International Food Research Journal 20(2): 835-842 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Properties of collagen from barramundi (*Lates calcarifer*) skin

[1,2,*]Jamilah, B., Umi Hartina, M. R., Mat Hashim, D. and [2]Sazili, A. Q.

[1]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[2]*Halal Products Research Institute, Putra Infoport Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

Article history

Received: 29 September 2011
Received in revised form: 9 November 2012
Accepted: 9 November 2012

Keywords

Collagen
Barramundi *(Lates calcarifer)*
enzyme-aided extraction
skin

Abstract

The properties of collagens from Barramundi (*Lates calcarifer*) skin obtained by acid solubilized (control), pepsin and papain aided extractions were investigated. The yields of collagens (dry weight basis) for acid solubilized, pepsin and papain aided extractions were 8.1, 43.6 and 44.0%, respectively. The collagens were generally colorless although collagens from the enzymes aided-extractions were slightly darker. Based on the e-nose evaluation, the collagens were considered odorless. The pH of all the collagens was in the vicinity of 3; however, those extracted with papain had significantly higher pH. The polypeptide profiles obtained in the SDS-PAGE analysis for pepsin extracted collagen were similar to those of acid solubilized collagens. Papain extracted collagen had distinctly different SDS-PAGE pattern. All the extracted collagens were of type 1 with apparent peptides molecular weight distribution of 37 to 250 kDalton. They had high solubility in pH 2 to 5 and increasing NaCl concentration up to 6%.

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Introduction

The interest of collagen as a functional ingredient and as a food supplement has been growing sharply within the last few years. Collagen has been traditionally extracted from the skins and hides of bovine and porcine in the past decades, however, collagen extraction from fish skin processing waste such as from the skins, bones is increasing in the need to recover valuable components from the waste. Religious constraints have also been the pushing factor. Fish skin collagens from Nile perch (*Lates niloticus*) skin (Muyonga *et al*., 2004); bigeye snapper (*Priacanthus tayenus*) skin (Kittipattanabawon *et al*., 2005; Nalinanon *et al*., 2007) and grass carp (*Ctenopharyngodon idella*) skin (Zhang *et al*., 2007) have been reported. These collagens were reported to have different characteristics from the mammalian collagen.

Collagen extraction is conventionally carried out using organic acids such as acetic, citric and lactic acid. Enzymatic pretreatment in collagen extraction by incorporating proteolytic enzymes non-specific for collagen such as pepsin, trypsin and pancreatin have been reported (Higheberger, 1961; Nishihara, 1962). Pepsin mainly of porcine origin has been reported to solubilise the collagen molecules from cross-linked fibrils by removing the telopeptides (intermolecular cross-links) regions (Nalinanon *et al*., 2007; Zhang *et al*., 2007). Solubility of collagen after enzymatic treatment may vary among fish species due to the differences in intrinsic structure of the tissues. The present outbreak of the swine flu may also create negative implication of such collagen. The use of papain, the plant-derived protease to aid the extraction of collagen from cardiovascular tissue in metabolism related study had been quite numerous. However, using papain to extract collagen as a food ingredient had hardly been reported except that of squid skin (Zheng *et al*., 2009).

Barramundi (*Lates calcarifer*) is a high value fish and it is one of the many fish species which is cultured in Malaysia and also found in other parts of the world. Presently, during filleting, the skin is discarded. Therefore, it is a potential source of raw material for collagen extraction. Therefore, the use of papain, was attempted in this study. The physico-chemical properties of the extracted collagen were then compared to that extraction by pepsin and mammalian collagens for comparison.

*Corresponding author.
Email: *jamilah@putra.upm.edu.my*

Materials and Methods

Materials

Barramundi skins were obtained from a local fish processing company in Selangor, Malaysia. The skins were washed under running tap water to remove superfluous materials and scales. Prior to the extraction of the collagen, the skins were cut into small pieces (0.5 cm x 0.5 cm) to facilitate the extraction process. Remaining skins were placed in polyethylene bags and stored at -20°C for up to two months if not used immediately.

