PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.324.332

Research Article
Characterization of the Acid- and Pepsin-Soluble Collagens from Haruan (*Channa striatus*) Scales

[1,2]Bagus Fajar Pamungkas, [2]Supriyadi, [2]Agnes Murdiati and [2]Retno Indrati

[1]Faculty of Fisheries and Marine Science, Mulawarman University, Kampus Gn. Kelua, Samarinda 75119, Indonesia
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia

Abstract

Background and Objective: The optimal use of haruan scales for preparing collagen is a promising method to increase value-added products and to protect the environment. The aim of this research was to characterize the acid- and pepsin-soluble collagens from haruan (*Channa striatus*) scales. **Materials and Methods:** The fish scales were subjected to an extraction with 0.5 M acetic acid and a digestion with 0.1% pepsin. The results are presented as the mean±standard deviation. **Results:** The yields of the acid-soluble collagen in the haruan scales (ASC-SH) and the pepsin-soluble collagen in the haruan scales (PSC-SH) were 1.44 and 2.94% (on a dry basis), respectively. ASC-SH and PSC-SH contained glycine as the major amino acid and had high imino acid contents (238 and 242 residues/1,000 residues, respectively). Based on the SDS-PAGE pattern, both ASC-SH and PSC-SH were identified as type I collagens containing α_1 and α_2 chains. β and γ components were also found in both collagens. The FTIR spectra indicated that both collagens had triple helical structures. The collagens were both soluble at acidic pH levels (1-4) and their solubility was low when the NaCl concentrations were above 3% (w/v). **Conclusion:** It was concluded that haruan scales could be an alternative source of collagen and that the characteristics of the collagens were slightly affected by the extraction process used in this study.

Key words: Acid-soluble collagen (ASC-SH), fish, haruan scale, pepsin digestion, pepsin-soluble collagen (PSC-SH)

Received: September 27, 2018 Accepted: December 12, 2018 Published: March 15, 2019

Citation: Bagus Fajar Pamungkas, Supriyadi, Agnes Murdiati and Retno Indrati, 2019. Characterization of the acid- and pepsin-soluble collagens from haruan (*Channa striatus*) scales. Pak. J. Nutr., 18: 324-332.

Corresponding Author: Retno Indrati, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia Tel: +62811267259 Fax: +62274589797

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Collagen, a part of the extracellular protein matrix, is a major component of various connective tissues, such as the skin, bones, tendons, blood vessels, cartilage and teeth[1]. Collagen plays an important role in maintaining the structure of various tissues and constitutes approximately 25-30% of the total protein[2,3]. At present, at least 29 types of collagen have been identified from various animal and human tissues[4]. Collagen has been widely used in the biomedical, pharmaceutical, cosmetic, food and packaging industries[5]. The main source of commercial collagen is usually the skin and bones of land-based animals, such as cows and pigs. However, these sources of collagen may present a concern among consumers because of the outbreak of bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and foot and mouth disease (FMD)[6]. In addition, the collagen obtained from pigs and cows cannot be used as a component of some foods for religious reasons[7]. Therefore, it is necessary to find other collagen sources as alternative raw materials.

Fish are one of the alternative raw materials suitable for collagen preparation because of their availability, the lack of disease transmission risk and the possibility of high collagen yields[8]. During fish processing, large quantities of byproducts, such as heads, bones, skin, scales and viscera, are generated. These byproducts constitute approximately 50-70% of the original raw materials and may cause environmental pollution[9]. Therefore, the optimal use of these byproducts, especially for preparing collagen, is a promising way to increase value-added products and to protect the environment.

Recently, studies on the isolation and characterization of the collagens extracted from fish byproducts, such as the bones and scales of black drum and sheepshead seabream[5], the scales, skins and bones of bighead carp[10], the scales of croceine croaker (*Pseudosciaena crocea*)[11],the scales of seabass[12] and the scales of snakehead (*Ophiocephalus argus*)[3], have been widely reported. These collagens from fish byproducts were mainly type-1 collagen with lower imino acid contents and denaturation temperatures[8].

Snakehead (*Channa striatus*), which has the local name "haruan", is a freshwater fish indigenous to many tropical and subtropical countries, including Indonesia. Haruan have been widely used for fresh consumption and are sometimes processed into salted fish and crackers. In the province of Kalimantan Timur, the average catches of haruan increased by 7.80% in the period of 2013-2014, with the catch volume reaching 5.141 t in 2014[13]. During gutting, a large amount of scales from this species is generated as byproducts and these scales could be used as an alternative raw material for collagen extraction. However, there is little information available about the extraction of collagen from the haruan scales. Thus, the aim of this study was to characterize the acid- and pepsin-soluble collagens from the haruan scales (*Channa striatus*).

MATERIALS AND METHODS

Materials: Fresh haruan scales (*Channa striatus*) were obtained from a traditional market in Samarinda, East Borneo. The scales were washed with chilled water, placed in polyethylene bags and frozen at -18°C. The frozen scales were placed in a container and transported to the Faculty of Agricultural Technology, Universitas Gadjah Mada and stored at -18°C until further use.

