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

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July 2012

Chairman: Professor Jamilah Bakar, PhD

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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 (R²> 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 ºC. The viscosity of barramundi and commercial mammalian collagens decreased rapidly with increasing temperature from 5 to 35 ºC, and then decreased at a slower rate from 40 to 50 ºC. 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.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

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 didapat 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 gysina, 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 ketelarutan 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 berkurangkan 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.

UMI HARTINA MOHAMAD RAZALI

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