Chemical reagents

All the chemicals used were of analytical grade. Pepsin (EC 3.4.23.1; 3260 U/mg) from porcine stomach mucosa and papain (EC 3.4.22.2; 30000 USP-U/mg) from Carica papaya were obtained from Merck (Darmstadt, Germany).

Extraction of collagen

The collagen was extracted according to Nalinanon *et al*. (2007). All the extraction processes for control (acid solubilized), pepsin and papain aided extraction (Jamilah *et al*., 2010) processes were performed at 4°C. Pepsin aided extraction was also carried for comparison. After alkaline pre-treatment and removal of fat, the skins were soaked in 0.5 M acetic acid with a solid/solvent ratio of 1:15 (w/v) and subjected to hydrolysis. Enzymatic hydrolysis was carried out with the addition of 20 kUnits of enzyme/g of skin for 24 h.

Moisture, ash, fat and crude protein

The moisture, ash, fat and crude protein content of the skin and the obtained collagens were determined according to AOAC (1995) standard procedures. Analyses were carried out in triplicates and calculated on dry weight basis of the skin.

Yield of collagen

The yields of collagens were calculated based on the weight of the skin.

Determination of pH

The pH of the extracted collagen was measured according to the British Standard Method BS 757 (British Standard Institute, 1975). A 1.0 % (w/v) collagen solution was prepared in distilled water and cooled to room temperature of about 25°C. The pH was measured with a glass electrode (Toledo 320 pH meter, Mettler-Toledo Instrument, Greifensee, Switzerland) which was standardized against pH 4.0 and 7.0 buffers. pH determination was carried out in triplicates.

Determination of color

Color measurements were made using Hunterlab Ultrascan Sphere Spectrocolorimeter (Minolta Cr-300 Series, US) and was reported as the 'L'- 'lightness', 'a'- 'redness' and 'b'- 'yellowness' values. The instrumental color was measured in triplicates.

Determination of odor

Odor characteristics of collagens were analyzed using the electronic nose, zNose™ (7100 Vapor Analysis System, Electronic Sensor Technology, New Bury Park, USA). The zNose™ system was equipped with a single, uncoated, high quartz surface acoustic wave (SAW) sensor. About 5 g of sample was weighed into a septa-sealed screw-cap bottle. The sample's vapor was pumped for 5 sec into the e-nose with a side-ported sampling needle. The column temperature was programmed to increase from 40 to160 °C at a rate of 5 °C per sec. The flow rate of the purified helium was fixed at 3 ml/min. The vapor pressure developed was detected as peaks and the peak height and areas correspond to the concentration of the volatiles. Each detected was express as a count (ct). Each sample was evaluated five times to obtain a stable odour profile.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed according to Laemmli (1970). The samples were dissolved in 0.02 M sodium phosphate containing 1 % sodium dodecyl sulphate and 3.5 M urea (pH 7.2). The mixtures were then centrifuged at 8500 g for 5 min at room temperature to remove undissolved debris. Solubilised samples containing 20 µg/µl of protein were mixed at 1:1 (v/v) ratio with the sample buffer (0.5M Tris-HCl, pH 6.8, containing 4 % SDS, 20 % glycerol). Gels were casted in Mini Protein unit (Bio-Rad Laboratories Inc., Richmond, CA, USA) and samples were loaded on the gel and a constant current of 15 mA/gel was passed through for 75 min. Gels were then stained with 0.05% (w/v) Coomassie Blue R-250 and destained overnight. Precision Plus ProteinTM Standards (Bio-Rad Laboratories, Inc., Hercules, CA, USA) (MW range of 10 kDa to 250 kDa) were used to estimate the molecular weights of peptides. Type 1 collagen (234112) from calf skin (Merck, Darmstadt, Germany) was also loaded for comparison.