Chemicals and reagents: NaOH, EDTA, CH_3COOH, NaCl, tris-hydroxymethyl aminomethane and pepsin from the hog stomach (EC3.4.23.1; powdered; ≥250 Units mg^{-1} solid)were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals for electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA, USA). All other reagents and chemicals were of analytical grade.

Extraction of the acid-soluble collagen (ASC): The collagen was extracted using a previously described method[12] with slight modification. To remove the noncollagenous substances, the fish scales were extracted with 0.1 M NaOH for 6 h at a solid-to-alkali solution ratio of 1:10 (w/v). The alkali solution was changed every 3 h. The scales were washed with cold water until a neutral pH of wash water was obtained. The scales were further demineralized with 0.5 M EDTA-2Na (pH 7.4) using a solid to solution ratio of 1:10 (w/v) for 24 h with continuous stirring. The mixtures were filtered through two layers of cheesecloth and washed with cold distilled water. The collagens in the scales were extracted with 0.5 M acetic acid at a sample/solution ratio of 1:10 (w/v) for 48 h with continuous stirring and the mixtures were filtered with two layers of cheesecloth. The collagen in the filtrate was precipitated by adding NaCl to a final concentration of 2.6 M. The resultant precipitate was collected by centrifugation at 15000 g and 4°C for 30 min (Eppendorf Centrifuge 5417 R, Hamburg, Germany) and then dissolved in a minimum volume of 0.5 M acetic acid. The solution was dialyzed (MWCO 12 kDa) against 30 volumes of 0.1 M acetic acid solution and deionized

water for 48 h. The resulting dialysate was freeze-dried and referred to as the "acid-soluble collagen from the scales of the haruan (ASC-SH)".

Extraction of the pepsin-soluble collagen (PSC): The residues of the scales after the ASC extraction were extracted with eight volumes of 0.5 M acetic acid containing 0.1% pepsin for 72 h with continuous stirring and the mixtures were filtered with two layers of cheesecloth. The filtrate was precipitated and dialyzed in the same way as for ASC as previously described. The collagen obtained with the aid of pepsin was referred to as the "pepsin-soluble collagen from the scales of the haruan (PSC-SH)". The yield of the extracted collagen was calculated based on the dry matter of the freeze-dried collagens in comparison with the dry weight of the samples.

Proximate analysis: The moisture, ash, protein and fat contents of the scales and collagen samples were determined according to the AOAC method[14].

Amino acid analysis: The samples were hydrolyzed in an inert atmosphere with 6 N HCl at 110°C for 24 h in the presence of 1% phenol (v/v). The hydrolysates were derivatized, dried and diluted with the sample diluents. The amino acid derivative samples were analyzed by LC-MS/MS (The Waters Xevo TQD, Milford, MA). The areas under the peak of each amino acid in the chromatogram were calculated and compared with the standard amino acids and reported as the number of residues per thousand amino acids.

Hydroxyproline content: The hydroxyproline contents of the ASC-SH and PSC-SH were determined by the method of Reddy and Enwemeka[15] with a slight modification. The samples were hydrolyzed with 6 N HCl for 6 h at 110°C. The filtrate (1 mL) was mixed with 1 mL buffered Chloramine T reagent (1.27 g Chloramine T in 20 mL 50% n-propanol and brought to 100 mL with acetate-citrate buffer) and the oxidation was allowed to proceed for 20 min at room temperature. The chromophore was developed with the addition of 1 mL Ehrlich's reagent solution and incubation for 20 min at 60°C in a water bath and the absorbance was measured at 550 nm using a UV-VIS spectrophotometer (Genesys 10S, China).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE): The electrophoretic patterns of ASC-SH and PSC-SH were visualized according to the method of Laemmli[16] with a slight modification, using a 7.5% resolving gel and a 5% stacking gel. The collagen samples (4 mg) were suspended in 1 mL 10% (w/v) SDS prior to incubation at 85°C for 1 h, followed by centrifugation at 10000 g for 15 min at room temperature (Eppendorf Centrifuge 5417 R, Hamburg, Germany). The protein concentration was determined according to the method of Lowry *et al.*[17]. The supernatants were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl pH 6.8, containing 25% glycerol, 10% SDS, 0.5% bromophenol blue and 5% βME) and boiled for 4 min. The samples (15 µg protein) were loaded onto the polyacrylamide gel and subjected to electrophoresis at a constant current of 180 V per gel using a Mini Protean unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid.

Fourier transform spectroscopy (FTIR): The infrared spectra of ASC-SH and PSC-SH were recorded in potassium bromide (KBr) disks with a Fourier transform IR spectrophotometer (Nicolet iS 10, Thermo Fisher Scientific Inc., USA). One milligram dry collagen was mixed with dry KBr (100 mg) and the mixture was pressed into a disk for the spectrum recording. The spectra in the range of 650-4000/cm with automatic signal gains were collected in 32 scans at a resolution of 4/cm.

