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CHARACTERIZATION OF ACID SOLUBLE COLLAGEN FROM THE SKIN OF SNAKESKIN GOURAMI (*TRICHOGASTER PECTORALIS*)

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ABSTRACT

The present study was aimed to isolate and characterized acid soluble collagen (ASC) from the skin of snakeskin gourami (*Trichogaster pectoralis*). ASC from gourami skin had a yield of 9.43% and 34.65%, based on wet and dry weight basis, respectively. The purity of ASC was superior with a distinct absorption peak at wavelength (WL) of 230.7 nm. Based on the electrophoretic pattern, gourami skin ASC was classified as type I collagen, as it comprised α1 and α2 as major components and higher molecular weight (MW) components γ, β were distinctly observed. ASC exhibited high T_{max} value of 33.43°C, which could correspond to its imino acids content of 188 residues/1000 residues. Fourier transform infrared (FTIR) spectrum and circular dichroism (CD) revealed that ASC extracted from gourami skin had greater structural integrity in its triple-helical form. Solubility of ASC was high at the pH range of 2-4 in which zeta potential exhibited highly positive charge. The highest solubility of ASC in the presence of NaCl was observed at 2% (w/v). Therefore, with all the characteristic features, ASC from snakeskin gourami skin can be a value-added product in the fish processing industry.

1. Introduction

Fish processing generates up to 70% of organic by-products, depending on species. Increasing interest has been paid from the fish processors to effectively utilize these by-products to produce value added products, especially collagen and gelatin (Kittiphattanabawon *et al*., 2019). Collagens with different properties have been extracted from bone, skin and scales of common carp (*Cyprinus carpio*) (Duan *et al*., 2009), skin of bigeye snapper (*Lutjanus lutjanus*) (Benjakul *et al*., 2010), scales of golden carp (*Probarbus jullieni*) (Ali *et al*., 2017) and the swim bladder of seabass (*Paralabrax* spp.) (Sinthusamran *et al*., 2013). Collagen is a major structural protein present in connective tissues (skin and bone) of animal. Collagen has wide range of applications ranging from cosmetic, biomedical, pharmaceutical, leather to film industries (Nalinanon *et al*., 2011; Ogawa *et al*., 2003). Fish skin being rich in collagenous protein, can serve as an excellent source for collagen production alternative to mammalian counter parts, which is mostly associated with several disadvantages such outbreak of mad cow disease and religions constraint, mainly

Islam and Judaism (Ali *et al*., 2018; Nalinanon *et al*., 2011).

Snakeskin gourami (*Trichogaster pectoralis*) is one of the common fish species of Thailand, habitat in shallow, sluggish or standing water with a lot of aquatic vegetation and in rice paddies and swamps. Due to its taste, it is highly valued for food and has been exported to other countries (Froese, 2014), and is one of the five most important aquacultured freshwater species in Thailand (Pongsri, 2005). Its flesh is appreciated for its good eating quality, and is eaten in the form of fried, grilled, fish soup or dried from (Froese, 2014). Due to its wide availability and acceptability, generation of its processing by-product, especially skin is in the significant amounts. This skin can be effectively utilized for the production of value-added products particularly collagen or gelatin which can be of highly significant as well as benefit the fish processers. Therefore, the objective of this study was to extract and characterize the acid soluble collagen (ASC) from the skin of snakeskin gourami in order to evaluate its possible use as an added value by-product for the fish processing industry.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Bovine hemoglobin, β-mercaptoethanol (β-ME), standard collagen type I were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trichloroacetic acid, disodium hydrogen phosphate, sodium citrate, acetic acid, Folin-Ciocalteu's phenol reagent and tris(hydroxylmethyl) aminomethane were obtained from Merck (Darmstadt, Germany). Coomassie Blue R-250 and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium dodecyl sulfate (SDS) and bovine serum albumin were obtained from Fluka (Buchs, Switzerland).

2.2. Preparation of fish skins

Freshly available snakeskin gourami (*Trichogaster pectoralis*) was purchased from the local market situated in Samut Prakan province of Thailand. Fish were packed in a polyethylene bags, loaded in ice with a sample:ice ratio of 1:2 (w/w) and transported to the Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok within 1 h. The skins were manually removed with a filleting knife and washed with cold distilled water. Cleaned skins were cut into small pieces (0.5 × 0.5 cm^2) using scissors. The prepared skins were placed in a polyethylene bag and stored at −20 °C until used for collagen extraction.