Determination of amino acid composition

The collagen was first hydrolysed and this was followed by derivatization prior to analysis. The barramundi skin collagens were acid hydrolysed with 6N HCl (5 ml) at 110 °C for 24 h. Upon completion

of the hydrolysis, the residues were added with 4 ml of internal standard (AABA) and were made up to 100 ml volume by deionised water. The derivatisation was carried out using AccQ-Fluor Reagent kit (Waters Co., Milford, USA). Standard solution or hydrolysed samples of 10 µl were topped up with 70 µl of borate buffer and 20 µl AccQ reagent. The mixtures were incubated at room temperature for 1 min and tranferred to auto sampler vials. After 10 min incubation at 55 °C, 5µl of the standards and samples were automatically injected into the HPLC column for peak separation.

HPLC analysis

Separations were achieved using a 3.9 x 150 mm AccQ Tag RP-column (Waters Co., Milford, USA) using a gradient run. The eluent system consisted of two components: AccQ Tag concentrate as Eluent A and 60 % acetonitrile as Eluent B. The gradient condition was programmed as follows: 100 % A in 0.5 min, 98 % A in 14.5 min, 90 % A in 4 min, 87 % A in 13 min, 65 % A in 2 min, 0 % A in 3 min, followed by 100% A for 13 min at the flow rate of 1 ml/min. Detection was achieved using fluorescence detector (FD) (Waters 2475, Waters Co., Milford, USA). Blank test, standard and samples were evaluated at E_{λ}=250nm and E_m=395nm. The determination of the amino acids was carried out using Waters Auto analyzer (Waters 2690/5, Waters Co., Milford, USA).

Determination of collagen solubility

The solubility of collagens at different pH and NaCl concentrations were evaluated according to the method of Montero *et al*. (1991). Collagen samples were dissolved in 0.5 M acetic acid with gentle stirring at 4 °C for 12 h to obtain the final concentration of 3 and 6 mg/ml. Eight ml of collagen solutions (3 mg/ml) were transferred to a centrifuge tube and the pH was adjusted with either 6 M NaOH or 6 M HCl to obtain a final pH ranging from 1 to 10. The solution was made up to 10 ml of distilled water, previously adjusted to the same pH as the sample solution tested. Five ml of collagen solutions (6 mg/ml) were mixed with 5 ml of cold NaCl in acetic acid (0-12 % (w/v), to obtain the final NaCl concentrations of 1-6 % (w/v). The solutions were stirred gently for 30 min at 4 °C and centrifuged at 10 000 x g for 30 min, also at 4 °C. Protein content (Lowry *et al*., 1951) in the supernatants was then determined.

Statistical analysis

All data collected were analyzed using Analysis of Variance (ANOVA) and Tukey's Comparison Test. The significance level among the means using statistical programme analyses package were also determined (Minitab Version 14, 2008).

Results and Discussion

Moisture, ash, fat and crude protein of fish skin

Not many reports are available on the moisture content of fish skin. Barramundi (*Lates calcarifer*) skin has a high moisture content of 68.5±0.4 % which was similar to the moisture content of nile perch (*Lates niloticus*) skin (68.4±0.6%) (Muyonga *et al*., 2004) and higher than that of bigeye snapper (*Priacanthus tayenus*) skin (64.08±0.1%) (Kittiphattanabawon *et al*., 2005). Higher moisture content (73.4%) was reported for the skin of brown backed toadfish (*Lagocephalus gloveri*) (Senaratne *et al*., 2006). The crude protein content of barramundi skin was 63.3±1.8% which was greater than the bigeye snapper skin (Kittiphattanabawon *et al*., 2005) and nile perch skin (Muyonga *et al*., 2004). However, the ash (0.84±0.1%) and fat contents (0.11±0.1%) in barramundi skin were lower than that of the Nile perch and bigeye snapper skins.

