



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND PHYSICOCHEMICAL PROPERTIES OF
COLLAGEN EXTRACTED FROM BARRAMUNDI (*Lates calcarifer*, Block)
SKIN***

UMI HARTINA MOHAMAD RAZALI

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By

UMI HARTINA MOHAMAD RAZALI

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Chairman: Professor Jamilah Bakar, PhD

Faculty: Faculty of Food Science and Technology

Collagens from barramundi (*Lates calcarifer*) skins were extracted by alkaline pre-treatment with mild acid extraction (0.5 M acetic acid); with and without (ASC) the addition of pepsin (PSC) and papain (PaSC). The use of papain was studied to replace the use of conventional enzyme, pepsin, in the collagen extraction process, which is of haram or non-halal origin. The collagens obtained were evaluated for their physico-chemical properties such as colour, odour, amino acid composition, molecular weight distribution and solubility characteristics. Optimization for collagen extraction using papain was carried out by Response Surface Methodology (RSM). The selected independent variables were papain concentration (10-50 kUnit/g) and extraction time (12-36 hr) with dependent variables of yield, hydroxyproline, total amino acid and imino acid content. Comparisons were then carried for the collagen obtained from the optimized process with commercial mammalian and tilapia collagens in their amino acid composition, thermal stability, viscosity and isoelectric point. The microstructures of the collagens were evaluated by scanning electron microscopy (SEM) while their secondary structures were

determined using Fourier Transform Infrared Spectroscopy (FTIR). Five-fold increments in yield were obtained by enzymatic extraction when compared to the non-enzymatic (8.1 %) extraction. Both PSC and PaSC produced high yields (~44 %). The protein content of the collagens was in the range of 60-85 %. The collagens were basically colourless although the enzymes aided-extractions were slightly darker. Collagens from papain treatment had the highest total amino acid content (728.54 mg/g), with pre-dominant amount of glycine, proline, alanine and arginine. Imino acid (pro+hyp) content of ASC, PSC and PaSC were 193, 198 and 195 residues/1000 residues, respectively. The SDS-PAGE pattern of PSC was similar to ASC; however, PaSC was distinctly different. All the extracted collagens were of type 1 with apparent polypeptides molecular weight distribution of 37 to 250 kDa. They had high solubility in pH of 2 to 5 and increasing NaCl concentration up to 2 %. Response Surface Analysis showed the significant ($p < 0.05$) in all reduced models with high overall coefficient of determination value ($R^2 > 0.8$). The optimized process was the combination of 30.4 kUnit/g of papain and at 24.5 hr of contact time. The predicted collagen yield, hydroxyproline, total amino acid and imino acid content under optimal conditions were estimated to be 58 %, 90 residues, 935 mg/g and 214 residues, respectively. The experimental values were 59.7 %, 89 residues, 948 mg/g and 209 residues, respectively. The imino acid content of barramundi collagen was higher than the commercial bovine collagen (202), but lower than observed for porcine collagen (220). T_{max} of barramundi collagen was found at 38.17 °C. The viscosity of barramundi and commercial mammalian collagens decreased rapidly with increasing temperature from 5 to 35 °C, and then decreased at a slower rate from 40 to 50 °C. Isoelectric point (pI) of barramundi collagen determined by zeta potential was at pH 6.01. Based on the FTIR analysis,

amide A, B I, II and III were detected for all collagens with slightly different IR regions.



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PENCIRIAN DAN CIRI-CIRI FIZIKOKIMIA KOLAGEN YANG DI EKSTRAK DARIPADA KULIT IKAN SIAKAP (*Lates calcarifer*, Blok)

Oleh

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Kolagen daripada kulit siakap telah diekstrak melalui pra-rawatan dengan alkali dan asid sederhana dengan dan tanpa (ASC) penggunaan pepsin (PSC) dan papain (PaSC). Papain dikaji untuk menggantikan penggunaan enzim yang biasa digunakan dalam pengekstrakan kolagen iaitu pepsin yang berasal daripada sumber haram. Kolagen yang diperolehi telah dikenalpasti ciri-ciri fiziko-kimianya seperti warna, bau, komposisi asid amino, berat molekul dan keterlarutan. Kondisi optimum untuk pengekstrakan kolagen telah dikaji dengan menggunakan kaedah respon permukaan (RSM). Pembolehubah yang dimanipulasi adalah kepekatan papain (10-50 kUnit/g) dan tempoh ekstraksi (12-36 jam) manakala, pembolehubah bertindakbalas adalah kadar perolehan, kandungan hydroxyprolina, jumlah asid amino dan asid imino. Perbandingan telah dibuat di antara kolagen yang diperolehi daripada keadaan optima dengan kolagen komersial mamalia dan tilapia untuk ciri komposisi asid amino, kestabilan terma, kelikatan dan titik isoelektrik. Struktur-mikro kolagen dikenalpasti melalui mikroskopi imbasan elektron (SEM) sementara struktur sekundernya pula ditentukan oleh Spektroskopi Fourier Transform Inframerah

