

Separation and some properties of collagens from *Ctenopharyngodon idella* scales

Nguyen Thi Kim An^{1,*}, Dam Xuan Thang¹, Nguyen Ngoc Thanh¹,
Nguyen Thi Huong¹, Le Thi Bich Thao²

¹Hanoi University of Industry, 298 Cau Dien Street, Tay Tuu Ward, Ha Noi, Viet Nam

²Institute of Biology, Vietnam Academy of Science and Technology (VAST),
18 Hoang Quoc Viet, Nghia Do Ward, Ha Noi, Viet Nam

*Email: annk_cnh@hau.edu.vn

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Abstract. Collagen was successfully extracted from scales of grass carp (*Ctenopharyngodon idella*) at room temperature (20–25 °C) using acid alone, as well as with pepsin or protease assistance. SDS-PAGE analysis revealed that acid-solubilized collagen (ASC) and pepsin-solubilized collagen (PSC) contained characteristic α -chains of approximately 120 kDa, consistent with type I collagen, whereas protease-solubilized collagen (PrSC) exhibited lower molecular weight chains (35–60 kDa). MS/MS spectrometry analysis confirmed that the isolated collagen was type I. Herein, the amino acid sequence of collagen isolated from grass carp cultivated in Viet Nam is reported for the first time, along with physicochemical properties, morphology, and elemental composition of the collagen. According to the research, grass carp scales represent a potential source for type I collagen extraction. Pepsin efficiently yields collagen with high purity and an intact triple-helix structure, while protease effectively produces collagen with lower molecular weight. These properties highlight the potential of both enzymes for various industrial and biomedical applications.

Keywords: *Ctenopharyngodon idella*, grass carp, fish scales, type I collagen, amino acid sequence.

Classification numbers: 1.1.1, 1.2.5, 1.4.3.

1. INTRODUCTION

Collagen is the most abundant protein found in all multicellular animals, serving as the main component of skin, muscles, tendons, cartilage, bones, blood vessels, ligaments, and cellular tissues. With a high protein content, collagen plays an important role in initiating and maintaining the interaction between cells and extracellular matrix in both vertebrates and invertebrates [1–3]. To date, at least 29 different types of collagen have been identified, among which types I, II, and III are the major and the most abundant [4, 5]. In particular, collagen type I is the primary component of mammalian connective tissue and animal protein, constituting about 30 % of all protein in the human body [6, 7].

Collagen type I is responsible for forming the structural fibrils of tissues [8]. Collagen type I also exhibits high biocompatibility with the human body and possesses properties such as the ability to absorb water, form gels, and stabilize emulsions, making it widely used as an

ingredient in cosmetics and medical biomaterials [6, 7]. In the medical field, it is used for implants such as artificial skin grafts, wound dressings, and nerve conduits due to its low immunogenicity [9, 10]. In cosmetics, its properties contribute to the development of creams and gels with high moisturizing action, as well as other activities such as anti-aging, anti-wrinkling, UV radiation protection, and wound healing [9]. Its inclusion in cosmetic formulations is attributed to its film-forming properties, which, when applied, cover the skin and decrease transepidermal water loss, thereby protecting the skin from corrosive elements [11]. Recent studies have highlighted the potential of by-products from the fish processing industry (such as scales, skin, swim-bladders, and bones) of both freshwater and marine fish as sustainable and abundant sources of collagen. These by-products can constitute 50–70% of the total catch weight and are rich in collagen [12, 13]. Consequently, the fish collagen segment is projected to grow and dominate the market at the fastest pace in the coming years due to its abundance and lower risk profile.

Grass carp *Ctenopharyngodon idella* (*C. idella*) is a freshwater fish species known for its delicious, high-nutritional-value meat and minimal small bones, making it highly favored by domestic consumers. Grass carp are widely cultivated across most provinces in Viet Nam due to their herbivorous nature, ease of farming, low nutritional requirements, and low production costs, while commanding high market prices. On average, a grass carp can reach weights of 1.5–2.5 kg within 7–8 months, and under optimal conditions, it can grow to a commercial weight of approximately 3–4 kg [14]. The body of grass carp is covered with large, hard scales that are biocomposites of highly ordered type I collagen fibers and hydroxyapatite $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ [15, 16]. However, these by-products are often discarded during processing despite being a rich source of collagen and minerals. As a result, our research team is dedicated to extracting and isolating collagen from fish scales while exploring enzymatic hydrolysis to create high-quality collagen and low molecular weight peptides, aiming to develop sustainable and cost-effective collagen sources for application in cosmetics and beauty products.