Determination of the collagen solubility: The solubilities of the collagens at the different pH and NaCl concentrations were determined by the method of Montero *et al.*[18] with a slight modification. ASC-SH and PSC-SH were dissolved in 0.5 M acetic acid to obtain a final concentration of 3 mg mL^{-1} and the mixtures were stirred at 4°C for 24 h. Then, the mixtures were centrifuged at 5000 g for 15 min at 4°C. The ASC-SH and PSC-SH solutions (8 mL) were transferred to a 50 mL centrifuge tube. The pH was adjusted with either 6 M NaOH or 6 M HCl to obtain the final pH ranging from 1-10. Then, the volume of solution was made up to 10 mL by deionized water and the solution pH was adjusted to the same pH as the collagen solutions. The solution was centrifuged at 15000 g for 30 min at 4°C. The protein content in the supernatant was measured and the relative solubility was calculated in comparison with that obtained at the pH, giving the highest solubility.

The ASC-SH and PSC-SH solutions (5 mL) were mixed with 5 mL NaCl in 0.5 M acetic acid at various concentrations to give final concentrations of 0, 1, 2, 3, 4, 5 and 6% (w/v). The mixture was stirred continuously at 4°C for 30 min, followed by centrifugation at 15000 g for 30 min at 4°C. The protein content in the supernatant was measured and the relative solubility was calculated as previously described.

Statistical analysis: All analyses were performed in triplicate. The results are presented as the Mean±the standard deviation.

RESULTS AND DISCUSSION

Yields and the proximate composition: The yields of ASC-SH and PSC-SH were 1.44 and 2.94% (on a dry basis), respectively (Table 1). The results showed that the yield of PSC-SH was higher than that of ASC-SH. Fish scales are biocomposites of collagen fibers with many crosslinked regions at the telopeptide and hydroxyapatite[19]. Galea *et al.*[20] reported that the crosslinks of collagen are stable to thermal and acid treatment. The use of 0.1% pepsin in this study could effectively solubilize the collagen from the haruan scales. The crosslinked regions at the telopeptide were cleaved by pepsin without damaging the integrity of the triple helix[21,22], resulting in increased collagen yields. The yield of ASC-SH was lower than that of the ASC from sardine scales (5% on a dry basis)[23] but higher than that of the ASC from silver carp scales (0.86% on a dry basis)[6]. The yield of PSC-SH was higher than that of the PSC from silver carp scales (2.32% on a dry basis)[6]. The differences in the yields might be attributed to the differences in fish species, biological conditions and the preparative methods for extracting the collagen[24,25].

The haruan scales contained moisture as a major component (56.73±0.41%), followed by protein (21.60±0.11%), ash (19.02±0.26%) and fat (0.26±0.12%). Compared with the raw material, ASC-SH and PSC-SH were considerably higher in protein (93.92±0.30 and 94.12±0.26%, respectively) but lower in ash (0.62±0.21 and 0.55±0.19%, respectively), fat (0.09±0.01 and 0.08±0.03%, respectively) and moisture (5.18±0.82 and 5.32±0.54%, respectively). The demineralization process with 0.5 M EDTA-2Na (pH 7.4) could have removed approximately 97% of the inorganic matter from the haruan scales, resulting in the lower ash content in the collagen. The higher protein and the lower moisture in the collagen indicated that the bulk of the collagen protein was obtained through the extraction process; meanwhile, a significant amount of water was released during the freeze-drying process. The results suggest that the methods used to extract collagen from the haruan scales were effective.

Amino acid composition: The amino acid compositions of ASC-SH and PSC-SH are expressed as residues per 1,000 total amino acid residues and are shown in Table 2. ASC-SH and PSC-SH had glycine as the major amino acid (280 and 282 residues/1,000 residues, respectively). The other amino acids of higher contents in ASC-SH and PSC-SH were proline (170 and 173 residues/1,000 residues, respectively), arginine (115 and 114 residues/1,000 residues, respectively), alanine (114 and 113 residues/1,000 residues, respectively), valine (73 and 67 residues/1,000 residues, respectively) and hydroxyproline (68 and 69 residues/1,000 residues, respectively). Serine, histidine and threonine levels were very low and cysteine was not detected. Muyonga *et al.* (2004)[26] reported that glycine is the most dominant amino acid in collagen and all members of the collagen family are characterized by triple-helical repeats of (Gly-Pro-Hyp)n[27]. The slight differences in the amino acid compositions between ASC-SH and PSC-SH might be due to the removal of some portions of the telopeptide region mediated by pepsin[28].