2.3. Extraction of acid soluble collagens (ASC)

Acid soluble collagen (ASC) was extracted following the method of Kittiphattanabawon *et al*. (2019) with slight modification. All processes were carried out at 4 °C under continuous stirring condition. Non-collagenous proteins were removed by soaking the prepared skin in 0.1 M NaOH at a skin/ alkali solution ratio of 1:15 (w/v). The mixture was continuously stirred for 6 h and the alkali solution was changed for every 2 h. The treated skins were then washed in cold water to achieve neutral pH. Followed by defatting in 10% butyl alcohol at a sample:solvent ratio of 1:10 (w/v) for 18 h, the solvent was changed for every 6 h. The defatted skin was then washed with cold water. Collagen was extracted by soaking the pretreated skin in 0.5 M acetic acid at a solid:solvent ratio of 1:15 (w/v) for 48 h. After extraction, the mixture was filtered using two layered cheese cloth and the residue was re-extracted under the same condition. Both, the extracts were combined, and collagen was precipitated by adding NaCl at a final concentration of 2.6 M in presence of 0.05 M Tris-HCl (pH 7.5). The resultant precipitate was collected by centrifuging at 15,000 *g* for 1 h (Heraeus Primo, Thermo Scientific, Germany) and was dissolved in a minimum volume of 0.5 M acetic acid,

followed by dialyzing against 0.1 M acetic acid for 2 days. Thereafter, dialyzed against distilled water for 2 days by a changing the solution for every 12 h. The dialysate was freeze dried and was referred to as "ASC".

Hydroxyproline content of ASC was determined according to the method of Nalinanon *et al*. (2007). The yield of ASC was calculated based on the dry weight of prepared skin using the following equation:

Yield (%) = (Weight of ASC (g)/Dry weight of prepared skin (g)) x 100 (1)

2.4. Characterization of ASC

2.4.1. UV absorption

The samples were solubilized in 0.5 M acetic acid to obtain a final concentration of 2 g/L. UV absorption spectra of collagens were measured using a spectrophotometer (V-730, Jusco Co., Japan). Prior to measurement, the baseline was set with 0.5 M acetic acid. The spectra were obtained by scanning the wavelength in the range of 200–300 nm with a scan speed of 50 nm/min at room temperature.

2.4.2. Sodium dodecylsulfate-polyacerylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970). The collagen samples were dissolved in 5% SDS and heated at 85 °C for 1 h. The mixtures were centrifuged at 4000 × g for 5 min to remove undissolved debris. The solubilized samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4% SDS, 20% glycerol) in the presence or absence of 10% β-mercaptoethanol. Samples (15 µg protein) were loaded onto a polyacrylamide gel (4% stacking gel and 7.5% separating gel) and subjected to electrophoresis at a constant current of 15 mA per gel, using an electrophoresis apparatus (AE-6440, Atto Co., Tokyo, Japan). After electrophoresis, the gel was stained with 0.25% Coomassie brilliant blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid then destained with 30% (v/v) methanol and 10% (v/v) acetic acid. Gels were imaged using a scanner (MFC-L2700DW, Brother, UK) and band intensities were quantified with the public domain digital analysis software, ImageJ (ImageJ 1.51t, National Institutes of Health, Bethesda, USA). High molecular weight marker (Sigma Chemical Co., USA) was used to estimate the molecular weight of proteins. Type I collagen from calf skin was used as a standard.

2.4.3. Amino acid composition

Collagens were hydrolyzed under reduced pressure in 4.0 M methanesulfonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115°C for 24 h. The hydrolyzates were neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.4 mL was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

2.4.4. Differential scanning calorimetry (DSC)

DSC was conducted using a differential scanning colorimeter model DSC 7 (Perkin Elmer, Norwalk, CT, USA). The denaturation temperature (T_{max}) and enthalpy (ΔH) calibrations were determined using indium. ASC were rehydrated in a 0.05 M acetic acid solution with a sample:solution ratio of 1:40 (w/v). The mixtures were allowed to stand for 2 days at 4 °C. The sample (5-10 mg) was accurately weighed into aluminium pans and sealed. The sample was scanned at 1 °C/min over the range of 20-50 °C using iced water as the cooling medium. An empty pan was used as the reference. The maximum transition temperature (T_{max}) was estimated from the thermogram. Total denaturation enthalpy (ΔH) was estimated by measuring the area of DSC thermogram.