2. MATERIALS AND METHODS

2.1. General

Fresh scales of grass carp (*C. idella*), obtained after fish processing, were collected in local markets in Tu Liem ward, Ha Noi, Viet Nam. The scales were thoroughly washed with fresh water, followed by distilled water, and were then stored in the freezer until use. Chemicals and enzymes, including pepsin (250 units/mg) and protease (15 units/mg), were provided by Sigma-Aldrich (USA), Bio-Rad (USA) and Merck (Germany). All reagents used were of analytical grade.

The nano liquid chromatography-tandem mass spectrometry (nano LC-MS/MS) system employed was a QSTAR® XL Hybrid LC/MS/MS system (Applied Biosystems/MDS Sciex, Ontario, Canada), which features a high-performance hybrid quadrupole time-of-flight mass spectrometer. The system was equipped with a nano-electrospray ionization (nano-ESI) source connected to a reverse-phase C18 column with a SilicaTip™ PicoTip® emitter (New Objective, USA). MS/MS spectra of the peptide mixture were recorded and analyzed using Mascot software (Matrix Science Inc., USA). Protein identification was performed by searching against the NCBIprot database.

2.2. Extraction and purification of collagen

The extraction of collagen from *C. idella* scales was conducted following the methods of Liu *et al.* [17] and Zhang *et al.* [18] with slight modifications [19]. In the first step, scales (100.00 g) were soaked in 15 volumes of 0.5 M NaOH (w/v) for 24 h to remove non-collagenous proteins and pigments. The scales were then thoroughly washed with cold distilled water until reaching a neutral pH. Next, the protein-free scales were decalcified by immersing them in 10 volumes (w/v) of a 1:1 (v/v) mixture of 0.5 M H₃PO₄ + 0.5 M HCl for 30 min, followed by washing with cold distilled water until the pH was close to 7. Subsequently, the demineralized scales were immersed in 15 volumes of 0.5 M acetic acid (w/v) under continuous stirring for 24 h to produce acid-solubilized collagen (ASC). Alternatively, scales were treated with 0.5 M acetic acid containing 1 % (w/v) pepsin to produce pepsin-solubilized collagen (PSC) or with 0.5 M acetic acid containing 1 % (w/v) protease to produce protease-solubilized collagen (PrSC). All procedures were carried out within a controlled temperature range of 4 °C to 35 °C to ensure optimal experimental conditions.

The supernatants were collected and cooled to 4 °C before salting out by adding NaCl to a final concentration of 2.0 M. After 1 h, the precipitates were centrifuged at 10,000 rpm for 30 min and then resolubilized in 0.5 M acetic acid. These salting-out and centrifugation steps were repeated three times for the purification of collagen. Afterward, the collagen solution was dialyzed using dialysis membrane against 0.1 M acetic acid for 24 h and then against deionized water for 24 h. Finally, the collagen solution was lyophilized using the Eyela freeze dryer FD550P (Tokyo Rikakikai Co., Ltd., Japan).

The extraction yield of dried collagen from *C. idella* scales was calculated using equation below:

$$\text{Yield of collagen (\%)} = \frac{\text{Weight of dried collagen (g)}}{\text{Weight of wet scale (g)}} \times 100$$

2.3. Characterization of extracted collagens

2.3.1. Fourier-transform infrared (FTIR) spectroscopy

Freeze-dried collagen samples were prepared by mixing with potassium bromide (KBr, Sigma-Aldrich, USA) in a 1:100 (w/w) ratio, followed by grinding into a fine powder. The resulting mixture was compressed into transparent pellets under high pressure. The compressed pellets were analyzed on a Tensor 27 Bruker FTIR spectrometer. FTIR spectra were recorded in the range of 4000–400 cm⁻¹ with a resolution of 2 cm⁻¹, averaging 32 scans in absorbance mode.

2.3.2. Field emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectroscopy (EDX)

The surface morphology of collagen samples was examined using a field emission scanning electron microscope (FESEM, S-4800, Hitachi, Japan). Solid samples were first coated with a thin layer of platinum using a sputter coater to enhance electrical conductivity and prevent charging effects during imaging. Images were captured at various magnifications to observe structural details at the microscale. For elemental analysis, energy-dispersive X-ray spectroscopy (EDX) was conducted with a Horiba 7593-H detector (Kyoto, Japan) integrated into the FESEM system. This setup enabled simultaneous morphological and compositional characterization of the samples. Data acquisition focused on identifying the presence and distribution of key elements associated with collagen.

