembekuan terus, pembekuan dengan air dan tanpa pembekuan. Untuk menyediakan WFP, langkah-langkah penyediaan yang terlibat adalah rawatan pembekuan, diikuti oleh penyediaan cincang filet dan pengeringan ketuhar untuk penyediaan WFP. Untuk penyediaan HPP, filet yang dicincang dipanaskan dengan air pada suhu 85 ^{o}C. Campuran ini kemudiannya menjalani proses pemisahan untuk mengasingkan lemak daripada protein sebelum proses pengeringan sejuk-beku untuk memperoleh serbuk protein. Daripada pemerhatian,pengutipan fillet ikan adalah tertinggi untuk ikan yang telah menjalani rawatan pembekuan dengan air. Ikan yang dirawat dengan pembekuan air juga

adalah lebih senang untuk dikendalikan mungkin kerana ikan kurang mengeluarkan lender. Selain itu, HPP dan WFP yang disediakan daripada ikan haruan yang dibekukan dengan air juga menghasilkan serbuk yang mengandungi protein dan abu yang lebih tinggi daripada serbuk protein yang disediakan melalui rawatan pembekuan yang lain. Justeru itu, pembekuan ikan dengan air dipilih sebagai langkah penyediaan untuk menganalisa ikan haruan yang diternak dengan diet lipid khas. Dalam bahagian kedua, analisa difokuskan terhadap serbuk protein haruan yang disediakan daripada haruan yang diternak dengan diet lipid khas (SFPP) disebabkan sumber ikan yang terhad. Keputusan menunjukkan bahawa SFPP mengandungi nisbah asid amino perlu yang lebih tinggi terutamanya Histidin (22.66 %). Ciri-ciri berfungsi SFPP juga adalah lebih rendah daripada serbuk telur putih. Kajian kalorimeter imbasan perbezaan terhadap cincang filet mentah ikan haruan mendedahkan bahawa denaturasi protein telah berlaku pada suhu serendah 54 °C. Kemungkinan besar, denaturasi protein telah mengakibatkan kehilangan ciri-ciri berfungsi SFPP. Kesimpulannya, SFPP yang kaya dengan asid amino telah berjaya disediakan. Walau bagaimanapun, penggunaan SFPP untuk tujuan ciri-ciri berfungsi adalah tidak sesuai. Pengunaan haba untuk pemisahan lipid daripada ikan mungkin telah mengakibatkan kehilangan ciri-ciri berfungsinya.

CHARACTERISTICS OF FISH PROTEIN POWDER PREPARED FROM SNAKEHEAD (CHANNA STRIATA) RAISED ON SPECIALISED LIPID DIET

ABSTRACT

Specially-fed Snakeheads (*Channa striata)* are snakehead fishes fed with diets high in lipids sourced from fish and soybean which have been shown to improve their reproduction efficiency. The objective of this study was to prepare specially-fed protein powder (SFPP) from these snakehead fish and evaluate it. To achieve this objective, a method has to be developed for the preparation of such powders. Therefore, this study was divided into two phases. As the supply of specially-fed Snakeheads was scarce, only commercial snakehead was utilised during phase I to develop preparation methods for whole fillet powder (WFP) and snakehead protein powder (HPP). Effects of freezing treatments (direct freezing, freezing with water or no freezing) before filleting on fillet harvesting yield were also investigated. To prepare WFP, after freezing treatments, fish were filleted, minced and oven dried. However, to prepare HPP, minced fillet were heated with water at 85 ^{o}C and the liquid extract was centrifuged to separate fat from protein. Freeze drying was used to obtain protein powder. Observations showed that fishes frozen with water yielded significantly higher ($p>0.05$) yield of fillets. Fish frozen with water had less slime on their body and were thus easier to handle. HPP and WFP obtained from fillets harvested from fish frozen with water also displayed significantly higher protein and ash and generally, most amino acid content were either significantly higher or not significantly different compared to powders

obtained via other freezing treatments. Therefore, preparation method involving freezing fish with water was chosen as a preparation step to study specially-fed snakeheads. In phase II, due to material scarcity, focus was solely on the analysis of SFPP prepared from fillets from specially-fed snakeheads. When compared to composition of HPP obtained from Phase I, analysis revealed that SFPP contained higher ratio of desirable essential amino acids, mainly histidine (22.66 %). Functional properties of SFPP were also inferior to those of egg white powder. Differential scanning calorimetry analysis of raw unprocessed fish fillet revealed that extensive protein denaturation had occurred at 54 °C and may have resulted in loss of functional properties of SFPP. In conclusion, SFPP was successfully prepared from specially-fed snakehead and was found to contain good levels of amino acids when compared to HPP prepared from commercial snakeheads. Unfortunately, SFPP is not suitable to be manipulated for functional purposes. The use of heat to remove fats from the fish might be the reason for this inferior property.