Yield of collagen

The yields (dry weight basis) of collagen from barramundi skins for both pepsin (PSC) and papain (PaSC) aided extraction were 5-fold higher (PSC – 43.6%; PaSC – 43.9%, respectively) than that of control (ASC) (8.1%) (Table 1). Hence, the use of the two enzymes had enhanced the extractability of the collagen from barramundi skin. The yield obtained for the ASC was similar to that reported for grass carp skin (8.0%) (Zhang *et al*., 2007) and slightly lower than that of the bigeye snapper skin (10.9%) (Kittipattanabawon *et al*., 2005). Pepsin was used in previous works to increase extractability of collagen from the raw materials. Nagai *et al*. (2002) and Zhang *et al*. (2007) had shown the efficiency of pepsin extraction for ocellate puffer fish and for grass carp fish, respectively. They reported collagen yield of 35% for ocellate puffer skin and 47% for grass carp skin. No significant difference was observed between the yield from pepsin solubilized collagen (PSC) and papain-solubilized collagen (PaSC). Thus, suggesting that papain could also be used to extract collagen and in fact, it would be a cheaper alternative enzyme. No reported work on collagen extraction from fish skin using papain was found for comparison.

Proximate analysis

The protein and moisture content of the extracted collagen were as shown in Table 1. The results showed that both collagens extracted with the aid of enzyme had significantly ($p<0.05$) higher protein

Table 1. The yield, protein, moisture, ash and fat content, pH and instrumental colour of collagen from barramundi skins

Properties	Fish skin	ASC	PSC	PaSC
Yield (% dry basis)	-	8.12±1.23^{a}	43.63±2.34^{b}	43.91±1.19^{b}
Protein content (%)	63.3±1.8	68.72±0.95^{a}	80.01±0.84^{b}	82.5±0.96^{c}
Moisture content (%)	68.5±0.4	12.38±1.23^{a}	11.55±0.47^{a}	11.12±0.31^{b}
Ash content (%)	2.95±0.1	1.28±0.05^{a}	0.95±0.03^{b}	0.93±0.10^{b}
Fat content (%)	0.11±0.1	0.04±0.01^{a}	0.09±0.03^{b}	0.06±0.02^{c}
pH at 25°C	7.13±0.5	3.41±0.01^{a}	3.44±0.01^{a}	3.93±0.04^{b}
Hunter colour values				
'L'	-	65.41±0.08^{a}	61.33±0.04^{b}	44.76±0.02^{c}
'a'	-	0.14±0.01^{a}	2.59±0.02^{b}	0.74±0.02^{c}
'b'	-	3.16±0.03^{a}	5.35±0.04^{b}	2.14±0.04^{c}

Values were means ± standard deviation of three replicates.
Values with the different superscripts within each row were significantly different ($p < 0.05$).
ASC: Acid-soluble collagen, PSC: Pepsin-soluble collagen, PaSC: Papain-soluble collagen

Table 2. Amino acid compositions of collagen from barramundi (*Lates calcarifer*) skins (mg/g sample)

Amino acid	Control	PSC	PaSC
Hyp	47.72±0.48^{c}	56.88±0.57^{b}	60.89±0.87^{a}
Asp	43.14±0.43^{b}	48.41±0.10^{a}	48.38±0.83^{a}
Ser	17.26±0.17^{b}	19.42±0.05^{a}	19.07±0.99^{a}
Glu	69.62±0.70^{b}	74.42±0.09^{a}	74.21±1.00^{a}
Gly	125.82±1.26^{c}	128.94±1.92^{b}	146.32±7.09^{a}
His	4.43±0.04^{c}	7.73±2.73^{b}	9.23±0.21^{a}
Arg	57.04±0.57^{b}	60.40±0.05^{a}	68.56±0.28^{b}
Thr	17.44±0.17^{c}	19.22±0.02^{a}	18.14±0.09^{b}
Ala	70.55±0.71^{c}	77.08±0.26^{a}	74.34±0.51^{b}
Pro	80.39±0.80^{b}	83.32±0.29^{a}	78.85±0.34^{c}
Cys	ND	ND	ND
Tyr	3.84±0.04^{a}	2.52±0.01^{b}	2.62±0.01^{b}
Val	18.49±0.18^{a}	18.35±0.13^{a}	18.49±0.05^{a}
Met	15.03±0.15^{a}	14.65±0.42^{a}	14.42±0.83^{a}
Lys	28.91±0.29^{b}	30.86±0.22^{a}	31.54±1.02^{a}
Ile	10.22±0.10^{a}	9.72±0.33^{b}	9.43±0.14^{b}
Leu	19.10±0.19^{a}	18.31±0.03^{b}	18.38±0.30^{b}
Phe	13.55±0.14^{a}	13.29±0.56^{ab}	12.73±0.17^{b}
Total	664.53	706.79	728.54