2.3.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE technique is used to separate proteins based on polypeptide chain length or molecular weight. SDS-PAGE was performed according to the methods of Laemmli *et al.* [20] using 12.6 % acrylamide gel (w/v). The samples were dissolved in 0.6 M Tris-HCl buffer (pH 6.8) containing 25 % (v/v) glycerol, 2 % (w/v) SDS, 5 % (v/v) β -mercaptoethanol and 0.1 % (w/v) Bromophenol blue. After electrophoresis, gels were visualized with Coomassie Brilliant Blue (CBB) staining solution [0.1 % (w/v) CBB, 30 % (v/v) methanol, 10 % (v/v) acetic acid].

2.3.4. Amino acid sequence analysis

Based on the SDS-PAGE electrophoresis results, the gel was washed, and 120 kDa protein bands were excised. The excised gel pieces were washed with solution of 50 % acetonitrile and 100 mM NH_4HCO_3 (solution A) at 30 °C until the CBB was completely removed. The gel pieces were then dehydrated with 100 % acetonitrile for 5 min and dried. Next, disulfide bonds between cysteine residues in the protein molecules were reduced using a reducing solution (10 mM dithiothreitol and 100 mM NH_4HCO_3) at 56 °C for 45 min. The solution was then cooled to room temperature. The gel pieces were alkylated with a solution of 50 mM iodoacetamide and 100 mM NH_4HCO_3 , incubated in the dark at room temperature for 45 min, followed by washing with solution A and dehydrating with 100 % acetonitrile, as previously described.

The gel pieces were dried and reconstituted in a hydrolysis solution (10 ng/ μL trypsin in 50 mM NH_4HCO_3 containing 10% acetonitrile). The samples were incubated on ice for 30 min before the addition of 50 mM NH_4HCO_3 , followed by overnight incubation at 30 °C. Formic acid was added to a final concentration of 1 % to stop the enzymatic reaction, and the supernatant was transferred to a clean Eppendorf tube. Peptides remaining in the gel were extracted using a solution containing 50 % acetonitrile and 5 % formic acid for 45 min with 5 min sonication; this process was repeated three times. Subsequently, the peptide extract in the combined supernatant was dried using a SpeedVac system (Massachusetts, USA). The resulting peptide mixture was purified in salt form using a Ziptip C18 column (Millipore, MA, USA) and subjected to mass spectrometry analysis using an LC-MS/MS system.

3. RESULTS AND DISCUSSION

3.1. Extraction of the collagens from *C. idella* scales

3.1.1. Effect of temperature on collagen yield

Fish collagen is thermally unstable and highly susceptible to denaturation when exposed to heat. Collagen from tropical fish tends to have a denaturation temperature in the range of 30–40 °C or higher, whereas collagen from cold-water fish typically denatures at around 20–25 °C [18, 21, 22]. Collagen isolated from tropical fish typically has a higher hydroxyproline content, which strengthens the triple helix structure of collagen and increases thermal stability [23].

Conventional collagen extraction methods are usually performed at 4–8 °C to prevent denaturation and maintain structural integrity [24–26]. These low temperatures are essential for preserving the native characteristics of collagen, however, maintaining such conditions during large-scale industrial operations requires specialized cooling systems and strict temperature control, leading to increased energy consumption and operational costs. Previous studies have revealed that collagen extraction from grass carp skin can be performed at higher temperatures, ranging from 10 to 40 °C. The findings showed that collagen yield increased significantly when

temperature was elevated from 5 to 20–25 °C but reduced at higher temperatures [27, 28]. Fish scales are biocomposites, composed of collagen matrix mineralized with a hydroxyapatite layer, requiring decalcification prior to extraction. Notably, extraction at room temperature (20–30 °C) is feasible and does not compromise collagen quality.

In this study, we investigated the collagen extraction process from *C. idella* scales within a temperature range of 4 °C to 35 °C (Figure 1a). The results showed that collagen yield increased as the temperature rose from 4 °C to 20 °C and 25 °C (yields of 1.78, 1.82 and 1.81 %, respectively). However, a significant decline in yield was observed at 30 °C and 35 °C (yields of 1.72 and 1.70 %, respectively). These results align with references [27, 28], which suggest that moderate increases in temperature enhance collagen solubility and yield. However, excessive heat at 30 °C and above leads to protein denaturation, reducing extraction yield. While the yield at 25 °C was slightly lower than at 20 °C, the difference was not statistically significant. Based on these results, a room temperature of 20–25 °C is optimal for collagen extraction from fish scales, ensuring both cost-effectiveness and efficiency.