2.4.5. Secondary structure

Fourier transform infrared (FTIR) spectra of collagen from the skin of snakeskin gourami was obtained using Bruker Model EQUINOX 55 FTIR spectrometer (Bruker, Ettlingen, Germany). Collagens samples were placed onto

the crystal cell. The spectra were acquired over the range of 4000-800 cm^{-1} with a resolution of 4 cm^{-1} for 32 scans against a background spectrum recorded from the clean empty cell at 25°C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

Circular dichroism (CD) spectra of collagen was determined by dissolving the samples in 0.5 M acetic acid to obtain a concentration of 0.5 mg/mL The spectra of all the solutions were recorded from the wavelength of 300–190 nm (Jasco J-815, Japan Spectroscopic Co., Japan).

2.4.6. Solubility of ASC

2.4.6.1. Effect of NaCl on collagen solubility

The samples were dissolved in 0.5 M acetic acid at 4 °C for 24 h to produce a final concentration of 6 mg/mL. Next, 5 mL of prepared solutions were mixed with 5 mL of 0.5 M acetic acid containing various concentrations of NaCl (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 20,000 *g* at 4 °C for 30 min. Protein content in the supernatant was measured by the method of Lowry *et al*. (1951), using bovine serum albumin as a standard. Relative solubility was calculated as above.

2.4.6.2.Zeta potential

ASC were dissolved in 0.5 M acetic acid at a concentration of 0.5 mg/mL. The mixture was stirred at 4 °C for 12 h. The zeta potential was measured using a zeta potential analyzer (ZetaPALS, Brookhaven Instruments Co., Holtsville, NY, USA). The zeta potential of ASC adjusted to different pH values with 1.0 M nitric acid or 1.0 M KOH using an Autotitrator (BI-ZTU, Brookhaven Instruments Co.) was determined. The isoelectric point was estimated from pH rendering zero zeta potential.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed on the data and differences between means were evaluated using the Duncan's multiple range test. SPSS Statistic Program was used for data analysis.

3.Results and discussions

3.1. Yield of ASC

The yield of ASC extracted from the skin of snakeskin gourami was 9.43% on a wet weight basis and 34.65% on a dry weight basis, similar results were reported by Jongjareonrak *et al*. (2005). Kittiphattanabawon *et al*. (2005) reported that the yield of ASC from the skin of brownstripe red snapper was 9% (wet weight basis) whereas collagen from skin and bone of bigeye snapper yielded 9.38%. The hydroxyproline content of the ASC extracted from the skin of snakeskin gourami was 23.2±1.24 mg/mL which was about 3 times higher than that of its fresh skin (7.22±0.6 mg/mL). The skin raw material could not totally solubilize by 0.5 M acetic acid, indicating the present of high molecular crosslink components in the skin matrix. The differences in extraction yield might be attributed to the differences in fish species, tissue structure and composition, biological conditions, and preparative methods (Ali *et al*., 2018; Jongjareonrak *et al*., 2005; Kittiphattanabawon *et al*., 2019; Sinthusamran *et al*., 2013).

3.2. UV absorption spectrum

Maximum UV absorption spectrum of ASC from snakeskin gourami was 230.7 ± 0.3 nm (Figure 1), which was similar to those of collagens from the skins of ornate threadfin bream (230 nm) (Nalinanon *et al*., 2011), channel catfish (232 nm) (Lui *et al*., 2007) and Rutilus Frisii Kutum (240 nm) (Naderi Gharagheshlagh *et al*., 2019). Generally, the absorption wavelength of protein is from tyrosine, tryptophan, and phenylalanine in the near UV region of 260-290 nm. Generally, the tyrosine and tryptophan residues are known to absorb UV light at 280 nm (Ali *et al*., 2017).

But the amount of tyrosine in ASC was 3 residues per 1000 residues. In addition, Edwards *et al*. (1997) found that ASC from *Oreochromis niloticus* skin showed a maximum absorption at 220 nm, which was related to the C=O, -COOH, -$CONH_2$ groups in polypeptides chains of collagen.

