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EXTRACTION AND CHARACTERIZATION OF AMINO ACIDS AND PROTEINS FROM WASTES OF THREADFIN BREAM, Nemipterus japonicus

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EXTRACTION AND CHARACTERIZATION OF AMINO ACIDS AND PROTEINS FROM WASTES OF THREADFIN BREAM, *Nemipterus japonicus* (Bloch, 1791)

By

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EXTRACTION AND CHARACTERIZATION OF AMINO ACIDS AND PROTEINS FROM WASTES OF THREADFIN BREAM, *Nemipterus japonicus* (Bloch, 1791)

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Chair : Prof. Shuhaimi Mustafa, PhD
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Fish such as *Nemipterus japonicus* is over-exploited in Malaysia and the amounts of this fish species processing wastes are staggering. The wastes are land-filled, ground and discarded or otherwise denied from human or animal consumptions, even though the wastes are still rich in proteins. This is due to the lack of methodologies to recover proteins from wastes from this species. Therefore, this study aimed at extraction of protein from the wastes of the threadfin bream, *Nemipterus japonicus* using pH shift solubilization and precipitation processes and evaluation of the influencing factors on protein extraction. Towards this objective, the wastes of head, skin and internal organs were collected and their nutritional composition evaluated. Furthermore, the ratio of waste: water for protein extraction was screened and the effect of pH, centrifugation speed and time on protein solubility rate, the amino acid composition and the molecular weight of proteins via SDS-PAGE were determined. Results demonstrated that moisture was the highest percentage (~70-78%) of all wastes. Highest protein content (19.67±1.10%), fat content (1.81±0.09%) and carbohydrate content (4.43±0.23%) were observed in skin, head and internal organs, respectively. The optimum ratio of waste: water for protein extraction is 1:9 for head, 1:8 for internal organs and 1:6 for skin. The protein extraction efficiently for all three samples were high at pH 3 and pH 12. Protein solubility increased with increased centrifugation speed up to 10,000 ×g, and there was no significant difference ($P>0.05$) between the protein solubility at 10,000 ×g and 20,000 ×g. Furthermore, there was significant difference ($P<0.05$) between protein solubility with increased centrifugation time. There is no significant difference ($P>0.05$) in protein yield between the alkaline and the acid process for heads and skin while protein yield in the alkaline and acid process for internal organ is significantly different ($P<0.05$). Alkaline process showed a remarkably higher amino acid content as compared to that of acid version.

Glutamic acid and lysine were found higher compared to other amino acids. The molecular weight of proteins isolated in this study were low (<100 KDa).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PENGEKSTRAKAN DAN PENCIRIAN ASID AMINO DAN PROTEIN DARIPADA SISA-SISA IKAN KERISI, *Nemipterus japonicus* (Bloch, 1791)

Oleh

NOORSULIYA RAIHAN BINTI YAHAYA

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Ikan seperti *Nemipterus japonicus* lebih dieksploitasi di Malaysia dan jumlah pemprosesan ikan spesies ini sangat meningkat. Sisa-sisa tersebut dipenuhi tanah dan dibuang atau tidak diterima sebagai keperluan makanan oleh manusia atau haiwan, walaupun sisa-sisa buangan masih kaya dengan protein. Ini adalah kerana kurangnya kaedah untuk memulihkan protein daripada sisa-sisa spesies ini. Oleh itu, kajian ini bertujuan untuk mengekstrakan protein daripada sisa buangan kerisi, *Nemipterus japonicus* menggunakan perubahan larutan pH dan proses pemendakan dan penilaian faktor-faktor yang mempengaruhi pengekstrakan protein. Berdasarkan objektif ini sisa-sisa daripada kepala, kulit dan organ dalaman dikumpulkan dan komposisi pemakanan sisa tersebut dinilai. Tambahan lagi, nisbah sisa: air untuk pengekstrakan protein telah diperiksa dan kesan terhadap pH, kelajuan sentrifugasi dan masa pada kadar kelarutan protein, komposisi asid amino dan berat molekul protein telah ditentukan melalui SDS-PAGE. Keputusan menunjukkan bahawa kelembapan adalah peratusan tertinggi (~70-78%) daripada semua sisa buangan. Kandungan protein tertinggi (19.67±1.10%), kandungan lemak (1.81±0.09%) dan kandungan karbohidrat (4.43±0.23%) diperhatikan dalam kulit, kapala dan organ dalaman. Nisbah optimum sisa: air untuk pengekstrakan protein adalah 1:9 untuk kepala, 1:8 untuk organ dalaman dan 1:6 untuk kulit. Pengekstrakan yang sesuai untuk ketiga-tiga sampel tersebut adalah tinggi pada pH 3 dan 12. Keterlarutan protein meningkat dengan peningkatan kelajuan sentrifugasi sehingga 10,000 ×g, dan tiada perbezaan signifikan ($P>0.05$) diantara kelarutan protein pada 10,000 ×g dan 20,000 ×g. Tambahan lagi, terdapat perbezaan signifikan ($P<0.05$) antara keterlarutan protein dengan peningkatan masa sentrifugasi. Tiada perbezaan yang signifikan ($P>0.05$) dalam hasil protein di antara proses alkali dan acid untuk kepala dan kulit manakala hasil protein dalam proses alkali dan acid untuk organ dalaman didapati perbezaan yang signifikan ($P<0.05$). Proses alkali menunjukkan kandungan asid amino yang lebih tinggi berbanding dengan versi

asid. Asid glutamik dan lisin didapati lebih tinggi berbanding asid amino yang lain. Berat molekul protein yang diasingkan dalam kajian ini adalah rendah (<100KDa).