3.1.2. Effect of enzymes on collagen yield

Enzymatic methods are widely recognized for improving extraction efficiency and product quality, as they promote selective hydrolysis without causing excessive damage to the collagen structure. Common enzymes used in collagen extraction include pepsin, trypsin, papain, alcalase, protease, etc. Pepsin, active under acidic conditions, is highly effective for extracting collagen type I and type II [3, 29–32]. Trypsin, a digestive enzyme, is less frequently used due to its potential to hydrolyze both collagen and other proteins. Papain, derived from papaya, efficiently breaks down non-collagenous proteins, aiding in the purification process. Alcalase, a microbial enzyme, is widely recognized for its efficiency in collagen extraction from sources like fish skin and scales [33, 34]. Protease plays a significant role in breaking down proteins and isolating collagen from complex biological materials [35].

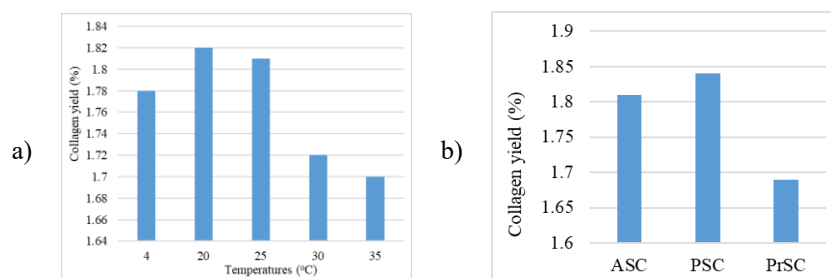


Figure 1. Effect of temperature (a) and enzyme types (b) to collagen yield.

In this study, collagen extraction was performed at a fixed temperature of 25 °C, with the addition of pepsin and protease enzymes to investigate their effects. The concentration of each enzyme was maintained at 1 %, a value determined based on a comprehensive review of the literature, which highlighted its effectiveness in balancing maximum yield with the preservation of collagen's structural integrity [36].

The results showed that the use of pepsin increased the collagen yield to 1.84 % (Figure 1b) and also improved the quality of the collagen compared to non-enzymatic method, as detailed in Section 3.2.2. This improvement was attributed to pepsin's ability to selectively degrade non-collagenous proteins while preserving the structural integrity of collagen, making it a preferred enzyme for collagen extraction. In contrast, the use of protease reduced collagen

yield to 1.69 % due to the cleavage of collagen molecules into shorter peptide chains that failed to precipitate during the salting-out step. Nevertheless, this method has the advantage of producing hydrolyzed collagen, which is characterized by shorter peptides which are more easily absorbed through the skin and digestive system compared to native collagen.

3.2. Characteristics of extracted collagen

3.2.1. IR spectra of collagen

The FTIR analysis of materials extracted from *C. idella* scales confirmed the presence of collagen through its characteristic absorption bands. The spectra of ASC, PSC and PrSC samples exhibited similar features, with distinct signals attributed to amide A, amide I, amide II and amide III, corresponding to the vibrational modes of collagen-specific functional groups [37].

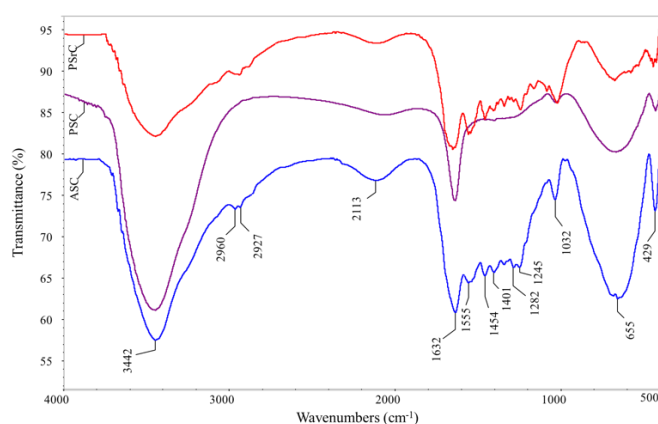


Figure 2. FTIR spectra of the ASC, PSC, PrSC extracted from *C. idella* scales.

