

OPTIMIZATION OF ENZYMATIC HYDROLYSIS CONDITION FOR AMINO
ACID AND TAURINE PRODUCTION FROM *THUNNUS TONGGOL* VISCERA
AS FISH PROTEIN HYDROLYSATES

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DEDICATION

For my husband
Mohd Nor Hazli Hassan,

My sons
Ahmad Qalifahmy, Ahmad Qairanfahmy, Ahmad Qairinfahmy and
Ahmad Qahfi Adha...
I love u and thank you

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To my life-partner, my husband Mohd Nor Hazli Hassan: because I owe it all to you. Many Thanks! My cheerleader, my sons: Thank you for being so understanding to Ibu..I love u all!!

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ABSTRACT

The common additives in cat food, particularly synthetic amino acid and taurine are commonly produced through chemical processes. This has triggered concern for long-term adverse health effects on cats. Hence, the demand for natural amino acid and taurine has increased. As a whole, this study aimed to produce amino acid and taurine in fish protein hydrolysates (FPH) from Longtail Tuna (*Thunnus tonggol*) viscera using bromelain and papain enzyme mixture. The main objective of this study was to optimize the enzymatic hydrolysis processing condition of *Thunnus tonggol* viscera towards high-quality FPH. The enzymatic hydrolysis process was done by combining the ground viscera with the enzyme mixture and this new mixture was then incubated to produce FPH. The FPH was first characterized for its physical properties, before it was optimized and compared to the commercial benchmarked fish flavour (BeFF). The optimization process was conducted using response surface methodology where the responses analyzed were the amount of total protein (%), total amino acid (g/100g) and taurine (g/100g). From the optimization analysis, compared to BeFF, the optimized FPH contained twice the amount of total amino acid and taurine which were 136.10 g/100g and 0.55 g/100g. It was also found that the optimized FPH contained 15.4 % of total protein. The physical properties of the optimized FPH was comparable to BeFF with pH 4.01 and viscosity 1962 cP. The sensory evaluation on the smell and aroma strength of the optimized FPH shows that both were well accepted. The optimal processing conditions that contributed to the high production of protein, amino acid and taurine were found to be at a temperature of 47.6 °C, incubation time of 22 hours and 0.97 % of enzyme concentration. Taken together, the current data showed that the optimization study has successfully produced a high-quality FPH with high amount of protein, amino acid and taurine content. This study provides a new perspective on the manufacturing process of natural amino acid and taurine, and it also demonstrates the potential of these product to be used as fish flavour in cat food.

ABSTRAK

Bahan tambah di dalam makanan kucing terutamanya asid amino dan taurina tiruan biasanya dihasilkan melalui proses kimia. Ini telah mencetuskan kebimbangan terhadap kesan mudarat dalam jangka masa panjang ke atas kesihatan kucing. Jadi, permintaan terhadap asid amino dan taurina semulajadi semakin meningkat. Secara keseluruhannya, kajian ini adalah bertujuan untuk menghasilkan asid amino dan taurina di dalam hidrolisat protein ikan (FPH) dari perut ikan Tuna Longtail (*Thunnus tonggol*) menggunakan campuran enzim bromelin dan papain. Objektif utama kajian ini adalah untuk mengoptimumkan keadaan proses hidrolisis enzim perut ikan *Thunnus tonggol* ke arah penghasilan FPH berkualiti tinggi. Proses hidrolisis enzim dilakukan dengan menggabungkan perut ikan kisar bersama campuran enzim dan seterusnya campuran baharu ini dieramkan bagi menghasilkan FPH. Pencirian fizikal ke atas FPH dilakukan terlebih dahulu sebelum dioptimasi dan dibandingkan dengan perisa ikan komersial (BeFF). Proses pengoptimuman dilakukan dengan menggunakan kaedah sambutan permukaan di mana tindakbalas yang dinilai adalah jumlah protein (%), jumlah asid amino (g/100g) dan taurina (g/100g). Daripada analisis pengoptimuman, dibandingkan dengan BeFF, FPH optimum mengandungi dua kali ganda jumlah asid amino dan taurina iaitu sebanyak 136.10 g/100g dan 0.55 g/100g. Didapati bahawa, FPH optimum mengandungi 15.4 % jumlah protein. Sifat-sifat fizikal FPH optimum adalah hampir sama dengan BeFF dengan pH 4.01 dan kelikatan 1962 cP. Penilaian deriaan ke atas bau dan kekuatan aroma FPH optimum mendapat keputusan penerimaan yang baik. Keadaan optima pemprosesan yang menyumbang kepada penghasilan tinggi protein, asid amino dan taurina adalah pada suhu 47.6 °C, tempoh pengeraman selama 22 jam dan kepekatan enzim sebanyak 0.97 %. Secara keseluruhannya, data dari kajian ini menunjukkan bahawa proses pengoptimuman telah berjaya menghasilkan FPH yang berkualiti tinggi dengan tinggi jumlah kandungan protein, asid amino dan taurina. Kajian ini juga memberi perspektif baharu tentang proses penghasilan asid amino dan taurina secara semulajadi dan ia juga menunjukkan potensi produk ini untuk digunakan sebagai perisa ikan dalam makanan kucing.