CHAPTER 1: INTRODUCTION

1.1 Introduction

The Dietary Guidelines for Americans 2010 recommends that each adult should consume 2-3 servings of fishes a week particularly fatty fishes as fishes are low in saturated fats but high in protein. Fishes are often consumed not only as a source of protein in our daily diet but have been known to be consumed for their medicinal values. Current fish products that have been commercially sold come mostly from marine fishes such as fish liver oils and shark cartilages (Haniffa *et al.,* 2014). Freshwater fishes on the other hand are rarely seen used as ingredients to make food products as they are deemed unfamiliar to consumers. Although they are unfamiliar, their significance and potential should not be overlooked and hence should be further investigated. In our study, we are focusing on one particular freshwater fish which is the snakehead (*Channa* striata) also known as *haruan* in the Malay language.

1.2 Background and Rationale

Malaysians have long been known to consume the white flesh of Snakehead for its benefits in healing of post-operative wounds (Baie and Sheikh, 2000; Wee and Tacon, 1982) and pain reduction (Zakaria et al., 2004) particularly for those who has just gone through surgeries or labour. This is because snakehead has been known to have an abundant level of arachidonic acid; an essential ω-6 fatty acid and key amino acids such as aspartic acid, glycine and glutamic acid (Zuraini et al., 2006) which are also the key ingredient for polypeptide formation responsible for the growth and

healing of wounds (Chyun *et al.* 1984; Heimann, 1982). Glycine is also a very important component of collagen in mammals (Szpak, 2011). Meanwhile, other researchers have also noted their antimicrobial and antifungal properties (Jais, 2007) and antinociceptive activity (Jais *et al.*, 1997).

However, current Snakehead supplies are not sustainable to cope with market's demand as many began to realise their medicinal properties causing an up rise in the commercialisation of products produced from this fish. Wild snakehead are also harder to find and catch as number of wild snakehead are dwindling day by day due to their natural habitats being destroyed by pollution (chemicals, pesticides, industrial wastes), global warming and overfishing of the fish. Farming of these fishes can be difficult as well due to slow growth and low survival rate caused by their cannibalistic behaviours (Boonyaratpalin *et al.*, 1985) which in turn lead to the rise in breeding cost.

Also, to date, products made from snakehead are only available in liquid form such as essence, which is for consumption purposes or in cream form, which is for external use for skin treatment only. Liquid form of snakehead extracts frequently poses problems of limited shelf life, physicochemical instability and restricted maneuverability due to presence of heavy weighted water (Hui *et al.*, 2010). Thus, preparation of the fish in powder form might be of interest to food industries. However, this can only happen when there is an increase in fish supply.

This has cause a previously supported research project from the School of Biological Sciences, Universiti Sains Malaysia to breed a new and "improved" species of snakehead which has been fed with a specialised lipid diet (Ghaedi *et al.*, 2014). This specialised lipid diet is thought to aid fish farmers reduce their breeding

cost while improving the health and diet of snakehead by improving the fishes' reproductive performances. Their current diet of chicken visceral and skin is not healthy and nutritious for the fish while small prawns and fishes can be expensive to purchase. It also causes foul smell in their breeding ground making their "home" an unsuitable and uncomfortable environment for them to thrive and breed. Thus, breeding of snakehead with this specialised lipid diet will be cheaper in the long run as they will give a higher yield while providing the fishes with the nutrition and conducive environment that they need to survive and breed. When the supply has increased, the fish can then be utilised to prepare dry powders which are expected to have longer shelf lives as well as ease in mobility when compared to current available products in the market. However, such powder has not been developed and evaluated for any potential application in food.

Thus, this research is divided into two phases. In phase I, studies will be conducted on commercial Snakehead to study the effects of various freezing pre-treatment on the filleting yield and the physicochemical properties and compositions of whole fillet powder (WFP) and snakehead protein powder (HPP). In phase II, using the best determined freezing pre-treatment from phase I, powders were then prepared from specially-fed snakehead provided by the School of Biological Sciences. Their compositions are evaluated against commercially-bred species whereas their functional properties were evaluated against egg white powders.

1.3 Objectives

The main intention was to prepare protein powders from the flesh of snakehead *Channa striata* raised on specialised lipid diet.

The other sub-objectives for each phase of the study are as listed below:-

(i) Phase I

a) To prepare and charaterise whole fillet powder (WFP) and haruan protein powder (HPP) using commercially bred snakeheads.

b) To study the effects of freezing pretreatment on the slime secretion and subsequently on the yield of the fillets harvested.

(ii) Phase II

a) To prepare and characterise haruan protein powder from snakeheads raised on specialised lipid diet (SFPP).

b) To study the effects of heat on the preparation of SFPP using differential scanning calorimetry (DSC)

1.4 Thesis Outline

The main content of this dissertation is on the development of protein powders from the flesh of specially fed *Channa striata* (Bloch 1793).