Additionally, Chi *et al.*[8] and Yu *et al.*[29] reported that the amounts of imino acids (proline and hydroxyproline) are important for the structural integrity of collagen because they are essential to the formation of intramolecular hydrogen bonds. Additionally, the imino acids contribute to the thermal stability of the helix structure of collagen, which is one of the most important characteristics needed for industrial application[30,31]. The imino acid contents of ASC-SH and PSC-SH were 238 residues/1,000 residues and 242 residues/1,000 residues, respectively, which were similar to those of the PSC from carp fish scales (231 residues/1,000 residues)[32] and Nile tilapia scales (245 residues/1,000 residues)[33] but higher than those of the PSC from bighead carp scales (156 residues/1,000 residues)[34], the ASC and PSC from spotted golden goatfish scales (186 and 189 residues/1,000 residues, respectively[28]), the ASC from croceine croaker scales (189 residues/1,000 residues)[35], the ASC and PSC from seabass scales (193 and

Table 1: Yield and proximate composition of the collagen

Component	Fish scales	Acid-soluble collagen (ASC-SH)	Pepsin-soluble collagen (PSC-SH)
Yield (%)	-	1.44±0.27	2.94±0.64
Moisture (% wet wt.)	56.73±0.41	5.18±0.82	5.32±0.54
Protein (% wet wt.)	21.60±0.11	93.92±0.30	94.12±0.26
Ash (% wet wt.)	19.02±0.26	0.62±0.21	0.55±0.19
Fat (% wet wt.)	0.26±0.12	0.09±0.01	0.08±0.03

The values are given as the Mean±standard deviation from a triplicate determination

Table 2: Amino acid composition of ASC-SH and PSC-SH (residues/1,000 residues)

Amino acid	ASC-SH	PSC-SH
Arginine	115	114
Histidine	3	3
Lysine	38	37
Phenylalanine	28	29
Isoleucine	27	27
Leucine	26	26
Tyrosine	9	9
Methionine	27	28
Valine	73	67
Proline	170	173
Glutamic acid	10	10
Aspartic acid	8	7
Cysteine	0	0
Threonine	4	3
Serine	2	2
Alanine	114	113
Glycine	280	282
Hydroxyproline	68	69

ASC-SH: Acid-soluble collagen from the haruan scales, PSC-SH: Pepsin-soluble collagen from the haruan scales

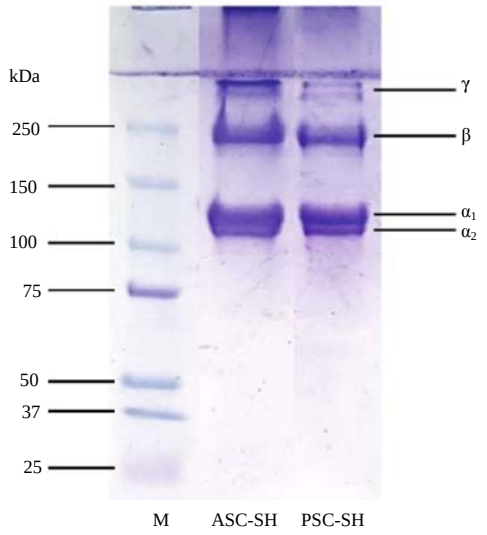

Fig. 1: SDS-PAGE patterns of the collagen from the haruan scales

M: Marker, ASC-SH: Acid-soluble collagen from the haruan scales, PSC-SH: Pepsin-soluble collagen from the haruan scales

195 residues/1,000 residues, respectively[12]), the ASC from tilapia scales (207 residues/1,000 residues)[36] and the PSC from Nile tilapia scales (187 residues/1,000 residues)[37]. The differences in the imino acid contents between the animals was dependent on the living conditions and habitat temperatures[24].

Gel electrophoresis: The SDS-PAGE patterns of ASC-SH and PSC-SH under reducing conditions are shown in Fig. 1. ASC-SH and PSC-SH had similar protein patterns that consisted of two α-chains; high molecular weight components, particularly β-chains (dimmers of α_1-chains) and γ-chains (trimers of α_1-chains). The α_1 and α_2 chains of ASC-SH and PSC-SH were 126 and 117 kDa, respectively. The results indicated that ASC-SH and PSC-SH were mainly composed of type 1 collagen, a heterotrimer containing two α_1-chains and one α_2-chain in the molecular form of $[\alpha_1 (I)_2\alpha_2(I)]$[27]. Yu *et al.*[29] reported that the band intensities of the α_1-chains were approximately two-fold higher than those of the α_2-chain. This pattern was similar to that of the type 1 collagen from silver carp[6], carp[32], lizard fish, gray mullet and yellow back seabream[38], seabass[12] and Nile tilapia[37] scales. In general, no differences were observed between the mobilities of the α-chains in ASC-SH and PSC-SH, indicating that the sites cleaved by pepsin were located very close to the terminal ends of the tropocollagen[39]. This result was consistent with a previous study reported by Zhang *et al.*[6] where the mobility of the α-chains in the ASC and PSC from silver carp scales were quite similar with the aid of pepsin. On the other hand, PSC-SH contained a much lower band intensity in its β-and γ-chains than was seen in those of ASC-SH. The results indicated that ASC-SH contained a higher proportion of inter and intra-crosslinks than PSC-SH. Zhang *et al.*[40] and Singh *et al.*[41] reported that pepsin cleaves the crosslink containing the telopeptide, so almost all β-and γ-chains were digested and removed by pepsin.

