UNIVERSITI PUTRA MALAYSIA

THE PRODUCTION OF THREADFIN BREAM (NEMIPTERUS JAPONICUS) PROTEIN HYDROLYSATE BY HYDROLYSIS WITH ALCALASE

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By

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Thesis submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

November 2001

~ To my friends ~

Kyaw Zay Ya, Amin Ismail (Dr), Anida, Shita, Leen, Nazri, Manichan, Mohsin, Yusep, Misnawi, Shida, En. Azman, Kak Jem, En. Halim and Mr Chan.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: Associate Professor Hajjah Jamilah Bakar, Ph.D.

Faculty: Food Science and Biotechnology

The optimization of hydrolysis conditions in terms of pH, temperature, enzyme-substrate (ES) ratio and hydrolysis time for the production of threadfin bream (*Nemipterus japonicus*) hydrolysate was studied. Alcalase showed a higher percent nitrogen recovery (% NR) than Flavourzyme:Protamex mixture. By using Alcalase, the optimum hydrolysis conditions were pH 8.5, 60°C, ES2% and 120 min hydrolysis time. At least twenty percent degree of hydrolysis (% DH) and 70% NR was achieved. The yield of spray-dried hydrolysate hydrolysed under these conditions was 4%. The hydrolysate was white in color, highly soluble over a wide pH range, high in protein and essential amino acids but low in fat. The hydrolysate also exhibited an improvement in foam ability in comparison to the threadfin bream muscle but the foam was unstable. Hydrolysis with Alcalase also produced hydrolysate with poor emulsifying properties. The major free amino acids in the hydrolysate were glutamic acid, aspartic acid, lysine, leucine and arginine in which glutamic acid was the dominant amino acid. Bitter amino acids methionine, valine, isoleucine, phenylalanine, leucine, arginine and tyrosine comprised of 42.34% of the total free amino acids. The inosine 5'-monophosphate (IMP) content was higher in the hydrolysate than that in the muscle. SDS-PAGE showed the presence of twenty peptide bands in the muscle having molecular weight between 7.2 to 87.6 kD and thirteen in the hydrolysate with molecular weight between 7.2 to 64 kD. For Sephadex G-75 fractions (FR), no peptide bands were seen in FR I, III, IV, VI, VII and VIII. FR II and V showed the presence of respectively five and seven peptide bands with molecular weight between 11.4 to 54 kD and 10.5 to 51.3 kD. Histamine content in the muscle was found to be 14.45 mgkg ^{1} but no histamine was detected in the hydrolysate. Sensory evaluation of the hydrolysate indicated that it was fishy in flavor and constitutes three basic tastes namely bitter, umami and salty with umami and bitterness as the dominating tastes.

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

PENGHASILAN HIDROLISAT PROTIN "KERISI" (*NEMIPTERUS JAPONICUS)* MELALUI HIDROLISIS DENGAN MENGGUNAKAN ALKALASE

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November 2001

Pengerusi: Profesor Madya Hajjah Jamilah Bakar, Ph.D.

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Persekitaran optima dari segi pH, suhu, nisbah enzim kepada substrat dan jangkamasa penghidrolisisan bagi menghasilkan hidrolisat 'Kerisi' (*Nemipterus japonicus)* telah dikaji. Didapati Alkalase menghasilkan peratus nitrogen (% NR) yang lebih tinggi berbanding campuran Flavourzyme:Protamex. Dengan menggunakan Alkalase, persekitaran optima yang diperolehi adalah pH 8.5, 60^{o}C, ES2% dan 120 minit. Sekurang-kurangnya 20% darjah hirolisis (% DH) dan 70% nitrogen (% NR) telah dihasilkan. Penghasilan hidrolisat kering melalui persekitaran ini adalah sebanyak 4%. Hidrolisat yang di hasilkan berwarna putih, mudah larut pada pH yang berbagai, tinggi kandungan protein dan asid amino asas tetapi rendah lemak. Hidrolisat juga menunjukkan penghasilan buih yang lebih baik dibandingkan isi ikan 'Kerisi' akan tetapi buih yang terbentuk adalah tidak stabil. Penghidrolisisan menggunakan Alkalase

juga menghasilkan hidrolisat yang mempunyai sifat-sifat emulsi yang lemah. Asid amino bebas di dalam hidrolisat terdiri daripada asid glutamik, asid aspatik, lisin, liusin dan arginin di mana asid glutamik merupakan asid amino utama. Asid amino pahit iaitu methionin, isoliusin, valin, fenalalanin, liusin, arginin dan tirosin merangkumi 42.34% daripada kandungan keseluruhan asid amino bebas. Kandungan inosin 5'-monophosphate (IMP) adalah lebih tinggi di dalam hidrolisat dibandingkan di dalam isi ikan. SDS-PAGE menunjukan terdapatnya duapuluh jalur peptida di dalam isi ikan dengan berat molekul di antara 7.2 dan 87.6 kD dan tigabelas jalur peptida dengan berat molekul diantara 7.2 dan 64 kD di dalam hidrolisat. Pecahan (FR) Sephadex G-75 menunjukan ketiadaan jalur peptida di dalam FR I, III, IV, VI VII dan VIII. FR II dan V menunjukan terdapatnya lima dan tujuh jalur peptida dengan berat molekul di antara 11.4 dan 54 kD serta 10.5 dan 51.3 kD. Kandungan histamin dalam isi ikan adalah 14.45 $mgkg^{-1}$ dan histamin tidak dapat dikesan di dalam hidrolisat. Penilaian deria mendapati bahawa hidrolisat mempunyai rasa dan bau hanyir di samping mempunyai tiga rasa asas iaitu pahit, umami dan masin di mana umami dan pahit merupakan rasa yang amat ketara.