In CHAPTER ONE, a general introduction on the background and the purpose for this research was discussed.

Studies on books, journals, articles and reviews are summarized in CHAPTER TWO where we discuss about the raw material: Snakehead (*Channa striata*), preserving the flesh quality, freezing pre-treatment and on fish muscle protein powders.

CHAPTER THREE consists of the studies conducted on the fillets from commercially-bred Snakehead fish (*Channa striata*) which has been subjected to various pre-freezing treatment. Color and composition of whole fillet powder (WFP) and snakehead protein powder (HPP) developed from these fleshes were evaluated and the most appropriate method was used for the following studies.

In CHAPTER FOUR, selected method of freezing was used on Snakehead fish (*Channa striata*) that has been fed with a special lipid diet formulated by a research team from the School of Biological Sciences of USM to prepare specially-fed snakehead protein powder (SFPP). SFPP was prepared as before and their characteristics such as color, compositions, functionality and thermal analysis were also evaluated.

In the final chapter, CHAPTER FIVE, an overall conclusion drawn from our study is given and any recommended possible future study was presented.

CHAPTER 2: LITERATURE REVIEW

2.1 Channa striata (Snakehead)

2.1.1 Taxonomy and Distribution of Snakehead

Belonging to the family of Channidae, "*haruan"* is a freshwater fish that is widely consumed throughout South-East Asia, China and India for its biomedical properties (Jais, 2007) and as dietary proteins (Gam *et al.,* 2005). The complete taxonomy of snakehead is as listed below.

Kingdom	: *Animalia*
Subkingdom	: *Bilataria*
Branch	: *Deutorostomia*
Phylum	: *Chordata*
Subphylum	: *Vertebrata*
Class	: *Actinopterygii*
Order	: *Perciformes*
Family	: *Channidae*
Genus	: *Channa*
Species	: *Striata*

Adapted from Haruan Research Group (2015)

About 30 species has been accounted for in the world with eight of them being native to Malaysia which includes *Channa micropeltes* (Toman), *Channa lucius* (Bujuk) and *Channa striata* (Snakehead) (Mohsin and Ambak, 1983; Wee, 1982; Rahim *et al.,* 2009). Various species of Channidae has also been discovered in neighbouring ASEAN countries primarily in Singapore, Indonesia, Thailand, Brunei, Cambodia, Vietnam, Laos, Myanmmar and Phillipines (Jais, 2007).

Snakeheads are usually found in lakes, pools, small rivers, swamps and shallow water bodies and have been known to thrive in harsh environmental conditions that have high concentration of ammonia or low dissolved oxygen (Qin *et al.,* 1997). However, studies has also shown that they can easily detect and sense contamination within their habitats and since then, they have acquired the skill to move from one location to another by "walking" on land in search of new clean waters (Jais, 1991).

2.1.2 Composition and Morphology of Snakehead

The FAO Fisheries and Aquaculture Department describes the snakehead's scaly body as elongated with broad but flattened head, having rounded caudal with oblique bars on its body, having only small teeth in a large mouth and possessing longer dorsal fin than its anal fin (www.fao.org). A detailed morphometric study of six selected populations in Peninsular Malaysia is also discussed by Rahim *et al.* (2009), which is important for the separation of races, species and population rating to allow for proper categorisation of various fish stocks.

This fish is highly and widely consumed as food like any other freshwater fish that is available in the market as a good source of protein and as Jais (2007) has described, it's almost boneless flesh is white (Figure 2.3) and has a meaty and tender taste. This versatile fish is usually steamed whole or filleted for its flesh and added into soups and porridges. Here in Malaysia as well, many consume snakehead as health food as many believed that it can help with post-natal and post-operative recoveries

Figure 2.1 Original description: *Ophicephalus striata* Bloch, 1793; image reversed from original pl. 359. Circular, Volume 1251 pg 115.

Plate2.1 Commercially bought *Channa striata.*

Plate2.2 Freshly filleted flesh of *Channa striata*.

Table 2.1 Fatty acid composition of the raw flesh of snakehead *Channa striata*.