TABLE OF CONTENTS

	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	vi
	LIST OF FIGURES	viii
	LIST OF SYMBOLS	x
	LIST OF ABBREVIATIONS	xi
	LIST OF APPENDICES	xii
CHAPTER 1	INTRODUCTION	1
	1.1 Introduction of Research	1
	1.2 Problem Statement	3
	1.3 Hypothesis	4
	1.4 Research Objectives	4
	1.5 Research Scope	5
CHAPTER 2	LITERATURE REVIEW	6
	2.1 Overview of Fish By-product in the Fishing Industry	6
	2.2 Methods in Recovering Bioactive Compound from Fish Wastes	8
	2.2.1 Chemical Hydrolysis	9
	2.2.2 Enzymatic Hydrolysis	10
	2.3 Fish Protein Hydrolysates for Fish Flavouring	13
	2.3.1 The Nutritional Value of FPH	16

2.4	Factors Affecting FPH Production	18
2.4.1	Type of Enzyme	18
2.4.2	Enzyme Concentration	22
2.4.3	Incubation Time	22
2.4.4	Temperature	23
2.4.5	The Mechanism of Protease	24
2.5	Introduction to Cat Diet and Cat Food	25
2.5.1	The Importance of Taurine for Cat	26
2.5.2	Development and Types of Commercial Cat Food	29
2.5.3	Nutrient requirement of cats	30
2.6	<i>Thunnus tonggol</i>	32
2.7	Optimization of Hydrolysis Using Response Surface Methodology (RSM)	33
2.8	HPLC for Amino Acid Determination	35
CHAPTER 3	METHODOLOGY	37
3.1	Research Framework	37
3.2	Raw Materials, Chemicals and Reagents	39
3.3	Determination of Nutritional Value	40
3.3.1	Determination of Total Protein	41
3.4	Determination of Total Amino Acid and Taurine	42
3.4.1	Preparation of Standard Solutions	42
3.4.2	Amino Acid Analysis	43
3.4.3	Performic Acid Hydrolysates Preparation	45
3.5	Physical Properties of FPH	46
3.5.1	Analysis of Viscosity	46
3.5.2	Test of pH	47
3.5.3	Analysis of Colour	47
3.5.4	Sensory Evaluation of Smell	48
3.6	Production of Fish Protein Hydrolysates as Fish Flavour	49
3.7	Screening on the Processing Condition	50
3.8	Optimization	51
3.9	Verification	54

3.9.1	Mineral Analysis	54
CHAPTER 4	RESULT AND DISCUSSION	55
4.1	Introduction	55
4.2	Production of FPH for Fish Flavour	56
4.2.1	The Physical Properties of FPH Formulations	58
4.2.2	Screening of Parameters of the Processing Condition	63
4.3	Optimization of Processing Condition of FPH	65
4.4	Null Hypothesis of Processing Parameters of Fish Protein Hydrolysates	67
4.5	Analysis of Total Protein, Taurine and Total Amino Acid by Response Surface Methodology (RSM)	73
4.5.1	Effect of Interaction of Temperature (°C), Time (Hour) and Concentration (%) on Total Protein Content (%)	81
4.5.2	Effect of Interaction of Temperature (°C), Time (Hour) and Concentration (%) on Taurine Content (g/100g)	84
4.5.3	Effect of Interaction of Temperature (°C), Time (hour) and Concentration (%) on Total Amino Acid Content (%)	87
4.6	Optimization of Processing Condition of FPH Using Response Surface Methodology	89
4.7	Verification	91
4.7.1	Proximate Analysis of Viscera, BeFF and Optimum FPH	92
4.7.2	Amino Acid Composition and Taurine Content	95
4.7.3	Physical Properties of BeFF and Optimized FPH	98
4.7.4	Mineral Analysis of Viscera, BeFF and Optimum FPH	100