Fourier transform spectroscopy (FTIR): The FTIR spectra of ASC-SH and PSC-SH exhibited characteristic peaks of amides I, II, III, A and B (Fig. 2). According to Sai, K.P. and M. Babu[42] the amide A band is associated with the N-H stretching frequency and occurs in a wave number range of 3400-3440 cm^{-1}. The absorption peaks of ASC-SH and PSC-SH were found at 3307 cm^{-1}. Doyle *et al.*[43] reported that when the NH group is involved with the H-bond in the peptide chain, the position starts to shift to lower frequencies. The amide B band positions of ASC-SH and PSC-SH were found at 2945 cm^{-1}, representing the asymmetrical stretching of CH_2[44]. The similar absorption peaks between ASC-SH and PSC-SH indicated that both collagens were involved in the hydrogen bonding between the free NH stretch and the polypeptide chain[28]. The amide I bands of ASC-SH and PSC-SH were found at a wave number of 1629 cm^{-1}. Payne and Veis[45] reported that the amide I band with the characteristic strong absorbance in the range of 1600-1700 cm^{-1} is mainly associated with the C = O stretching vibration or the H-bond coupled with COO^-. Amide II is responsible for the combination of the NH in-plane bend and the CN stretching vibration[46]. Amide II of PSC-SH was found at a lower wave number (1539 cm^{-1}) compared with that of ASC-SH (1548 cm^{-1}), suggesting a higher proportion of

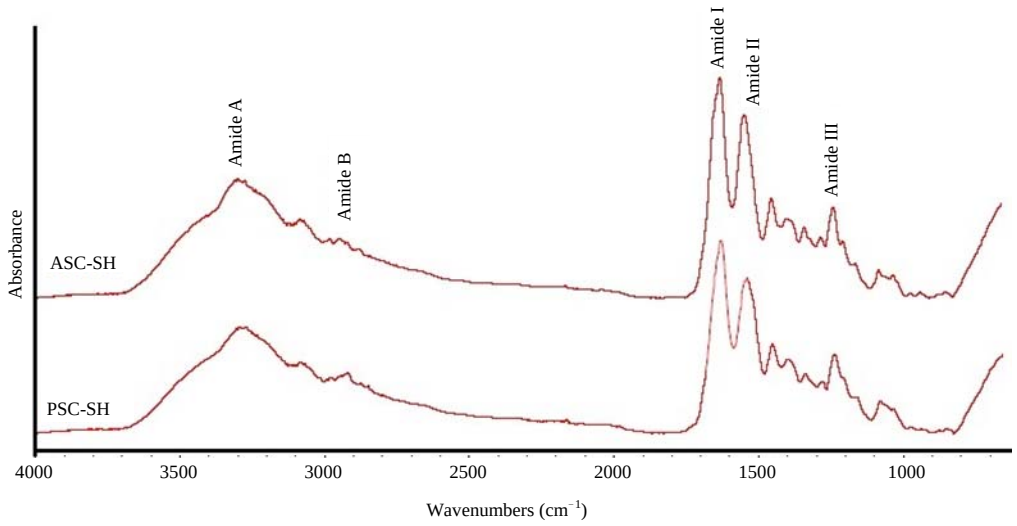

Fig. 2: FTIR spectra of ASC-SH and PSC-SH from the haruan (*Channa striatus*) scales

hydrogen bonds in PSC-SH. Nalinanon *et al.*[47] reported that pepsin was able to cleave the nonhelical domain of the telopeptide regions, increasing the proportion of the triple helix of PSC-SH that was stabilized with the H-bond. The amide III bands of ASC-SH and PSC-SH were found at wave numbers of 1238 and 1237 cm^{-1}, respectively. The amide III peak is associated with the intermolecular interactions in collagen, involving C-N stretching and N-H in-plane bending from the amide linkages as well as absorptions arising from the wagging vibrations from the CH_2 groups of the glycine backbone and proline side-chains[48], indicating that hydrogen bonds were involved in ASC-SH and PSC-SH. De Guzzi Plepis *et al.*[48] also used the intensity ratio between the amide III band and the 1450 cm^{-1} band to indicate the triple-helical structure of collagen. From the results, the absorption ratio between the peak height of amide III and the peak of the 1450 cm^{-1} band in ASC-SH and PSC-SH was 0.85. The value was approximately 1.0, indicating that the triple helix of both ASC-SH and PSC-SH still existed and that a high extent of the intermolecular structure was maintained.