Values are presented as the mean ± SD of duplicates samples.
Values with the different superscripts within each row were significantly different ($p < 0.05$).
ND- not detected
ASC: Acid-soluble collagen, PSC: Pepsin-soluble collagen, PaSC: Papain-soluble collagen.

contents than the control. The protein content of PaSC was significantly higher ($p<0.05$) than PSC. The protein content of the collagen obtained from the control (68.71%) was lower than the collagen from bigeye snapper skin (94.0%) obtained by the same extraction method (Kittiphatanabawon *et al*., 2005), but higher than the protein content reported for hake skin collagen (33.5%) (Montero *et al*., 1990). These differences could probably be due to genetic differences. All collagens had significantly lower ash and fat contents than the skin.

pH

The pH of collagens from barramundi skin was in the acidic range and is typical for acidic extracted collagens, however, PaSC was the least acidic among the three (Table 1). The PaSC was significantly ($p<0.05$) less acidic than the control and PSC. No significant difference ($p>0.05$) was observed between the control and PSC.

Color

Color of freeze-dried collagens obtained from the Hunterlab Ultrascan Sphere Spectrocolourimeter is shown in Table 3.1. The 'L' (lightness) value of the control, PSC and PaSC showed significant ($p<0.05$) differences, of which the control was the lightest. A high 'L' value indicates a whiter attribute of the samples. Lower 'L' value observed for all samples suggested that there was carry-over of the skin pigment during extraction, which contributes to the darker appearance. Further step to decolorize the collagens may be needed, if required for commercial application. There were also significant differences ($p<0.05$) in 'a' (redness) and 'b' (yellowness) values among the collagens. PSC and PaSC were slightly more colored than the control. Reports on the color properties of other sources of collagens are limited for comparison.

Odor properties

The odor patterns of collagen samples are as shown in the chromatogram (zNose™) (Figure 1) and the fingerprint (VaporPrintTM) (Figure 2). The chromatogram showed the vaporized derivatives of the condensates due to the separation of the volatiles from the non-volatiles for the operating temperature of the surface acoustic wave (SAW) crystal used in the study.

The zNose™ chromatogram showed sixteen detectable volatiles for the control and PaSC, while eighteen peaks were observed for PSC. However, the odor pattern of all the collagens were similar but of different intensities. Figure 2 shows the VaporPrintTM of the odor distribution of the samples which provided the fingerprints for odor concentration and characteristic pattern of the respective collagens. PaSC had odour pattern of lower intensity than the PSC, thus suggesting that the papain-aided extraction could result in collagen with less detectable odour.

SDS-PAGE profile

Figure 3 shows the SDS-PAGE pattern of various collagens from barramundi skin and type 1 collagen from calf skin. Three major bands were easily visible for the control, which was similar to those reported in other fish collagens such as from brown backed toadfish, Baltic cod and grass carp (Senaratne *et al*., 2006; Zhang *et al*., 2007). Four major bands with one band of high intensity were obtained for PSC, while eight visible bands were observed for PaSC. The numerous apparent peptide bands in the relatively lower molecular weight (MW) range (37 – 75 kDa)

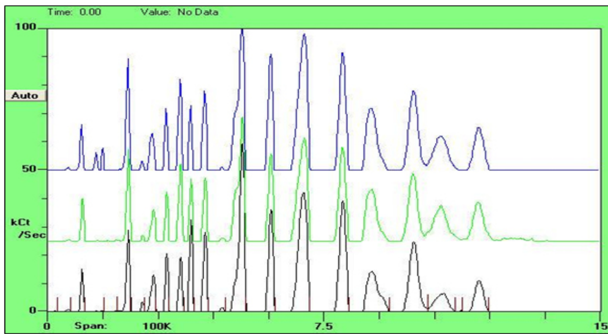

Figure 1. Chromatogram of odour properties of collagen from barramundi skin
ASC: Acid-soluble collagen, PSC: Pepsin-soluble collagen, PaSC: Papain-soluble collagen