CHAPTER 5	CONCLUSION	102
5.1	Conclusion	102
5.2	Recommendation	103
REFERENCES		104

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Overall nutritional composition of fish viscera (Ghaley <i>et al.</i> , 2013)	7
Table 2.2	Previous study on the development of FPH from fish by-products	12
Table 2.3	Minimum nutrient requirement for a cat in adult and growing or reproductive stage (European Pet Food Industry Federation, 2011; Vogt <i>et al.</i> , 2010)	31
Table 3.1	Screening of parameters and conditions for FPH production	51
Table 3.2	Independent variables, their coded and actual level	52
Table 3.3	Central composite design for optimization of enzymatic hydrolysis of <i>Thunnus tonggol</i> viscera developed from CCD	53
Table 4.1	Formulation of FPH for fish flavour development	56
Table 4.2	The physicochemical properties of each FPH formulation	58
Table 4.3	The mean value of sensory evaluation of FPH developed	61
Table 4.4	Analysis of variance for aroma attributes	62
Table 4.5	Analysis of variance for the strength of aroma attributes	62
Table 4.6	Screening of hydrolysis processing condition	64
Table 4.7	The processing condition range of FPH obtained from OFAT design	65
Table 4.8	Experimental design for optimization of FPH	66

Table 4.9	Analysis of variance for total protein	68
Table 4.10	Analysis of variance for taurine	68
Table 4.11	Analysis of variance for total amino acid	68
Table 4.12	Summary of ANOVA for total protein	69
Table 4.13	Summary of ANOVA for taurine	70
Table 4.14	Summary of ANOVA for total amino acid	72
Table 4.15	Coefficient estimate of the quadratic model for total protein	73
Table 4.16	Coefficient estimate of the quadratic model for taurine	75
Table 4.17	Coefficient estimate of the quadratic model for total amino acid	76
Table 4.18	Comparison between predicted and experimental responses at optimum conditions	91
Table 4.19	Proximate analysis of raw viscera and benchmark FPH	93
Table 4.20	The amino acid in BeFF and viscera	97
Table 4.21	The pH, viscosity and colour results of BeFF fish flavour	98
Table 4.22	Minimum mineral daily requirement for the adult cat and the mineral content of viscera, BeFF and optimized FPH	101

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Hydrolysis of protein (Tavano, 2013)	9
Figure 2.2	Chemical structure of bromelain enzyme (Sigma Aldrich, 2018)	20
Figure 2.3	Chemical structure of papain enzyme (PubChem, 2018)	21
Figure 2.4	Mechanism action of cysteine protease enzyme (Rao <i>et al.</i> , 1998)	24
Figure 2.5	Chemical structure of taurine	27
Figure 2.6	Formation of taurine from methionine and cysteine in the body (De Luca <i>et al.</i> , 2015)	27
Figure 2.7	Longtail tuna (<i>Thunnus tonggol</i>)	32
Figure 2.8	<i>Thunnus tonggol</i> viscera	33
Figure 2.9	The chromatographic profile of amino acid and taurine in FPH	36
Figure 3.1	Flow chart of research activities	38
Figure 3.2	Flow chart of amino acid standard preparation	43
Figure 3.3	Flow chart of sample preparation (Waters, 2006)	44
Figure 3.4	Flow chart of performic acid hydrolysis (Waters, 2006)	46
Figure 4.1	The colour comparison of F1 (left-control; no enzyme) and F11 (right-formulation with enzyme)	60
Figure 4.2	Predicted values versus actual values for total protein (%)	78