Fatty acid	Zuraini *et al.* (2006)	Jais *et al.* (1994)
C14:0 (Myristic acid)	ND	1.38 ± 0.32
C16:0 (Palmitic acid)	30.39 ± 0.23	26.90 ± 0.23
C18:0 (Stearic acid)	15.18 ± 0.15	10.30 ± 0.52
C20:0 (Arachidic acid)	ND	-
*C16:1 (Palmitoleic acid)	2.98 ± 0.07	4.96 ± 0.54
*C18:1 (Oleic acid)	12.04 ± 0.54	15.01 ± 0.19
*C18:2 (Linoleic acid)	8.34 ± 1.01	0.78 ± 0.07
*C18:3 (Linolenic acid)	ND	1.22 ± 0.16
*C20:4 (Arachidonic acid)	19.02 ± 0.78	12.70 ± 0.70
*C20:5 (Eicosapentanoic acid)	ND	1.29 ± 0.07
*C22:6 (Decosahexanoic acid)	15.18 ± 1.12	16.43 ± 0.71

Values are % of the total fatty acid ± SD. Only selective fatty acids were listed out. ND, not detected; (-), not determined; (*), unsaturated fatty acids.

However, till this day, most research conducted on this fish was on the amino and fatty acid content of its raw flesh (Jais, 2007; Zuraini *et al.*, 2006). Many studies has revealed high presence of poly-unsaturated fatty acids (PUFAs) such as arachidonic acid (AA) but almost undetectable or very low levels of eicosapentaenoic acid (EPA) in the flesh of the snakeheads. Zuraini *et al.* (2006) analysed the fatty acid content of three *Channa* spp. and observed highest level of AA in *C. striata* (19%) as compared to other *Channa* spp. like *C. micropeltes* (4.71%) and *C. lucius* (12.41%) while EPA was not detected in *C. striata* and *C. lucius* but at low levels in *C. micropeltes* (3%). Furthermore, level of AA were also higher than other Malaysian fishes such as Belut Sawah (15.17%), Jelawat (2.83%), Kap Rumput (15.51%) and Siakap (9.07%) as reported by Endinkeau and Tan (1993). Zuraini *et al.* (2006) did not detect EPA in *C. striata* and these levels were also lower than EPA levels of other fishes such as Belut Sawah (2.96%),

Jelawat (2.68%), Kap Rumput (2.75%) and Siakap (5.12%) as reported by Endinkeau and Tan (1993). The composition of these two fatty acids, AA and EPA were in accordance with the wound healing properties of this snakehead fish and will be explained further details in section 2.1.3.

Amino acids are important biological compounds needed by human to ensure on-going metabolic processes. Besides these fatty acids, snakehead is also known to exhibit high levels of amino acids. Amino acids such as aspartic acid, glycine and glutamic acid have very important key role in the wound healing process (Chyun and Griminger, 1984). Zuraini *et al.* (2006) again observed that amino acid glutamic acid (21.7%), aspartic acid (11.4%) and glysine (4.3%) were also highest in C.*striata* when compared to *C.micropeltes* and *C.lucius*. The same author also explains that glycine, alanine, proline, arginine, serine, isoleucine and phenylalanine are all essential amino acids needed for polypeptide formation and are very important for tissue re-growth and healing with glycine being the main key ingredient of human skin collagen. Similarly, Jais *et al.* (1994) and Zakaria *et al.* (2007) too observed glycine being one of the most abundant amino acid in *C. striata* at 9.77% and 35.77% respectively although it also contains all the other important amino acids. Thus, having proven that *C. striata* contains all these important amino acids, it is no wonder this fish is famous for its wound healing properties.

Table 2.2 Amino acid composition of snakehead *Channa striata*.

Amino acid	% of total protein ± SD	
	Zuraini *et al.* (2006)	**Jais *et al.* (1994)**
Aspartic acid	11.4 ± 0.12	4.19 ± 0.60
Glutamic acid	21.7 ± 0.90	8.46 ± 1.06
Serine	4.8 ± 0.03	4.01 ± 0.21
Glycine	4.3 ± 0.19	9.77 ± 0.96
Histidine	1.2 ± 0.02	3.24 ± 0.15
Arginine	5.9 ± 0.15	2.34 ± 0.38
Threonine	4.2 ± 0.06	6.10 ± 0.43
Alanine	5.8 ± 0.73	8.40 ± 0.52
Proline	3.2 ± 0.21	9.17 ± 1.33
Tyrosine	3.6 ± 0.14	3.36 ± 0.38
Valine	4.2 ± 0.06	4.14 ± 0.44
Methionine	3.4 ± 0.11	5.99 ± 0.25
Cystine	0.9 ± 0.15	3.83 ± 0.17
Isoleucine	3.8 ± 0.25	5.54 ± 0.46
Leucine	7.5 ± 0.85	10.51 ± 0.83
Phenylalanine	4.3 ± 1.20	5.04 ± 0.62
Lysine	9.7 ± 0.57	5.89 ± 1.11

Values are % of the total amino acid ± SD.

2.1.3 Wound Healing Properties of Snakehead

Thanks to old-fashioned believes by local midwives and nurses which has been proven without a doubt over the course of time, therapeutic values of snakehead has been put to work in post-operated patients and caesarian destined mothers. It is said that by incorporating snakehead into their diets, it may speed up the recovery of wounds.