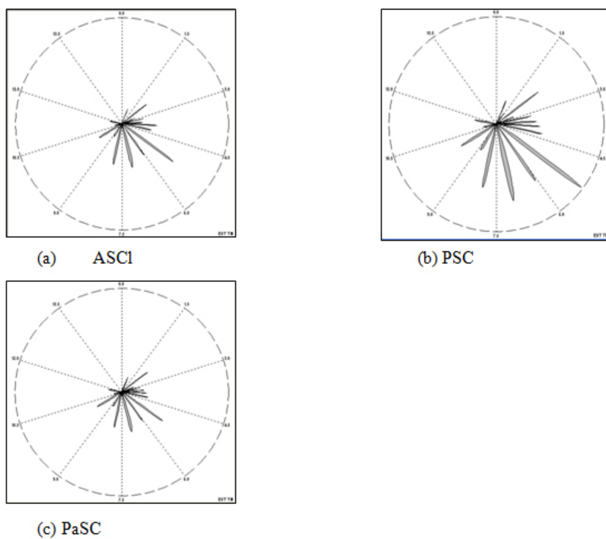

Figure 3.2. VaporPrintTM of collagens from barramundi skin
(a) Acid-soluble collagen, (b) Pepsin-soluble collagen, (c) Papain-soluble collagen

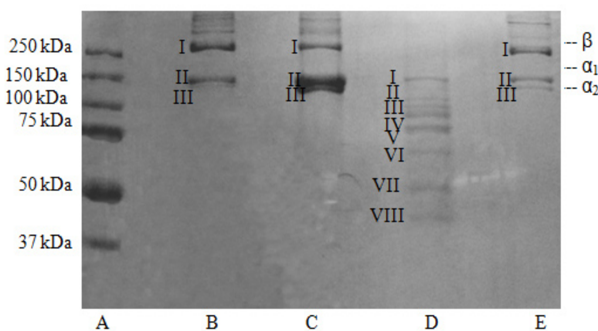

Figure 3. SDS-PAGE patterns of collagens from the skin of barramundi extracted using non-enzymatic and enzymatic treatment. (A) Molecular weight markers, (B) acid-soluble collagen, (C) pepsin-soluble collagen, (D) papain-soluble collagen, (E) calf skin collagen

in the PaSC may indicate that papain exhibited a stronger hydrolytic reaction towards collagen matrix in the barramundi skin.

The control and PSC contained two distinct α-chains (α1 and α2) and a β-dimmer as the major components. These electrophoretic patterns were similar to those of calf skin collagen. In general, the major type of collagen found in fish skins was Type 1 collagen, which consisted of two identical α1- chains and α2-chains (Yata *et al*., 2001; Montero *et al*., 1990). Only the α1- and α2-chains were visually observed in PaSC but no distinct β-component could be obtained. Nalinanon *et al*. (2008) suggested that the degradation of peptide bands with the concomitant formation of low molecular weight (MW) bands was the results of hydrolytic action of the protease, which cleaved the peptides not only at the telopeptide region but also within tropocollagen. Hence, indicates that papain was a very strong protease for the collagen extraction. In order to retain the β-component in PaSC, perhaps we could control or modify the extraction process parameters such as enzyme concentration, contact time, pH and temperature. The molecular weight pattern of collagen is related to its quality; formation of lower molecular weight polypeptides may explain that the collagen has been hydrolysed, whereas the presence of β- and α- components indicates its purity.