Figure 4.3	Predicted values versus actual values for taurine (g/100g)	78
Figure 4.4	Predicted values versus actual values for total amino acid	78
Figure 4.5	Externally studentized residuals versus run number for total protein (%)	80
Figure 4.6	Externally studentized residuals versus run number for taurine (g/100g)	80
Figure 4.7	Externally studentized residuals versus run number for total amino acid (g/100g)	81
Figure 4.8a, b, c	Response surface plot of the interaction between AB, AC and BC towards total protein content (%) in FPH.	82
Figure 4.9a, b, c	Response surface plot of the interaction between AB, AC and BC towards taurine (%) in FPH.	85
Figure 4.10a, b, c	Response surface plot of the interaction between AB, AC and BC towards total amino acid (%) in FPH.	88
Figure 4.14	Point of optimized conditions for maximum responses	90
Figure 4.15	The physical appearance of optimized FPH (left) and BeFF (right)	100

LIST OF SYMBOLS

%	-	Percentage
° C	-	Degree Celcius
μL	-	Microliter
μm	-	Micrometre
μmole	-	Micromole
mL	-	Millilitre
v/v	-	volume / volume
mm	-	Millimetre
rpm	-	Rotation per minute
cP	-	Centipoise
mg	-	Milligram
μg	-	Microgram
g	-	Gram
±	-	Plus minus
R ²	-	Coefficient of multiple determination
α	-	Alpha
MW	-	Molecular weight

LIST OF ABBREVIATIONS

BeFF	-	Benchmark Fish Flavour
FPH	-	Fish protein hydrolysates
HPLC	-	High Performance Liquid Chromatography
ANOVA	-	Analysis of variance
RSM	-	Response Surface Methodology
CCD	-	Central Composite Design
AOAC	-	Association of Official Analytical Chemists
ICPMS	-	Inductively Coupled Plasma Mass Spectrometry
SPSS	-	Statistical Package for the Social Sciences
F1 – F11	-	Formulation 1 – Formulation 11
IU	-	International Unit
P	-	Probability
M	-	Mean
SD	-	Standard deviation

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	Example of sensory evaluation form for fish protein hydrolysate (FPH)	119
Appendix B	Technical Data Sheet of Commercial Benchmark Fish Flavour	120
Appendix C1	Graph of interaction between two responses (AB, AC, BC respectively) towards total protein	121
Appendix C2	Graph of interaction between two factors (AB, AC, BC respectively) towards taurine content	123
Appendix C3	Graph of interaction between two factors (AB, AC, BC respectively) towards total amino acid content	125
Appendix D	Example of HPLC chromatogram of amino acid and taurine in fish protein hydrolysates	127

CHAPTER 1

INTRODUCTION

1.1 Introduction of Research

Increased in income generations among consumers have encouraged pet adoption especially cats as in many homes around the world. Additionally, the trend of domesticating exotic cats such as Bengal, Persian, Sphynx, British Shorthair continued to grow positively over the years throughout countries in Asia. As a result, the growth of commercial cat food has increased by 5.54% from 2016 and 2017 and are predicted to be in progressive trend in the forthcoming year (Mordor, 2018). Moreover, pet industry players are offering a variety of choices for pet food products in term of value for money and nutrition thus adding more to its positive growth.

All cat food products must meet a certain nutrient requirement in ensuring that the cat obtained its balanced diet which is important for its health and fitness. One of the most important nutrient for a cat is taurine and amino acids as it helps in function and maintaining its vision, reproduction system, growth, and other metabolic functions (Ripps and Shen, 2012; Verbrugghe and Bakovic, 2013). During food manufacturing, all the ingredients are cooked and process in such a temperature and condition resulting some of its essential such as amino acid, taurine, and vitamins were lost due to processing heat or oxidation. Currently, to fortify the food again with its essential nutrient the common practices adopted by the manufacturer is to add synthetic taurine and amino acid into its final product. These practices have

caused concern among pet owner about the safety issue on the usage of synthetic additives to cats health and wellbeing.

Animal proteins are rich in amino acid and taurine and it can be yield by many hydrolysis methods. Fish waste is an abundant source of amino acid and taurine and hydrolysis of this protein waste will produce fish protein hydrolysates. Fish waste usually consists of fish viscera, head, bones, skin, scales, and frames (Martínez-Alvarez *et al.*, 2015; Klomklao, 2008). Processes such as base and acid precipitation, enzymatic hydrolysis, resins, and others have been studied for many years and were utilized to produce many types of fish protein hydrolysate (FPH) (Benjakul *et al.*, 2014; Roslan *et al.*, 2014). This FPH are highly valuable in fertilizer, food, and feed industry, pharmaceutical and nutraceutical as well as cell and microbial cultivations (Chalamaiah *et al.*, 2012; Yarnpakdee *et al.*, 2015).