Various studies have shown positive effects on patients' recovery during post-operative duration (Jais *et al.*, 1994; Wee, 1982). Baie and Sheikh (2000)

demonstrated that application of cetrimide cream made with snakehead extracts on wounds resulted in greater tensile strength on wounds. These effects may have been attributed through the impeccably high levels of wound healing amino acids namely aspartic acid, glutamic acid and glycine (Chyun *et al.* 1984). A key component of human skin collagen, glycine interacts with aspartic acid and glutamic acid to generate polypeptides needed for growth and healing (Chyun *et al.* 1984; Heinmann, 1982).

Also present in high levels is essential fatty acid arachidonic acid, 20:4ω6 (Jais *et al.*, 1994; Zuraini *et al.*, 2006). Arachidonic acid is the most important prostaglandins precursor which induces platelet aggregation and endothelial cell adhesion which in turn initiates blood clotting (Bowman and Rand, 1980), stimulating blood cell responses to injury. On the other hand, low or almost undetectable EPA suggest that it is highly unlikely to interfere with blood clothing as EPA are active in antithrombotic activity and prostaglandin inhibition (Gibson, 1983).

2.1.4 Difficulty in Breeding Snakehead and Specialised Lipid Diet

Nevertheless, with the increase of human activity in many of the fishes' natural habitats where they breed, feed and reproduce, it is no surprise that numbers of wild fishes are quickly decreasing (Ambak and Zakaria, 2010). Farming of these fishes is also considered expensive and difficult because of its slow growth and very low survival rate due to its cannibalistic behavior (Boonyaratpalin *et al.*, 1985). Furthermore, being carnivorous, snakehead is listed as a pest in Malaysia and hence no attention was given on the culturing and farming of this fish (Jais, 2007) even

though snakehead has a high market price. So, snakehead will face extinction if demand continues to grow but only depended upon the catch of wild snakeheads.

Hence, a team of researchers from the School of Biological Sciences, Universiti Sains Malaysia (USM) developed feed fortified with lipid thought to improve reproductive performance of these fishes (Ghaedi *et al.,* 2014) easing cultivation of the fish. The best suited lipid level for feed was determined by feeding a group of fish with diets of different levels of lipid over a period of time before calculating their weight gain and also collecting tissue samples for testing. When there is an increase in supply, the fish can therefore be utilised to make into a more readily accessible form such as protein powders with longer shelf life. But till this very day, no such powder has been evaluated for any potential application in food.

2.2 Preserving Flesh Quality and Freezing Pre-treatment

2.2.1 Spoilage upon death

Fishes decay very quickly once killed mainly from two causes; (i) autolysis and (ii) bacterial decomposition. When fishes are alive, the naturally occurring bacteria and enzymes do not cause any degradation to its own flesh. However, when a fish dies, condition in the fish becomes unfavourable for enzymatic activities and their metabolic reaction slows down. These enzymes then begin self digestion or autolysis on the fishes' tissues. Invasion of bacteria into fish muscle also begins resulting in muscle decomposition and early quality loss of fresh fish which would have otherwise been prevented in living fishes due to their own metabolic reactions and defense mechanism (Lakshmanan, 2000). Aside from those two, chemical changes within fish muscle also cause degradation of fish

quality as oxidation of fats in the flesh begins immediately after removal from water (Olafsdóttir *et al.*, 1997).

2.2.2 Slime production

Mucus, also known as slime(s) are high molecular-weight watery substances that are made up mainly of water and gel forming macromolecules produced by the goblet cells (Shepard, 1994). Similarly, snakehead slimes are made up of water (95%), glycoproteins and fatty acids (Jais *et al.* 1998). Slime is also the primary physical barrier that prevents the entry of bacteria into the fish as it contains antibodies and bacteria-killing enzymes (Rottmann *et al.,* 1992).

As mentioned earlier, snakehead has a mucus layer that also helps them to thrive in waterless conditions. Balasubramanian *et al.* (2011) mentioned that fishes that need to survive challenging environments tend to develop mucus on its epidermal layer. Other scientist has also recorded slime secretion by snakehead which act as a protective barrier between the fish epidermal layer and it's surroundings when exposed to stressful conditions as a form of defense mechanism (Jais *et al.* 1998, Jais *et al.* 1997). Unfortunately, these slimy layer increases the difficulty in handling of the fish as the slime makes them slippery to touch.

2.2.3 Freezing Pre-treatment

Freezing of fishes immediately after killing has long been practiced as a way of preserving the quality of their flesh as spoilage rate is dependent upon temperature. If temperature of the fish was increased from 0 °C to 5 °C, rate of spoilage would have doubled (Lakshmanan, 2000). Hence, if the fishes are properly handled after killing by freezing them immediately, spoilage rate would be delayed.