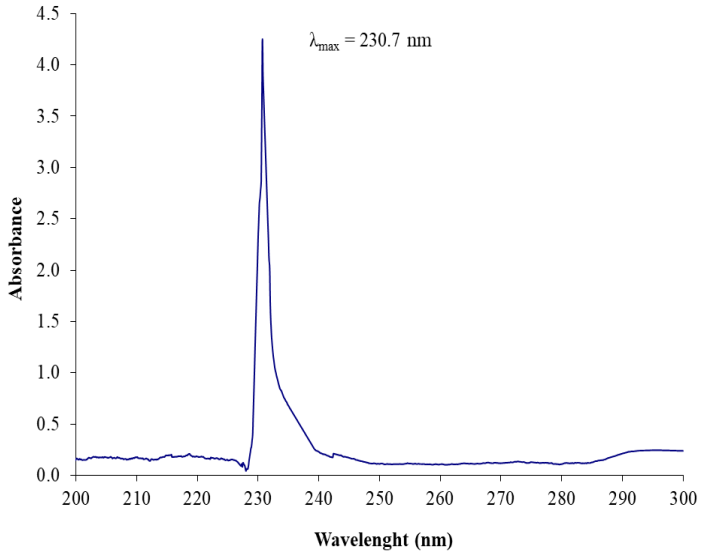


Figure 1. UV absorption spectrum of ASC from the skin of snakeskin gourami.

3.3. Protein patterns

Protein patterns of ASC and skin of snakeskin gourami are shown in Figure 2. The result revealed that both the samples contained α1-chain and α2-chain at a ratio of approximately 2:1, suggesting that type I collagen was the major protein component in the skin. It was noted that ASC mainly contained the highest band intensity of β-chains and γ-chain,which is consistent with findings reported for type I collagen from the skins of hake and trout (Montero *et al*., 1990), golden carp (Ali *et al*., 2018) and bigeye snapper (Kittiphattanabawon *et al*., 2005). Collagen from the swim bladder of seabass was also reported to be type I collagen (Sinthusamran, *et al*., 2013). The ratio between α1-chain and α2-chain of snakeskin gourami was 2.37, which is similar to that from skin of ornate threadfin bream (2.18) (Nalinanon *et al*., 2011). Band intensity ratios of ASC from snakeskin gourami skin (Figure 2A), Ornate threadfin bream, Deep-sea redfish, Black drum fish and calf skin (Table 1) show high population of cross-linked chains (γ and β). The results are in accordance with collagen from the skin of Ornate threadfin bream (2.18) and Deep-sea redfish (2.47) reported by Nalinanon *et al*. (2011) and Wang *et al*. (2007). ASC from the skin of snakeskin gourami found band intensity ratio of β/(α1 + α2) was 1.18 that was lower than in ASC from the skin of Ornate threadfin bream (1.39), and black drum fish (1.59) (Nalinanon *et al*., 2011; Ogawa *et al*., 2003). The different band intensity ratios of α1/α2, β/(α1 + α2), γ/(α1 + α2) and HMC/(α1 + α2) were specifically observed in collagen and could identify the proportion of monomer chain and cross-linked chain**.**