(FTIR). Perolehan sebanyak 5 kali ganda telah didapati daripada pengestrakan menggunakan enzim berbanding dengan tanpa penggunaan enzim. Kedua-dua PSC dan PaSC menghasilkan kadar perolehan yang tinggi (~44%). Kandungan protein dalam kolagen adalah di antara 60-85 %. Secara umumnya, kolagen yang didapati adalah tidak berwarna walaupun kolagen daripada pengekstrakan enzim kelihatan sedikit gelap. Kolagen daripada rawatan papain mempunyai jumlah asid amino yang paling tinggi (728.54 mg/g) dengan kandungan dominan glysina, prolina, alanina dan arginina. Kandungan asid amino untuk ASC, PSC dan PaSC adalah 193, 198 dan 195 residu/1000 residu, masing-masing. Corak SDS-PAGE bagi kolagen daripada PSC adalah sama dengan ASC tetapi kolagen daripada PaSC adalah jelas berbeza. Semua kolagen yang telah diekstrak adalah jenis I yang mempunyai berat molekul polipeptida di antara 37 hingga 250 kDa. Kolagen yang diekstrak mempunyai ketelantaran yang tinggi pada pH 2 hingga 5 dan dengan peningkatan kepekatan NaCl sehingga 2%. Analisis respon permukaan telah menunjukkan kadar yang ketara ($p < 0.05$) dalam semua model dengan penentuan nilai pekali yang tinggi ($R^2 > 0.8$). Proses yang optima adalah kombinasi kepekatan papain pada 30.4 kUnit/g dan tempoh pengekstrakan selama 24.5 jam. Kadar perolehan, kandungan hydroxyprolina, jumlah amino asid dan imino asid yang diramalkan pada kondisi optimum adalah 58 %, 90 residu, 935 mg/g dan 214 residu, masing-masing. Nilai yang didapati daripada eksperimen adalah 59.7%, 89 residu, 948 mg/g dan 209 residu, masing-masing. Kandungan asid imino dalam kolagen siakap adalah lebih tinggi berbanding dengan kolagen lembu komersial (202) tetapi lebih rendah daripada kolagen babi komersial (220). Nilai T_{max} kolagen siakap didapati pada 38.17 °C. Kelikatan kolagen siakap dan mamalia komersial berkurangan secara mendadak dengan penambahan suhu daripada 5 hingga 35°C, dan kemudian

berkurang pada kadar yang perlahan daripada 40 hingga 50 °C. Titik isoelektrik (pI) bagi kolagen siakap yang ditentukan oleh 'zeta potential' adalah pada pH 6.01. Berdasarkan analisis FTIR, amida A, B, I, II dan III telah dikenalpasti dalam semua kolagen, dengan zon FTIR yang berbeza.



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I certify that an Examination Committee has met on « « « to conduct the final examination of Umi Hartina binti Mohd Razali on her Master of Science thesis entitled 'Characterization and physico-chemical properties of collagen extracted from barramundi (*Lates calcarifer*) skin ' in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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Date : 20 July 2012



TABLE OF CONTENTS

| | Page |
|---|-------------|
| ABSTRACT | ii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | vi |
| APPROVAL | vii |
| DECLARATION | ix |
| LIST OF TABLES | xii |
| LIST OF FIGURES | xiii |
| LIST OF ABBREVIATIONS | xvi |
| CHAPTER | |
| 1 INTRODUCTION | 1 |
| 2 LITERATURE REVIEW | |
| 2.1 Collagen | 5 |
| 2.2 Structure and properties of collagen | 6 |
| 2.2.1 Types and molecular structure of collagen | 9 |
| 2.2.1.1 The fibril-forming collagen (Types I, II, III, V and XI) | 9 |
| 2.2.1.2 The FACIT collagen (Type IX, XII, XIV) | 12 |
| 2.2.1.3 Microfibrillar collagen (Type VI) | 12 |
| 2.2.1.4 Short chains collagen (Type X and VIII) | 13 |
| 2.2.1.5 Basement membrane collagen (Type IV) | 13 |
| 2.2.2 Fibril structure of collagen | 14 |
| 2.2.3 Amino acid composition | 16 |
| 2.3 Sources of collagen | |
| 2.3.1 Mammalian sources | 18 |
| 2.3.2 Aquatic sources | 19 |
| 2.4 Extraction of collagen | |
| 2.4.1 Acid extraction | 21 |
| 2.4.2 Enzymatic extraction | 22 |
| 2.5 Collagen from aquatic sources | |
| 2.5.1 Extractability | 23 |
| 2.5.2 Optimization of fish collagen extraction | 29 |
| 2.5.3 Characterisation of fish collagen | |
| 2.6 Applications of collagen | 44 |
| 2.7 Collagen perspective | 47 |
| 3 EFFECTS OF PEPSIN AND PAPAIN ON EXTRACTION OF COLLAGEN FROM BARRAMUNDI (<i>Lates Calcarifer</i>) SKINS | |
| 3.1 Introduction | 48 |
| 3.2 Materials and methods | |
| 3.2.1 Materials | 50 |
| 3.2.2 Chemicals reagents | 50 |
| 3.2.3 Extraction of collagen | 50 |