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

<	Less than
>	More than
%	Percentage
µL	microliter
×g	Number of times the gravitational force
AOAC	Association of Analytical Communities
BSA	Bovine Serum Albumin
cm	Centimetre
FPH	Fish Protein Hydrolysates
FPI	Fish Protein Isolates
g	gram
GC-MS	Gas Chromatography-Mass Spectrometry
h	hours
H_2O	water
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
kDa	Kilo Dalton
M	Molarity
mA	milliamps
mg	milligram
min	minute
mL	milliliter
NaOH	Sodium hydroxide
pH	potential of Hydrogen
pI	Isoelectric point
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
UV-VIS	Ultraviolet-Visible
v/v	volume per volume
w/v	weight per volume
	Number of times the gravitational force

CHAPTER 1

INTRODUCTION

According to the World Health Organization, essential nutrients are vital for growth, disease prevention and also for a good health. It can be divided into two categories which are macronutrients and micronutrients. One of the major elements is protein. A part from egg, protein also can be consumed through fish in daily life dietary. Due to this reason, fish industries have become a massive economic source of food worldwide with an estimation of one billion people dependent over fish production, processes and trading in order to survive (Oosterveer, 2008). According to Dekkers *et al.*, (2011), the fish processing industry produces more than 60% wastes, which comprises viscera and roes, head, skin, frames, fins, trimmings, and only 40% fish products are made for human consumption. These large quantities of fish wastes which contains high amount of protein are either thrown away back into the sea, causing serious pollution and disposal problems or processed into low market-value products such as animal feed, fish meal and fertilizer (Hsu, 2010). However, proteins content has not been efficiently extracted and characterized.

Threadfin bream (*Nemipterus spp.*) is often caught by accident in shrimp trawlers because of its small size and sorting difficulty, thus threadfin bream has been labelled as a 'trash' fish (Normah & Nur Anati, 2015). However, nowadays threadfin bream is perceived as a fundamental raw material in the use of tropical surimi productions (Wiriyaphan *et al.,* 2015). According to Karthikeyan *et al.*, (2006), approximately 19% of protein is found in the flesh of a threadfin bream.

In food industry, the flesh is highly demanded but the heads, skins and internal organs of threadfin bream were discarded in large quantity. These wastes can be processed into commercially valuable food ingredients. Fish head presence of high protein content with good amino acid balance and bioactive peptides and fish skin is a rich source of collagen and gelatin. In addition, internal organs is also considered as a potential source that may have some valuable properties for the manufacturing of protein hydrolysates (Chalamaiah *et al.,* 2012). Nowadays, there are a lot of research activities regarding fish protein properties in order to improve waste from fish that can be transforming into valuable product other than fish fillets. This, will not only resolve environmental matters such as pollution, but could also have a great and positive impact on the world's economy (Rustad *et al.,* 2011).

Up until now, there have been several developed methods focusing on protein isolation which involves treatment by using heat, extraction using solvent, hydrolysis by using acid or enzyme, repeated water washing and refining as well as the combination of these various methods (Shaviklo, 2015). Nevertheless, these methods are found to have less commercial viable due to their negative influence in terms of functionality and nutritional qualities of the products (Hultin & Kelleher, 2002).

Two processes including alkaline or acid solubilization and isoelectric precipitation of proteins are known to have productions of highly functional and stable protein isolates (Worawan & Manat, 2016). These processes auger well for the purpose of protein extraction, from the waste of numerous species such as sardine (Cortes-Ruiz *et al.,* 2001), herring (Undeland *et al.,* 2002), Pacific whiting (Kim *et al.*, 2003), catfish (Kristinsson *et al.,* 2005), and Atlantic croaker (Kristinsson & Liang, 2006). However, it can be concluded that no report has been published on the potential application of these methods for the production of functional proteins from threadfin bream (*Nemipterus* spp.) heads, skins and internal organs. Thus, it is not surprising that the substance produces the second largest source of fish waste product, constituting important raw materials for surimi productions as well as fish crackers or 'keropok' – a popular snack in the East coast of Malaysian (Yongsawatdigul & Hemung, 2010; Wiriyaphan *et al.,* 2015).

As acid and alkali-aided processes are efficient and inexpensive methods for the extraction of protein from fish wastes. The objective of this study was to apply different solubilization, either at alkaline and acidic pHs, for protein concentrate production from threadfin bream wastes and evaluate their amino acids profiles, nutritional properties and protein recovery yield. Results from this study are expected to provide important information on protein isolation using the pH-shift process with the potential to improve the value of fish wastes and increase their use for human consumption.

With the realization on the economic importance of the much sought-after fish protein isolate in the food industry, the general objective of this study was directed towards the protein extraction from fish wastes, it is hypothesized that protein yield and extraction efficiency could be increased by pH-shift method. Based on the above hypothesis the specific objectives of the study in this thesis were to:

1. To optimize the extraction of protein from *Nemipterus japonicus* wastes using pH-shift method.
2. To identify the profiling of protein and amino acid of extracted protein from *Nemipterus japonicus* wastes.

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