Amino acid composition

Total amino acid content (mg/g) of the control, pepsin soluble and papain soluble collagens were as shown in Table 2. The total amount of amino acids found in PaSC, PSC and control were in descending order. All amino acids were detected in collagens, except for cysteine. The total amino acid compositions of enzyme-aided extractions were 6 – 8% higher than the non-enzymatic process. PaSC had significantly higher ($p<0.05$) amino acid content than PSC. The significant different in amino acid content between PSC and PaSC could be due to the different intermolecular action of the two proteases as observed in the peptide patterns of the respective collagens in SDS-PAGE (Figure 3).

All collagens obtained in this study were rich in glycine (more than 18%), however the values were considered low as compared to other reported fish collagens which had 25-30% of glycine content (Kittiphatanabawon *et al*., 2005; Senaratne *et al*., 2006; Zhang *et al*., 2007). They were low in histidine, tyrosine and isoleucine. High contents of proline, alanine, glutamic acid, arginine, hydroxyproline and aspartic acid, in descending order, were observed in all collagen samples. This pattern was also observed in collagen from other reported fish species such as in bigeye snapper and brown backed toadfish (Kittipattanabawon *et al*., 2005; Senaratne *et al*., 2006). Hydroxyproline is unique amino acid found in collagen, and was used as a measure of the yield and efficiency of the collagen extraction process

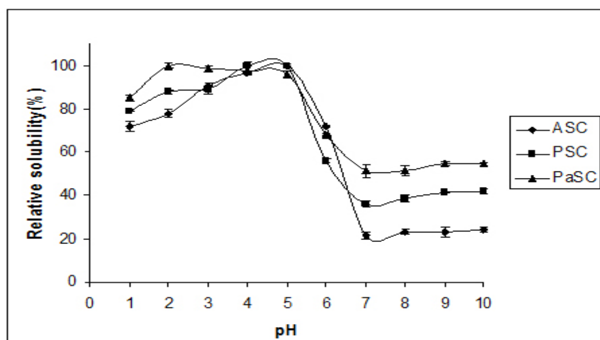

Figure 4. Solubility of ASC (control), PSC and PaSC from barramundi skin in 0.5 M acetic acid at different pH

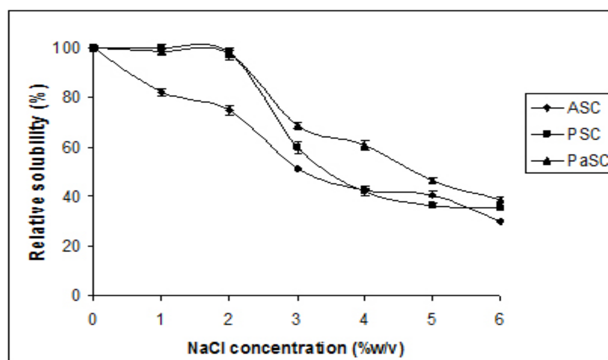

Figure 5. Solubility of ASC (control), PSC and PaSC from barramundi skin in 0.5 M acetic acid at different NaCl concentrations

(Nalinanon *et al*., 2007; Skierka and Sadowska, 2007). The amount of hydroxyproline for ASC (47.72 mg/g), PSC (56.88 mg/g) and PaSC (60.89 mg/g) were significantly different ($p<0.05$) (Table 2), with PaSC had the highest hydroxyproline content.

Glycine were reported to occur in every third residue in the collagen of all skin matrixes, forming a tri-peptide unit Gly-X-Y, except for the fourteen amino acids from the N-terminus and the first ten or so from the C-terminus (Foegeding *et al*., 1996). The tri-peptide sequence is known to be occupied mostly with proline and hydroxyproline, the imino acids that are responsible for the stability of the collagen helices. Increased amount of imino acids content throughout the collagen matrix formed a more stable collagen (Wong, 1989). The imino acid (hyp+pro) contents of collagens from barramundi skin in 1000 residues of amino acid were shown in Table 3. The imino acid content of barramundi collagens was similar to reported fish skin collagens, but lower than that of mammalian collagens (215-220 residues/1000 residues) (Zhang *et al*., 2007). Collagen with high imino acid content is desirable for industrial application due to high thermal stability. Although the amino acid composition of collagen always relate to the origin of the raw material, nonetheless, the characteristics of the extracted collagen is also affected by the amino acid distribution, which could be optimized.