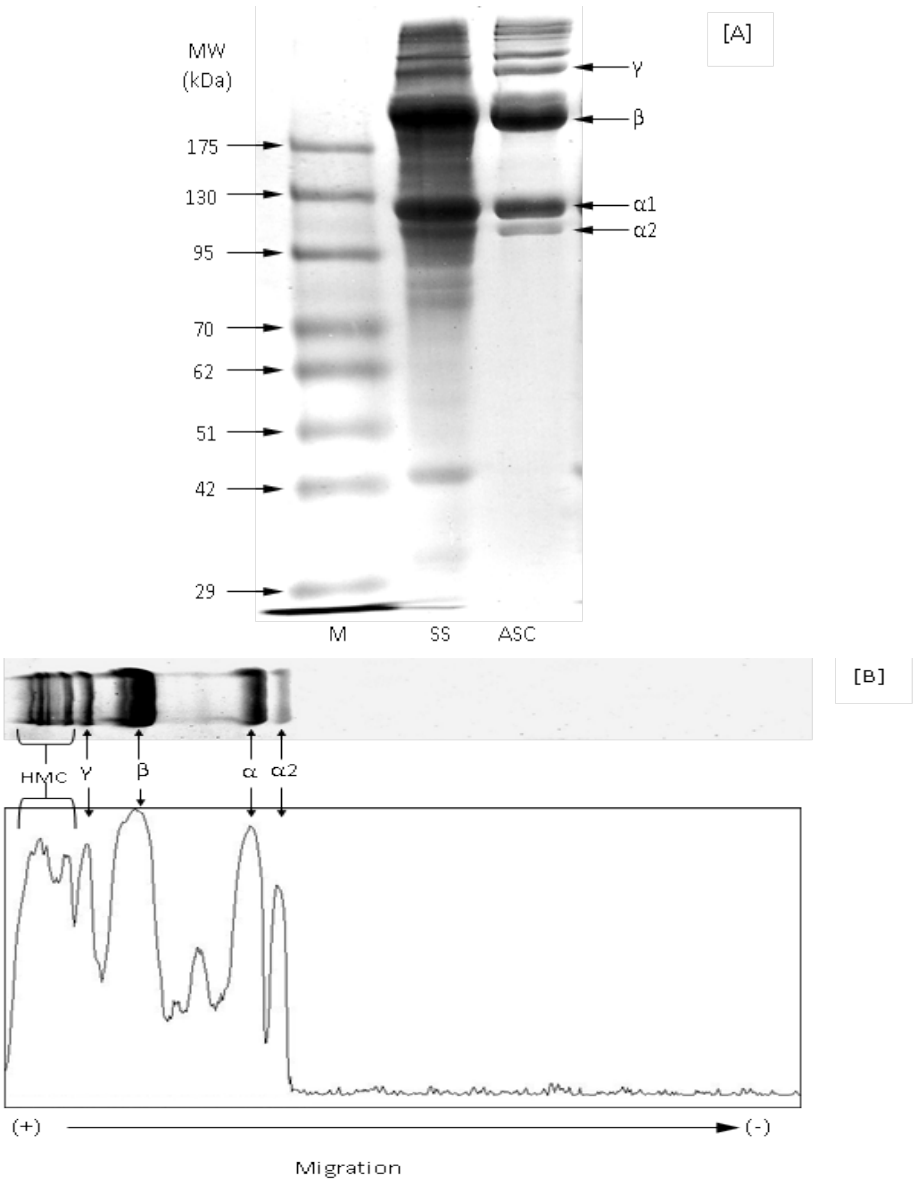


Figure 2. Protein patterns of the skin of snakeskin gourami (SS) and its acid soluble collagen (ASC) counterpart [A] and densitogram of ASC [B]. M denotes high-molecular weight protein markers. HMC is high molecular weight component.

Table 1. Band intensity ratios of collagens from the skin of snakeskin gourami (ASC) in comparison with ornate threadfin bream, deep-sea redfish, black drum, and calf skin.

Components	Band intensity ratio				
	ASC	Ornate threadfin bream[(a)]	Deep-sea redfish[(b)]	Black drum[(c)]	Calf [(c)]
α1/α2	2.37	2.18	2.47	ND	ND
β/(α1 + α2)	1.18	1.39	1.52	1.59	1.26

γ/(α1 + α2)	0.45	0.33	1.1	1.32	0.75
HMC/(α1+ α2)	1.15	0.62	ND	ND	ND

D = Not determined.

Sources :(a) Nalinanon *et al.* (2011); (b) Wang *et al.* (2007) and (c) Ogawa *et al.* (2003).

3.4. Amino acid composition

The amino acid composition of ASC was expressed as "residues per 1000 total amino acid residues" (Table 2). Type I collagen and ASC from the skin of snakeskin gourami were rich in glycine, alanine, proline, and hydroxyproline and had low or no residues of cysteine, tyrosine and hydroxylysine (Singh *et al.*, 2011). Glycine was the most abundant compound with 332 and 313 units of the total amino acids present in type I collagen and ASC, respectively. Generally, glycine in collagen represent almost one third of the total residues and occurs every third residue in collagen except for the first 14 amino acid residues from N-terminus and the first 10 residues from the C-terminus (Foegeding, 1996). Both collagens had proline (130 and 115 residues/1000 residues), alanine (108 and 124 residues/1000 residues) and hydroxyproline (95 and 73 residues/1000 residues). Alanine was the second most abundant amino acid in all collagens. All collagens contained no cysteine and negligible tryptophan. The imino acid (proline and hydroxyproline) content of type I collagen and ASC were 225 and 188 residues/1000 residues, respectively. ASC was relatively higher than those of ASCs from ballon fish skin (179 residues/1000 residues) (Huang *et al*., 2011), cod skin (179 residues/1000 residues), carp skin (179 residues/1000 residues) (Duan *et al*., 2009), swim bladders of seabass (128 residues/1000 residues) (Kaewdang *et al*., 2014) but was slightly lower than cobia skin (203 residues/1000 residues) (Zeng *et al*., 2012), brownbanded bamboo shark skin (204 residues/1000 residues) (Kittiphattanabawon *et al*., 2010), striped catfish skin (206 residues/1000 residues) (Singh *et al*., 2011). The imino acid content was used to determine the thermal stability of collagen and the formation of junction zones via hydrogen boding (Sinthusamran *et al*., 2013). The pyrrolidine rings of proline and hydroxyproline impose restrictions on the conformation of polypeptide chains and help to strengthen the triple helix (Wang *et al*., 2007).