Furthermore, it has good functional properties such as high solubility in a wide range of pH and temperature, good emulsifier, high stability, good flavour and strong aroma making it a favourable choice over its original proteins (Zapata *et al.*, 2018). Some studies also have shown that FPH has good antioxidant properties by oxygen scavenging radicals and metal-chelating ability (Arason *et al.*, 2009). Due to this, FPH has also been utilized as one of the ingredients or as a flavouring agent in producing good and high-quality pet food (Herpandi *et al.*, 2011). It has been found can improve the taste and flavour in dry kibbles and also wet food. Henceforth, based on the statistic given by Euromonitor International (2015), the demand of safe, low cost and natural seafood flavour is increasing due to the increasing demand and production of pet food in Malaysia.

1.2 Problem Statement

Cat needs a diet with a balanced amount of protein, fat, minerals and carbohydrate. Another important essential nutrient for a cat to maintain its health is amino acid and taurine. A good commercial cat food must at least meet the minimum daily nutrient requirement for a cat. Synthetic amino acid and taurine is usually being used as an additive in pet food to compensate for the nutrient loss during processing as well as a nutrient requirement by the cat. The synthetic additives are produced using strong chemicals such as ammonia and sulphuric acid which might cause long-term health effect to cats. Because of this, reducing the use of these synthetic additives in cat food has been the aim of the researchers and the manufacturer and this can be done by producing natural amino acid and taurine from its ingredients based.

On the other hand, the fish processing industry has produced a large volume of fish waste that can pollute the environment by the river and land contamination as well as loss of economic values if not handled properly. These wastes can be utilized to produce FPH containing valuable high-quality protein. In addition, FPH has been used in many pet foods and it can also a great source of amino acid and taurine. In response to both synthetic additive and fish waste problem, this study proposed enzymatic protein hydrolysis as a method to produce high-quality fish protein hydrolysates. The study also suggested the use of papain and bromelain enzyme mixture for the enzymatic hydrolysis process as it has been found to be effective in producing high-quality FPH. The mixture of papain and bromelain is also cost-effective and its synergistic effect is desirable for the production of amino acid and taurine. The optimization of enzymatic hydrolysis for production of taurine and amino acid from *Thunnus tonggol* viscera and the study its processing condition of temperature, duration and enzyme concentration has yet been done before. Thus, these parameters were chosen as the controlled parameter and its effect on the quality of protein hydrolysate produced was studied.

1.3 Hypothesis

Fish protein hydrolysate made from *Thunnus tonggol* has a high content of amino acid and taurine. The FPH from *Thunnus tonggol* can be developed into fish flavour for cat food flavouring (palatant). Optimization in the processing conditions of enzymatic hydrolysis of temperature, enzyme concentration and duration of hydrolysis will produce high quantity and quality of amino acids and taurine in fish protein hydrolysates.

1.4 Research Objectives

The objectives of the research are as follows:

1. To produce fish protein hydrolysate from *Thunnus tonggol* viscera.
2. To optimize the enzymatic hydrolysis processing parameters of *Thunnus tonggol* viscera for production of high-quality amino acid and taurine content in fish flavour.

1.5 Research Scope

To achieve the research objective, five scopes have been outlined in this research

1. Determination of the nutritional value of *Thunnus tonggol* viscera and the benchmark FPH. The physical properties such as pH, viscosity, colour and smell quality of BeFF was also determined.
2. Production of FPH by enzymatic hydrolysis of *Thunnus tonggol* viscera using papain and bromelain enzyme.
3. Screening on the processing condition of enzymatic hydrolyses such as temperature, enzyme concentration and time of hydrolysis was done using one factor at a time method (OFAT) to determine the minimum and maximum value range.
4. Optimization of enzymatic hydrolysis processing conditions for total protein, taurine, and total amino acid in fish flavour production. The optimization of the formulation was carried out based on three different parameters which are temperature, enzyme concentration and hydrolysis time using response surface methodology of Central Composite Design (CCD) from Design Expert 7.0 software.
5. Comparison of nutritional value, physicochemical properties, mineral content and sensory on the smell of optimized fish flavour with viscera and BeFF.

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