Solubility of collagen at different pH and NaCl concentrations

Effect of pH

Figure 4 shows the effect of pH on the solubility of the extracted collagens. The maximum solubility for control, PSC and PaSC were obtained at pH 5, pH 4 and pH 2, respectively, and they were significantly different. The enzymatic pre-treatment resulted in the shifting of the pH of maximum solubility towards more acidic values. The collagens were more soluble in the acidic pH range of 1-5. A sharp drop in the solubility of the collagens was observed and reached the minimum when the pH was increased to pH 7. PaSC exhibited the highest solubility in the acidic pH range and pH exceeding 7.

The effect of pH on the collagen solubility is associated with its isoelectric point (pI), where the overall charge on protein is near zero. Results obtained suggested that the pIs of the collagens could be expected to be in the neutral or alkaline pH ranges, as seen by the lowest solubility in these pH ranges. Foegeding *et al*. (1996) reported that collagen from bovine and porcine had pIs of pH 6-9. Characteristic solubility of bovine and porcine collagens is not available for comparison.

The significant difference in the solubility of the papain and the pepsin extracted collagens may suggest that papain was highly reactive in cleaving the high molecular weight components of the collagen, as evident by the peptide pattern of the various collagens (Figure 3). Collagens that are highly soluble in acidic pH ranges can be easily formulated into fruit juices. They are normally formulated in acidic range of pH 3-4, however, they may be less suitable for biomedical applications since the pH range suggested is from 4-8.5 as suggested by Lu *et al*. (2007).

Effect of NaCl concentration

Figure 5 shows the relative solubility of the extracted collagens from barramundi skin as affected by the different NaCl concentration. The solubility of collagen decreased in the presence of increasing NaCl concentration. (r^2 = 0.95, 0.88 and 0.93 for control, PSC and PaSC, respectively). A significant difference ($p<0.05$) in the solubility was observed between the acid-solubilzed collagen and the enzymatic-aided extracted collagens (PSC and PaSC) at the NaCl concentration of 1-2% (w/v). However, the solubility of PSC and PaSC, was relatively unchanged up to 2% NaCl concentration and gradually decreased thereafter. The effect of different ionic strength on collagen solubility could be explained by the salting in and salting out phenomena of protein. At low salt concentration, the protein solubility usually increased

slightly (salting-in) but at high salt concentration, the solubility of proteins drop sharply (salting-out). The salting- out effect increased hydrophobic interaction and competition of salts ion in water, which resulted in protein precipitation (Vojdani, 1996). The results obtained showed that the collagens obtained from enzymatic-aided extraction had greater solubility than the control collagen. These findings were similar to those of Jongjareonrak *et al*. (2005) and Nalinanon *et al*. (2007) on the solubility of fish collagens extracted by the acid and pepsin treatment. Non-enzymatic treated collagen normally composed of intact collagen molecular structure with presence of intermolecular cross-links (Jongjareonrak *et al*., 2005). Foegeding *et al*. (1996) stated that as the cross-linking in the collagen molecule increase, it become less soluble in solvent such as salt and acid solutions. It was suggested that the greater solubility of the later collagen was due to the proteolytic action of the protease in altering collagen structure and reducing the chain length of the collagen (Nalinanon *et al*., 2007). Greater relative solubility of papain-extracted collagen than the pepsin-extracted collagen, especially at higher NaCl concentration (4-6% (w/v)) found in this study may suggest that PaSC was more stable than PSC in the presence of NaCl.

Conclusion

This study has proven that barramundi (*Lates calcarifer*) skin can be a viable source of commercial collagen and papain can be used to enhanced the extraction process. Collagen extracted from the skin of barramundi by papain aided extraction had higher amino acid content with distinguisable polypeptides pattern from collagens extracted using pepsin. The extracted collagen was odorless, colorless and had good solubility in the acidic range.

Acknowledgements

This research work was supported by Universiti Putra Malaysia Research University Grant Scheme (RUGS) No. 91022.

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