| | | |
|----------|---|----|
| 3.2.4 | Analyses | |
| 3.2.4.1 | Proximate compositions | 51 |
| 3.2.4.2 | Yield of collagen | 51 |
| 3.2.4.3 | Determination of pH | 51 |
| 3.2.4.4 | Determination of colour | 52 |
| 3.2.4.5 | Determination of odour | 52 |
| 3.2.4.6 | Determination of molecular weight | 53 |
| 3.2.4.7 | Determination of amino acid composition | 53 |
| 3.2.4.8 | Determination of collagen solubility | 54 |
| 3.2.4.9 | Statistical analyses | 55 |
| 3.3 | Results and discussion | |
| 3.3.1 | Moisture, ash, fat and crude protein of fish skin | 55 |
| 3.3.2 | Yield of collagen | 56 |
| 3.3.3 | Proximate Compositions | 56 |
| 3.3.4 | pH of collagen | 57 |
| 3.3.5 | Colour properties | 57 |
| 3.3.6 | Odour properties | 58 |
| 3.3.7 | SDS-PAGE profile | 61 |
| 3.3.8 | Amino acid compositions | 63 |
| 3.3.9 | Solubility at different pH and salt concentration | |
| 3.3.9.1 | Effect of pH | 67 |
| 3.3.9.2 | Effect of NaCl concentration | 68 |
| 3.4 | Conclusion | 70 |
| 4 | OPTIMIZATION OF COLLAGEN EXTRACTION FROM BARRAMUNDI (<i>Lates calcarifer</i>) SKINS USING RESPONSE SURFACE METHODOLOGY (RSM) | |
| 4.1 | Introduction | 71 |
| 4.2 | Materials and methods | |
| 4.2.1 | Materials | 73 |
| 4.2.2 | Chemical reagents | 73 |
| 4.2.3 | Extraction of collagen | 73 |
| 4.2.4 | Experimental design | 73 |
| 4.2.5 | Determination of yield | 74 |
| 4.2.6 | Determination of amino acid composition | 74 |
| 4.2.7 | Analysis of data | 74 |
| 4.2.8 | Optimization of collagen extraction | 75 |
| 4.2.9 | Verification of models | 76 |
| 4.3 | Results and discussion | |
| 4.3.1 | Fitting the response surface model to significant independent variables | 77 |
| 4.3.2 | Yield of collagen | 83 |
| 4.3.3 | Hydroxyproline content | 85 |
| 4.3.4 | Total amino acid content | 87 |
| 4.3.5 | Imino acid content | 89 |
| 4.3.6 | Optimization procedure for predicting an optimum conditions of collagen extraction from barramundi (<i>Lates calcarifer</i> skin) | 91 |
| 4.3.7 | Verification of the final reduced models | 93 |
| 4.4 | Conclusion | 95 |

| | | |
|----------|--|-----|
| 5 | PHYSICO-CHEMICAL PROPERTIES OF COLLAGEN FROM BARRAMUNDI (<i>Lates Calcarifer</i>) SKINS | |
| 5.1 | Introduction | 96 |
| 5.2 | Materials and methods | |
| 5.2.1 | Materials | 97 |
| 5.2.2 | Chemical reagents | 97 |
| 5.2.3 | Proximate compositions | 98 |
| 5.2.4 | Determination of amino acid composition | 98 |
| 5.2.5 | Differential scanning calorimetry (DSC) | 98 |
| 5.2.6 | Determination of viscosity | 99 |
| 5.2.7 | Determination of isoelectric point (pI) | 99 |
| 5.2.8 | Fourier transform infrared spectroscopy (FTIR) | 100 |
| 5.2.9 | Scanning electron microscopy (SEM) | 100 |
| 5.2.10 | Statistical analysis | 100 |
| 5.3 | Results and discussion | |
| 5.3.1 | Proximate compositions | 101 |
| 5.3.2 | Amino acid compositions | 102 |
| 5.3.3 | Thermal stability | 106 |
| 5.3.4 | Viscosity | 108 |
| 5.3.5 | Isoelectric point | 109 |
| 5.3.6 | Fourier transform infrared spectroscopy (FTIR) | 112 |
| 5.3.7 | Electron microscopy observation | 119 |
| 5.4 | Conclusion | 121 |
| 6 | CONCLUSION AND RECOMMENDATIONS | 122 |
| | REFERENCES | 125 |
| | APPENDICES | 141 |
| | BIODATA OF STUDENT | 154 |
| | LIST OF PUBLICATIONS AND ACHIEVEMENT | 155 |