Collagen solubility: The solubility of ASC-SH and PSC-SH, as affected by pH, is shown in Fig. 3a. ASC-SH and PSC-SH showed high solubility in very acidic pH values (1-4) and reached a maximum at pH 3. A sharp decrease in the solubility was observed at pH values above 4. The solubility of ASC-SH reached its minimum at pH values of 7 and 8 (5%), while PSC-SH reached its minimum at pH 7 (8%). However, both collagens showed a slight increase in solubility when the pH was higher than 8. This result was in accordance with a previous study conducted by Matmaroh *et al.*[28] and

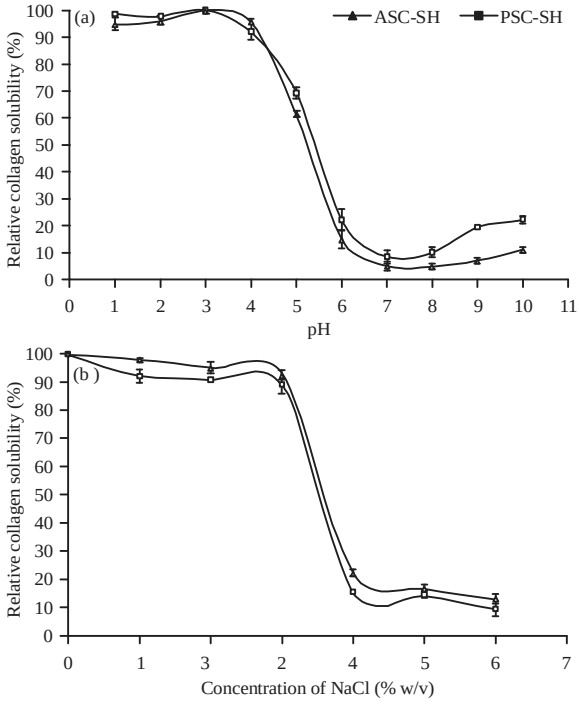

Fig. 3(a-b): Relative solubility of the acid-soluble collagen (ASC-SH) and pepsin-soluble collagen (PSC-SH) from the haruan (*Channa striatus*) scales at different (a) pH and (b) NaCl concentrations
The bars represent the standard deviation (n = 3)

Chuaychan *et al.*[12] whom reported that collagen had low solubility in the neutral and slightly alkaline pH ranges. Jongjareonrak *et al.*[22] reported that low solubility could be attributed to the hydrophobic interactions among collagen

molecules. A slight increase in the solubility at pH levels above 8 was probably due to the repulsion forces between the chains, as the pH was higher than the pI[29]. PSC-SH showed higher solubilities than ASC-SH at all of the pH levels tested. The results indicated that PSC-SH might possess a lower degree of crosslinking than ASC-SH[22]. This finding was consistent with the SDS-PAGE pattern of collagen, which showed that the level of high molecular weight crosslinks of ASC-SH were higher than that of PSC-SH (Fig. 1).

The solubility of ASC-SH and PSC-SH showed asimilar pattern, with only a slight difference at various NaCl concentrations (Fig. 3b). The solubility of ASC-SH and PSC-SH remained at a high level in the presence of NaCl up to 3% (w/v) and sharply decreased when the NaCl concentration was 4% (w/v), after which the solubility remained at a constant low level. The result was similar to the solubility of collagen from the scales of tilapia[36] and nile tilapia[37]. The decrease in the solubility of ASC-SH and PSC-SH could be described as being due to a "salting out" effect at relatively high NaCl concentrations[31]. The salting out effect enhances the hydrophobic–hydrophobic interactions between protein chains and increases the competition with ionic salts for water, which results in protein precipitation[22,49]. Moreover, PSC-SH showed a higher solubility than ASC-SH at all NaCl concentrations tested. Jongjareonrak *et al.*[22] reported that the aid of pepsin in collagen extraction might induce the partial hydrolysis of high MW crosslinked molecules, resulting in a greater solubility of PSC-SH. In addition, the differences in the amino acid compositions and structures of ASC-SH and PSC-SH might result in such differences[29].

CONCLUSION

Both acid-soluble collagen and pepsin-soluble collagen were successfully extracted from the haruan scales. Pepsin digestion was able to increase the yield of collagen by 103%. The amino acid composition, SDS-PAGE and FTIR confirmed that ASC-SH was mainly composed of type I collagen and had a higher content of high-molecular weight crosslinks than did PSC-SH. ASC-SH and PSC-SH showed high solubility at acidic pH levels (1-4) and had higher solubility when the NaCl concentration was lower than 3%. Therefore, it is possible to use haruan scales for processing as an alternative source of collagen for industrial purposes.

SIGNIFICANCE STATEMENT

This study shows that haruan scales could be an alternative source of collagen based on the amino acid composition, SDS-PAGE and FTIR. In future studies, the utilization of these collagens for biomedical purposes will also be examined.

ACKNOWLEDGMENTS

The authors wish to acknowledge the financial support of the Doctoral Program from the Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education of the Republic of Indonesia, via Beasiswa Pendidikan Pascasarjana Dalam Negeri/ BPPDN with grant number 1420/E.4.4/2014.