Table 2. Amino acid composition of acid soluble collagen (ASC) from the skin of snakeskin gourami and calf skin type I collagen (residues/1000 residues)

Amino acid	Type I collagen	ASC
Alanine	108	124
Arginine	49	55
Aspartic acid/asparagine	45	49
Cysteine	0	0
Glutamic acid/glutamine	73	75
Glycine	332	313
Histidine	5	6
Isoleucine	12	12
Leucine	24	27

Lysine	27	28
Hydroxylysine	7	6
Methionine	6	11
Phenylalanine	12	16
Hydroxyproline	95	73
Proline	130	115
Serine	34	33
Threonine	16	28
Tyrosine	3	5
Tryptophan	0	0
Valine	20	24
Total	**1000**	**1000**
Imino acids *	225	188
* Imino acids include proline and hydroxyproline.		

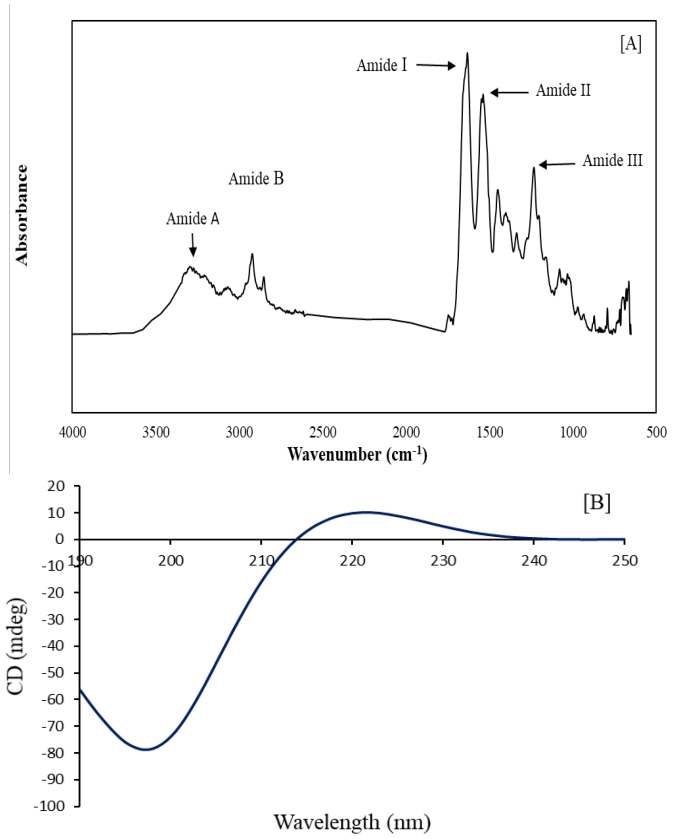


Figure 3. Fourier transform infrared (FTIR) [A] and circular dichroism (CD) [B] spectra of ASC from the skin of snakeskin gourami.