However, snakehead was also observed to secrete slime when subjected to cold stress upon killing (Jais *et al.* 1998). Hence, to overcome the slime problem and ease the handling processes, previous researchers have placed the fish in bags filled with equal weight of distilled water and freezing them to a low ambient temperature of ≥ 0 °C (Hui *et al.,* 2010). Interestingly, by doing so, the slime secretes out and into the water, easing the handling processes. The migration of slime may have occurred due to differences in concentration of solutes because during freezing, ice crystals forms and solute concentration changes as water freezes out resulting in fish tissue dehydration (Shenouda, 1980; Careche *et al.*, 1999).

2.3 Fish Muscle Protein Powders

Fish muscle are not favourable to be used as a raw food ingredient as fish muscle are decayable and chemical composition varies drastically even among fishes of the same species and thus posses problems for the food processing industries (Kristinsson and Rasco, 2000). According to FAO Fisheries and Aquaculture

Department, proteins obtained from fish muscle tissues may be divided into three main categories:

(i) Structural proteins

Most of the proteins from fish are made up of this category. It constitute about 70-80% of the total protein content of fish. These proteins are usually soluble in salt solutions of high ionic strength of about 0.5M. Examples of structural proteins are actin, myosin, tropormyosin and actomyosin. These muscles are important for muscle movements in fishes.

(ii) Sarcoplasmic proteins

About 25-30% of the total protein content of fish is made up of this category. These proteins are usually soluble in neutral salt solutions of low ionic strength of < 0.15M. Examples of sarcoplasmic proteins are myoalbumin, globulin and enzymes but most of them are made up of enzymes involved in cell metabolism.

(iii) Connective tissue proteins

About 3% of the protein in teleostei and about 10% in elasmobranchii is made up of this category. Example of connective tissue protein is collagen. Collagen in fish is much more sensitive to heat destruction or decomposition but has fewer and more flexible crosslinks as compared to collagen from warm blooded vertebrates.

Fish muscle protein powders are known to have high biological value. During these recent years, studies on production of powdered form of fishes have been of interest as fish muscles are usually high in amino acid and are easily digestible protein. Furthermore, powdered forms of proteins are lighter in weight and therefore, are more mobile and are more stable due to low water activity (Hui *et al.*, 2010). Currently, various powders exist and are usually classified according to their lipid and protein content (Table 2.3).

Table 2.3 The various classifications of protein powders.

Type	**Classification**	**Source**
Fish Protein Isolate (FPI)	- More pure than FPC - Protein content of > 90 % - Fat content of < 1 %	Shaviklo, 2006
Fish Protein Concentrate (FPC)	Type A - A virtually odourles and tasteless powder having a maximum total fat content of 0.75 % Type B - A powder having no specific limits as to odour, flavor, having fishy flavor and a maximum fat content of 3 % Type C - Normal fish meal produced under satisfactory hygienic conditions	Food and Agriculture Organization of the United Nations (FAO), 1969
Fish Protein Hydrolysate (FPH)	- Peptides of different length broken down chemically or enzymatically from proteins	Kristinsson and Rasco, 2000
Fish Protein Powder (FPP)	- Food grade powder product intended for human consumption	U.S. Food and Drug Administration (FDA)
Fish Meal	- Very cheap FPC but not intended for human consumption - Used for making animal feeds - Fish meal made under hygienic condition is also known as FPC-Type C	FAO, 1969

To date, only liquid snakehead essence (for consumption purposes) and cream (for external application on the epidermal layer) are available commercially in the market. These products possess many challenges such as limited shelf life and difficulty in transportation due to bulkiness of liquid contained within the product. Therefore, if the protein extract maybe manipulated into dry powder form, the shelf life of snakehead protein maybe extended due to removal of water and subsequently reduce the bulkiness. Currently, only few researchers have successfully prepared powder form of protein extracted from the flesh of snakehead. Hui et al. (2010) reportedly prepared K-carrageenan encapsulated snakehead extracts by utilising a spray dryer while most other researcher prepared liquid extracts by using the traditional preparation method of cooking snakehead flesh in a pressure cooker (Jais *et al.* 1994, Dahlan-Daud *et al.*, 2010).

2.3.1 Effects of Drying on Quality of Fish Powders

One particular method of obtaining fish proteins is through drying. Fish drying has long been practiced and were traditionally produced through low cost sun drying or smoking (Doe, 1998) which requires heating as a drying step. However, methods of drying has become more industrialized to cope with market's demand and so, drying chambers or even ovens are now used instead of the traditional methods (Doe, 1998). Another method of drying is through freeze drying or lyopholisation which subject samples under very low temperature and vacuumed conditions, resulting in less oxidations and chemical modification to food ingredients (Chen *et al.,* 2013). Hence, if production cost is not an issue, freeze drying is preferable over high temperature oven drying for perishable and heat sensitive

proteins such as those from fish (Shaviklo, *et al.*, 2010) since this method of drying can be quite costly.