REFERENCES

1. Morimura, S., H. Nagata, Y. Uemura, A. Fahmi, T. Shigematsu and K. Kida, 2002. Development of an effective process for utilization of collagen from livestock and fish waste. Process Biochem., 37: 1403-1412.
2. Lee, C.H., A. Singla and Y. Lee, 2001. Biomedical applications of collagen. Int. J. Pharm., 221: 1-22.
3. Liu, W., G. Li, Y. Miao and X. Wu, 2009. Preparation and characterization of pepsin solubilized type I collagen from the scales of snakehead (*Ophiocephalus argus*). J. Food Biochem., 33: 20-37.
4. Liu, Q., B.H. Kong, Y.L. Xiong and X.F. Xia, 2010. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. Food Chem., 118: 403-410.
5. Ogawa, M., R.J. Portier, M.W. Moody, J. Bell, M.A. Schexnayder and J.N. Losso, 2004. Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). Food Chem., 88: 495-501.
6. Zhang, J., R. Duan, C. Ye and K. Konno, 2010. Isolation and characterization of collagens from scale of silver carp (*Hypophthalmichthys molitrix*). J. Food Biochem., 34: 1343-1354.
7. Huang, C.Y., J.M. Kuo, S.J. Wu and H.T. Tsai, 2016. Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion-hydro-extraction process. Food Chem., 190: 997-1006.
8. Chi, C.F., B. Wang, Z.R. Li, H.Y. Luo, G.F. Ding and C.W. Wu, 2014. Characterization of acid soluble collagen from the skin of hammerhead shark (*S phyrna lewini*). J. Food Biochem., 38: 236-247.
9. Kittiphattanabawon, P., S. Benjakul, W. Visessanguan, T. Nagai and M. Tanaka, 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chem., 89: 363-372.

10. Pan, B.Q., W.J. Su, M.J. Cao, Q.F. Cai, W.Y. Weng and G.M. Liu, 2012. IgE reactivity to type I collagen and its subunits from tilapia (*Tilapia zillii*). Food Chem., 130: 127-133.
11. Wang, B., Y.M. Wang, C.F. Chi, H.Y. Luo, S.G. Deng and J.Y. Ma, 2013. Isolation and characterization of collagen and antioxidant collagen peptides from scales of croceine croaker (*Pseudosciaena crocea*). Mar. Drugs, 11: 4641-4661.
12. Chuaychan, S., S. Benjakul and H. Kishimura, 2015. Characteristics of acid-and pepsin-soluble collagens from scale of seabass (*Lates calcarifer*). LWT-Food Sci. Technol., 63: 71-76.
13. Anonymous, 2015. Statistical data of aquaculture fishery in 2009-2013. Ministry of Marine and Fishery of Indonesia, Indonesia
14. AOAC., 2005. Official Method of Analysis of the Association of Official Analytical of Chemist. Association of Official Analytical Chemist, Arlington, VA., USA.
15. Reddy, K. and C.S. Enwemeka, 1996. A simplified method for the analysis of hydroxyproline in biological tissues. Clin. Biochem., 29: 225-229.
16. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
17. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
18. Montero, P., F. Jimenez Colmenero and J. Borderìas, 1991. Effect of pH and the presence of NaCl on some hydration properties of collagenous material from trout (*Salmo irideus* Gibb) muscle and skin. J. Sci. Food Agric., 54: 137-146.
19. Zhang, Y., W. Liu, G. Li, B. Shi, Y. Miao and X. Wu, 2007. Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (*Ctenopharyngodon idella*). Food Chem., 103: 906-912.
20. Galea, C. A., B.P. Dalrymple, R. Kuypers and R. Blakeley, 2000. Modification of the substrate specificity of porcine pepsin for the enzymatic production of bovine hide gelatin. Prot. Sci., 9: 1947-1959.
21. Sato, K., T. Ebihara, E. Adachi, S. Kawashima, S. Hattori and S. Irie, 2000. Possible involvement of aminotelopeptide in self-assembly and thermal stability of collagen I as revealed by its removal with proteases. J. Biol. Chem., 275: 25870-25875.
22. Jongjareonrak, A., S. Benjakul, W. Visessanguan and M. Tanaka, 2005. Isolation and characterization of collagen from bigeye snapper (*Priacanthus macracanthus*) skin. J. Sci. Food Agric., 85: 1203-1210.
23. Nomura, Y., H. Sakai, Y. Ishii and K. Shirai, 1996. Preparation and some properties of type I collagen from fish scales. Biosci. Biotechnol. Biochem., 60: 2092-2094.
24. Regenstein, J.M. and P. Zhou, 2007. Collagen and Gelatin from Marine by-Products. In: Maximising The Value of Marine By-Products, Shahidi, F. (Ed.)., Woodhead Publishing Limited, UK., pp: 279-303.
25. McCormick, R.J., 2009. Collagen. In: Applied Muscle Biology and Meat Science, Du, M. and R.J. McCormic (Eds.)., Taylor and Francis, USA., pp: 129-148.
26. Muyonga, J.H., C.G.B. Cole and K.G. Duodu, 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem., 85: 81-89.
27. Foegeding, E.A., T.C. Lanier and H.O. Hultin, 1996. Characteristics of Edible Muscle Tissues. In: Food Chemistry, Fennema, O.R. (Eds.). 3rd Edn., Marcel Dekker, New York, pp: 902-906.
28. Matmaroh, K., S. Benjakul, T. Prodpran, A.B. Encarnacion and H. Kishimura, 2011. Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*). Food Chem., 129: 1179-1186.
29. Yu, D., C.F. Chi, B. Wang, G.F. Ding and Z.R. Li, 2014. Characterization of acid-and pepsin-soluble collagens from spines and skulls of skipjack tuna (*Katsuwonus pelamis*). Chinese J. Natural Med., 12: 712-720.
30. Huang, Y.R., C.Y. Shiau, H.H. Chen and B.C. Huang, 2011. Isolation and characterization of acid and pepsin-solubilized collagens from the skin of balloon fish (*Diodon holocanthus*). Food Hydrocolloids, 25: 1507-1513.
31. Jamilah, B., M.U. Hartina, D.M. Hashim and A.Q. Sazili, 2013. Properties of collagen from barramundi (*Lates calcarifer*) skin. Int. Food Res. J., 20: 835-842.
32. Zhang, F., A. Wang, Z. Li, S. He and L. Shao, 2011. Preparation and characterisation of collagen from freshwater fish scales. Food Nutr. Sci., 2: 818-823.
33. El-Rashidy, A.A., A. Gad, A.E.H.G. Abu-Hussein, S.I. Habib, N.A. Badr and A.A. Hashem, 2015. Chemical and biological evaluation of Egyptian Nile Tilapia (*Oreochromis niloticas*) fish scale collagen. Int. J. Biol. Macromol., 79: 618-626.
34. Liu, D., L. Liang, J.M. Regenstein and P. Zhou, 2012. Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (*Hypophthalmichthys nobilis*). Food Chem., 133: 1441-1448.
35. Wang, B., L. Li, C.F. Chi, J.H. Ma, H.Y. Luo and Y.F. Xu, 2013. Purification and characterisation of a novel antioxidant peptide derived from blue mussel (*Mytilus edulis*) protein hydrolysate. Food Chem., 138: 1713-1719.
36. Chen, S., H. Chen, Q. Xie, B. Hong and J. Chen *et al*., 2016. Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenops ocellatus*). Food Hydrocolloids, 52: 468-477.
37. Zeng, S., J. Yin, C. Zhang, Z. Jiang, W. Wu and X. Zhao, 2016. Physicochemical properties of pepsin-solubilized collagen extracted from Nile Tilapia (*Oreochromis niloticus*) scale. J. Aquat. Food Prod. Technol., 25: 656-665.