3.5. Thermal stability of ASC

The T_{max} and ΔH of ASC estimated from DSC thermogram were 33.43 °C and 1.15 J/g, respectively. Thermal stability of collagen was governed by pyrrolidine rings of proline and hydroxyproline and partially by hydrogen bonding through the hydroxyl group of hydroxyproline (Benjakul *et al*., 2010). However, the T_{max} value might also be determined by the conformation and amino acid sequence of collagen (Ali *et al*., 2018). T_{max} values of ASC from snakeskin gourami were higher than those previously reported for collagens from several fish species. T_{max} of ASC from the skin of snakeskin gourami was in accordance with those of cold-water fish such as cod (15 °C), Alaska pollack (16.8 °C), Japanese seabass (30 °C), skip jack tuna (29.7 °C) and ayu (29.7 °C) (Sinthusamran *et al*., 2013; Yu *et al*., 2014) or collagen from the skin of tropical fish such as brownstripe red snapper (31.5 °C), bigeye snapper (30.4 °C), black drum fish (34.2 °C), sheepshead seabream (34 °C) (Jongjareonrak *et al*., 2005; Kittiphattanabawon *et al*., 2010; Ogawa et al., 2003). The differences in Tmax amongst collagens from different species were correlated with different imino acid contents, body temperature and environmental temperature (Duan *et al*., 2009; Kittiphattanabawon *et al*., 2005).

3.6. Secondary structure of ASC

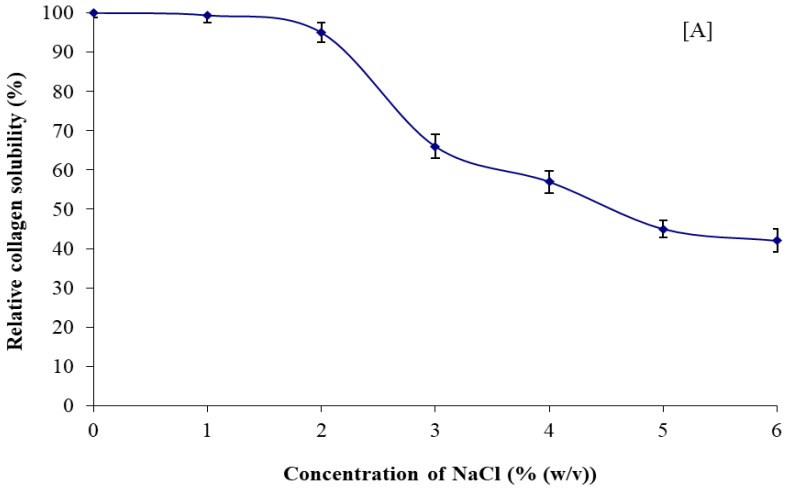
The FTIR spectrum of ASC from the skin of snakeskin gourami exhibited characteristic peaks of amide A and B, as well as amide I, II, III (Figure 3A). The major peaks found in ASC were similar to those of collagens isolated from other fish species (Kaewdang *et al*., 2014; Kittiphattanabawon *et al*., 2010; Li *et al*., 2020). The amide A was found at a wave numbers of 3288 cm^{-1} and associated with N-H stretching vibration, which occurs in the wave number range of 3400-3440 cm^{-1} (Purna Sai and Babu, 2001). Also, when the NH group of a peptide is involved in a hydrogen bond, the position is shifted to a lower frequency (Ali *et al*., 2018). The amide B was found at a wave number of 3067 cm^{-1}, associated with the asymmetrical stretch of CH_2 (Muyonga *et al*., 2004). The wave number of amide I, amide II and amide III bands are directly associated with the configuration of collagen. The amide I band with the characteristic strong absorbance in the range of 1600-1700 cm^{-1} was mainly related to the C=O stretching vibration along the polypeptide backbone, and it could be a sensitive marker of peptide's secondary structure (Sinthusamran *et al*., 2013). The amide I band of ASC was found at the wavenumber of 1631 cm^{-1}. The amide II band of ASC was situated at a wave number of 1546 cm^{-1}, while the amide III band of ASC was located at wave number 1232 cm^{-1}. The amide II and amide III bands represent N-H bending vibration and C-H stretching, respectively (Naderi Gharagheshlagh *et al.*, 2019). As a consequence, ASC was still conserved in its secondary structure. The CD spectrum of ASC scanned in the range of 190-250 nm is shown in Figure 3B. The CD curves showed a rotatory maximum at 222 nm and minimum at 197 nm, and a consistent crossover point at about 214 nm, which is a typical characteristic of triple helical conformation of collagen (Ikoma *et al*., 2003). CD spectra represents backbone configuration of protein through absorption regions of peptide linkage (Ogawa *et al*., 2004). ASC exhibited distinct positive and negative absorbance for a native collagen, while denatured collagen has a more distorted spectrum, reflected by the disappearance of a positive peak at 221 nm as well as negative peak shifts to lower absorption than 198 nm as reported by Ali *et al*. (2017). The results confirmed that ASC had high structural integrity without denaturation.