As mentioned in previous section, fish proteins are quite sensitive and easily damaged. Drying can have diverse effects on fish proteins particularly on its functional properties. Cordova-Murueta *et al.* (2007) saw a change in protein composition as well as decrease in solubility and proteolytic activity of nine different species of fish proteins due to dehydration stress. Whether the proteins were derived through heat or freeze-drying methods, this stress has lead to changes in protein structure leading to the denaturation of proteins. However, some researchers have successfully increase protein function through the addition of additives. Shaviklo *et al.* (2010) used sucrose as a lyoprotectant to protect proteins of saithe (*Pollachius* virens) from the adverse effects of drying.

2.3.2 Functional Properties

Besides having nutritional properties, proteins are also known for providing non-nutritional functional characteristics such as solubility, gelation, foaming and emulsification. (Table 2.4) These functional properties are some of the key properties of proteins which are important during food product formulation.

Like other commonly used protein such as soy or egg white protein, fish muscle protein may exhibit functional properties. Previous studies have shown that various fish protein powders too have functional properties (Table 2.5) but none has been recorded for snakehead thus far.

Table 2.4 Defining some of the functional properties of proteins.

Functional properties	Definition	Source
Solubility in NaCl	The amount of protein that is soluble in 3% NaCl solution	Santana *et al.*, 2001
Gelation	Ability to convert fluids into solid through macromolecular network formation	Foegeding and Davis, 2011
Foaming	Ability to form interfacial foams and film	Foegeding and Davis, 2011
Emulsification	Ability to aid in forming or stabilising newly made emulsions	Liceaga-Gesualdo and Li-Chan, 1999

Table 2.5 Protein powders made from various fish muscles that has been shown to exhibit functional properties

Fish Source	Drying Method	Functional properties	Source
Arrowtooth Flounder (*Atheresthes stomias*) and Herring (*Clupea harengus*)	Freeze-dried	Emulsifying and fat adsorption capacity	Sathivel *et al.*, 2004
Herring (*Clupea harengus*)	Freeze-dried	Emulsifying stability and foaming capacity	Liceaga-Gesualdo and Li-Chan, 1999
Red Salmon (*Oncorhynchus nerka*)	Freeze-dried	Solubility, fat adsorption, water adsorption and emulsification stability	Kristinsson and Rasco, 2000
Alaska Pollock (*Theragra chalcogramma*)	Freeze-dried	Solubility, fat adsorption and emulsion stability	Sathivel and Betchel, 2006
Carp (*Catla catla*)	Oven dried	Foaming capacity and stability	Elavarasan *et al.*, 2014

CHAPTER 3: PHYSICO-CHEMICAL PROPERTIES AND COMPOSITION OF COMMERCIALLY-BRED SNAKEHEAD FISH (*CHANNA STRIATA*) WHOLE FILLET POWDER (WFP) AND SNAKEHEAD PROTEIN POWDER (HPP) PREPARED WITH PRE-FILLETING FREEZING TREATMENT

3.1 Materials

Commercial snakehead were acquired and purchased from Jusco Bandar Perda, Penang, Malaysia. Only fishes weighing between 250 g to 450 g were used (*n* = 30).

3.2 Pre-treatment and Preparation of Snakehead

Fishes were packed into foam containers and filled with water just enough to cover the fishes. Containers were sealed and breathing holes were prepared on the boxes' cover by puncturing holes onto the cover with a sharp and pointed utensil. Fishes were then transported back to the lab for further processing where they were randomly assigned to three groups (ten for control, C; ten for freezing with distilled water, FW; and ten for directly freezing without distilled water, DF). Fishes were killed by burying the fishes in ice slurry (Blessing *et al.*, 2010) (Plate 3.1).

Plate 3.1 Fish were killed in ice slurries before weighing.

3.2.1 Freezing Pre-treatment

Two types of freezing conditions were used. The fishes were either frozen with distilled water (FW), frozen directly without water (DF) or freshly processed without freezing (control, C). All fishes were weighed and measured individually and their weights and total length were recorded.

In FW samples, each fish was placed into a clear plastic bag, filled with equal weight of distilled water and sealed with a commercial portable sealer machine and placed in - 20 ºC freezer for approximately 8 h. This was then transferred to a refrigerator (4 - 6 ºC) to thaw overnight. For DF samples, the steps were similar to that of FW except that no distilled water was added before putting the fish in the freezer.