38. Okazaki, E. and K. Osako, 2014. Isolation and characterization of acid-soluble collagen from the scales of marine fishes from Japan and Vietnam. Food Chem., 149: 264-270.
39. Ogawa, M., M.W. Moody, R.J. Portier, J. Bell, M.A. Schexnayder and J.N. Losso, 2003. Biochemical properties of black drum and sheepshead seabream skin collagen. J. Agric. Food Chem., 512-513: 8088-8092.
40. Zhang, F., Z. Wang and S. Xu, 2009. Macroporous resin purification of grass carp fish (*Ctenopharyngodon idella*) scale peptides with *in vitro* angiotensin-I converting enzyme (ACE) inhibitory ability. Food Chem., 117: 387-392.
41. Singh, P., S. Benjakul, S. Maqsood and H. Kishimura, 2011. Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). Food Chem., 124: 97-105.
42. Sai, K.P. and M. Babu, 2001. Studies on *Rana tigerina* skin collagen. Comparat. Biochem. Physiol. Part B, 128: 81-90.
43. Doyle, B.B., E.G. Bendit and E.R. Blout, 1975. Infrared spectroscopy of collagen and collagen like polypeptides. Biopolymers, 14: 937-957.
44. Abe, Y. and S. Krimm, 1972. Normal vibrations of crystalline polyglycine I. Biopolymers, 11: 1817-1839.
45. Payne, K.J. and A. Veis, 1988. Fourier transform ir spectroscopy of collagen and gelatin solutions: Deconvolution of the amide I band for conformational studies. Biopolymers, 27: 1749-1760.
46. Barth, A. and C. Zscherp, 2002. What vibrations tell about proteins. Quart. Rev. Biophys., 35: 369-340.
47. Nalinanon, S., S. Benjakul, W. Visessanguan and H. Kishimura, 2007. Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). Food Chem., 104: 593-601.
48. De Guzzi Plepis, A.M., G. Goissis and D.K. Das Gupta, 1996. Dielectric and pyroelectric characterization of anionic and native collagen. Polym. Eng. Sci., 36: 2932-2938.
49. Bae, I., K. Osatomi, A. Yoshida, K. Osako, A. Yamaguchi and K. Hara, 2008. Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes. Food Chem., 108: 49-54.