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Au/g	Anson Unit per gram
ES	enzyme-substrate ratio
% NR	percent nitrogen recovery
% DH	percent degree of hydrolysis
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
D	Dalton
kD	kilo Dalton
RH	Relative Humidity
ID	Internal Diameter
RP	Reverse Phase
QDA	Quantitative Descriptive Analysis
IMP	Inosine 5'- monophosphate
FR	Fraction
meq/g	microequivalent per gram
nm	Nanometer
FAO/WHO	Food and Agriculture Organization/World Health Organization
HPLC	High Pressure Liquid Chromatography

CHAPTER I

INTRODUCTION

Hydrolysis of protein by the application of enzyme to produce fish protein hydrolysate is another tool for recovering protein from underutilized fish species (Hoyle and Merritt 1994; Quaglia and Orban 1987a; Shahidi et al. 1994). Hydrolysate is a modified form of protein consisting of a mixture of peptides of varying sizes and can be partially concentrated to form a liquid, spray-dried or freeze-dried hydrolysate to obtain a product with a highly concentrated protein (Skanderby, 1994). Hydrolysate is produced for use as milk replacer, food ingredients, flavor enhancer or as protein supplement (Rebeca et al. 1991; Yanez et al. 1976). The potential source of raw material for fish protein hydrolysate production are underutilized fish, by-catch as well as wastes from processing operation such as head, tail, bone, skin and viscera (Kim et al. 1994; Kim et al. 2000).

Fish protein hydrolysate can be prepared by the hydrolysis of either the fatty or non-fatty fish species. However, the preparation of hydrolysates from fatty-fish species requires either the removal or stabilization of fat prior to the hydrolysis process (Ritchie and Mackie 1982; Hoyle and Merritt 1994; Liceaga-Gesualdo and Li-Chan 1999). Although enzymatic hydrolysis of fish protein produces hydrolysate with high protein content and excellent solubility, bitterness and fishy off-flavor are not an uncommon problem in fish protein hydrolysate (Shahidi et al. 1995; Hevia and Olcott 1977; Hoyle and Merritt 1994). Several studies have been carried out to overcome these problems such as partial deodorization by passing through steam containing orthophosphoric acid and ethanol extraction (Hoyle and Merritt 1994; Venugopal and Lewis 1981).

By-catch refers to the non-targeted fish species that are caught incidentally with the targeted species such as the shrimp or commercial fish. They are heterogeneous in composition consisting of species that are very small, bony, dark flesh and toxic while others having strong flavor and unattractive appearance and texture. These factors caused sorting difficulties and due to some of the aforementioned factors in addition to the wide market price gap in comparison to the shrimp price, the fish gain less popularity and therefore are often discarded at sea (Venugopal and Shahidi 1995; Wan Ismail and Abdullah 1983). The wastage of by-catch has been a major problem in the world fisheries. Statistics between 1988 to 1990 indicated that out of 77 million tons global marine catches, 35% were discarded by-catch which were mostly from the shrimp trawlers and Malaysia's shrimp trawler was listed as one of the top twenty fisheries with the highest recorded discard (Alverson et al. 1994). In the study of species classified as by-catch, threadfin bream was listed among the largest species identified (Wan Ismail and Abdullah, 1983). In a more recent study reported by Matsushita and Ali (1997) during the on-deck sorting selectivity studies in Kuala Kedah, Malaysia showed that threadfin bream was grouped as small or low-price ('trash fish') in addition to slender shad (*Ilisha elongata*) and squid. In Malaysia, by-catch are used in fish meal production, some have been cured into dried salted fish or used in fish noodles production for human consumption (Wan Ismail and Abdullah, 1983). They have also been used for marinated and breaded product in Brunei, surimi production in Thailand and as mechanically deboned such as in India (Mariani et al. 1996; Suwanrangsi 1988; Venugopal and Shahidi 1995).

Studies regarding fish protein hydrolysate involved the optimization of the hydrolysis conditions, physico-chemical and functional properties, its potential use in fish sauce production, removing fishy off-flavor and determining the nature of bitter components (Diniz and Martin 1996; Shahidi et al. 1997; Beddows and Ardeshir 1979; Hoyle and Merritt 1994, Hevia and Olcott 1977). The production of threadfin bream (*Nemipterus japonicus*) hydrolysate has not been carried out thus far. Therefore, the purposes of this study are 1) to compare the activity of Alcalase and Flavourzyme:Protamex mixture on the hydrolysis of threadfin bream and to determine the optimum hydrolysis conditions of the selected enzyme or enzyme mixture namely the pH, temperature, enzyme-substrate ratio and reaction time, 2) to determine the physico-chemical, functional properties and sensory characteristics of Alcalase produced threadfin bream hydrolysate and 3) to study the taste and intensity characteristics of the hydrolysate (five basic tastes namely bitter, sweet, sour, salty and umami).