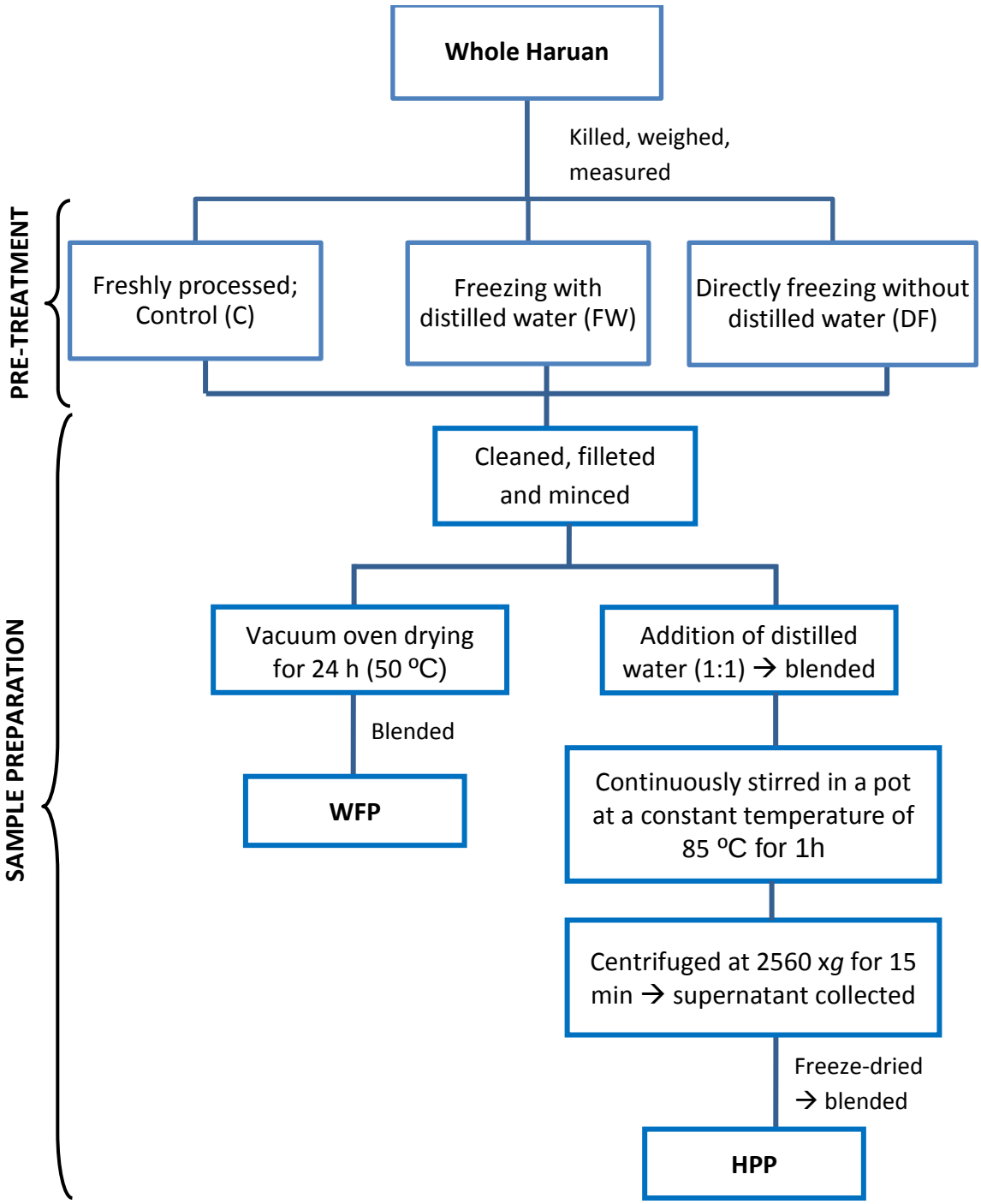

Figure 3.1 Flow chart depicting overall procedure for production of Whole Fillet Powder (WFP) and Snakehead Protein Powder (HPP). Prepared samples were then stored in an air tight container and kept in a fridge (4 ºC) until further analysis.

3.2.2 Filleting

Each fish was rinsed under running tap water prior to filleting. To maximize yield with minimum amount of bones during filleting, each fish was cut lengthwise along the back bone from the neck all the way to the tail (Zakaria *et al.,* 2007) (Plate 3.2). Skins were then removed and only fish flesh were put on ice to maintain freshness of the fillets. Fillets were cleaned with distilled water, patted dry with kitchen towel and placed in a pre-weighted container. Weights of fillet harvested were recorded. Fillet yield was calculated based on the formula below (Powell *et.al.,* 2008) (1).

$$\text{Fillet Yield \%} = \frac{WH}{WW} \times 100 \qquad (1)$$

where WH represents weight of fillets harvested in g and WW represents weight of whole fish in g.

Plate 3.2 Filleting of Snakehead by cutting the fish lengthwise along the back bone from the neck all the way to the tail.