3.7. Solubility of ASC

The effect of NaCl on collagen solubility is shown in Figure 4A. The high solubility of ASC in 0.5 M acetic acid was maintained in the presence of NaCl up to 1-2%. Solubility of ASC decreased gradually when the NaCl

concentration exceeded 2% and was also reduced when the NaCl concentration was 6%. This result is in accordance with the reports on the solubility of collagens from the skins of yellowfin tuna, dusky spinefoot, sea chub, eagle ray, red stingray, yantai stingray, brownstripe red snapper, bigeye snapper and striped catfish in acetic acid solution generally decreased with an increase in NaCl concentration (Jongjareonrak *et al*., 2005; Kittiphattanabawon *et al*., 2005). This effect could be due to the "salting out" of collagen, which occurred at relatively high concentration of NaCl (Singh *et al*., 2011). According to Matmaroh *et al*. (2011), at low concentrations of NaCl, salt ions are bound weakly to the charged groups on the protein surfaces without affecting the hydration shell on those domains. These results support the use of collagen from fish scales and skin as an alternative source for use in food, pharmaceutical and nutraceutical industries.

The zeta potential is a key indicator of the stability of colloidal dispersions. ASC from snakeskin gourami at different pH levels is shown in Figure 4B. At pH 2-6, collagen sample were positively charged, and negatively charged between a pH of 7-10 with the net charge of zero at pH of 6.23. When pH values were above or below pI values of the proteins, the repulsion between the protein chains increased resulting in a high net charge and the solubility of protein molecules. When the net charge of a protein was zero, hydrophobic-hydrophobic interactions increased, resulting in protein precipitation and aggregation (Ali *et al*., 2018). Collagens extracted from various fish have been shown to exhibit different pI values. For example, the pI value of collagen from striped catfish skin was 4.27 (Singh *et al*., 2011), collagen from spotted golden goatfish scales had a pI of 4.96 (Matmaroh *et al*., 2011), and the pI of bamboo shark skin collagen was 6.12 (Kittiphattanabawon *et al*., 2010). The slight differences in pI between collagens from various fish species might be caused by the slight difference in their amino acid sequences and distribution of amino acid residues.



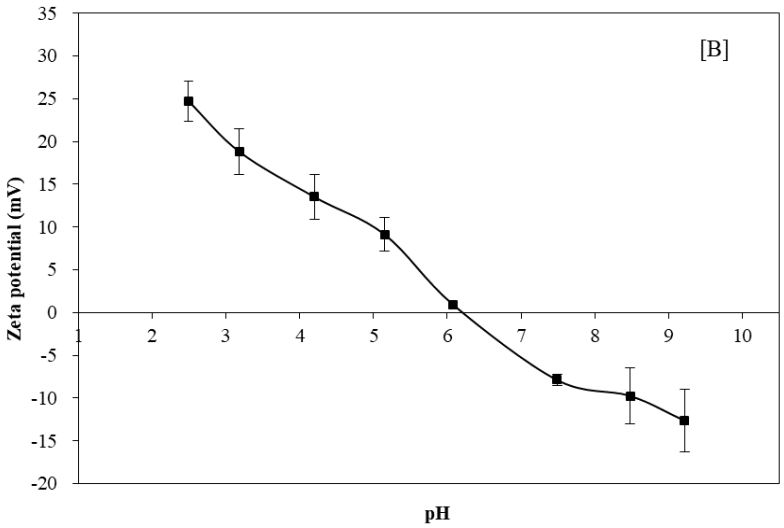


Figure 4. Solubility at different NaCl concentrations [A] and Zeta potential at different pHs [B] of ASC from the skin of snakeskin gourami.

4. Conclusions

Collagen from the skin of snakeskin gourami was simply extracted by using acetic acid. Based on SDS-PAGE and amino acid composition, ASC was characterized to be type I collagen. The structural integrity of ASC was well preserved as determined by FTIR and circular dichroism. The solubility of ASC was depended on pH and NaCl concentration. Since collagen has a wide range of commercial uses, and based on obtained results, ASC from skin of snakeskin gourami can be a promising means of alternative source to produce collagen mainly from the fish processing industries.

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