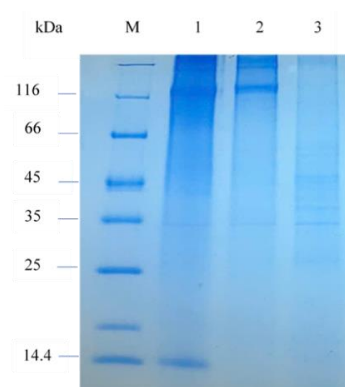


Figure 3. SDS-PAGE profile of collagens from *C. idella* scales. Lane M, marker protein; lane 1: ASC; lane 2: PSC; lane 3: PrSC.

The prominent peaks corresponding to type I collagen functional groups were identified in all three collagen samples. The amide A band, observed between 3400 and 3500 cm^{-1} , appeared at a lower wavenumber than the typical free N-H vibrations, indicating the presence of N-H stretching vibrations involved in intermolecular hydrogen bonding. The amide I, amide II and amide III vibrations were detected at approximately 1632, 1555 and 1245 cm^{-1} , respectively. These absorption bands in the obtained ASC, PSC and PrSC were consistent with those of collagens isolated from other sources [38–40]. However, in the FTIR spectrum of PSC, the amide B vibration (at approximately 2927 cm^{-1} , asymmetrical CH_2 stretching) exhibited significantly reduced intensity, alongside decreased absorption intensities in the Amide II and Amide III regions (Figure 2). Furthermore, in the IR spectra of PSC and PrSC, the vibration at 655 cm^{-1} , associated with out-of-plane bending of C-H and N-H bonds in the triple-helical structure of collagen, exhibited a reduced intensity compared to the spectrum of ASC. This is likely due to enzymatic hydrolysis, which disrupted the triple-helical structure of collagen.

3.2.2. SDS-PAGE patterns

The SDS-PAGE patterns of ASC, PSC, and PrSC extracted from *C. idella* scales, as shown in Figure 3, revealed distinct molecular characteristics. Both ASC and PSC exhibited molecular masses of approximately 120 kDa, containing two α -chains (α_1 and α_2), which are characteristic

of type I collagen, as previously reported [40]. In the SDS-PAGE pattern of ASC, an additional band at 14.4 kDa was observed, while no such bands were detected below the α -chains in the pattern of PSC. This suggests that PSC, extracted using the pepsin-soluble method, preserves the fundamental collagen structure while hydrolyzing small molecular weight proteins or peptides, resulting in higher-quality collagen. These findings are consistent with previous studies, underscoring pepsin as an effective enzyme for the extraction of collagen from fish by-products [29–32].

In contrast, the SDS-PAGE pattern of PrSC lacked the type I collagen band but displayed electrophoretic bands corresponding to small molecular weight proteins or peptides with masses ranging from 35 to 60 kDa. This is likely due to the proteolytic action of protease, which cleaves peptide bonds within collagen molecules. These results highlight the potential application of protease in producing low molecular weight, collagen-derived peptides that are easily absorbed.

3.2.3. FESEM morphology of collagen

FESEM analysis revealed that the morphological structures of ASC, PSC, and PrSC extracted from *C. idella* scales were similar, exhibiting consistent microstructural characteristics. Shown in Figure 4 is the FESEM micrograph of PSC, which represents a higher-purity collagen variant. It can be seen that the collagen had the typical fibril structure with fibers concentrated to form fiber bundles similar to collagens obtained from other fish [21, 41]. At low magnification (500x, scale bar 100 μ m), collagen exhibits a highly porous, interconnected network with voids of varying sizes. At higher magnifications (5000x, scale bar 10 μ m), distinct collagen fibers are visible, forming an organized fibrillar network with smooth surfaces and interconnections, highlighting its mechanical strength and flexibility. At ultra-high magnifications (20,000x and 30,000x, scale bars 2 μ m and 1 μ m), the nanoscale details of the fibrils are observed, showing tightly packed arrangements and smooth textures, indicating high purity, uniformity, and structural integrity. The morphological characteristics of collagen confirm its suitability for applications such as tissue regeneration, wound healing, drug delivery systems, and scaffolds. Its high porosity, fibrillar alignment, and mechanical stability suggest excellent potential for mimicking the extracellular matrix, promoting cell adhesion and proliferation [22, 31, 37, 41, 42].

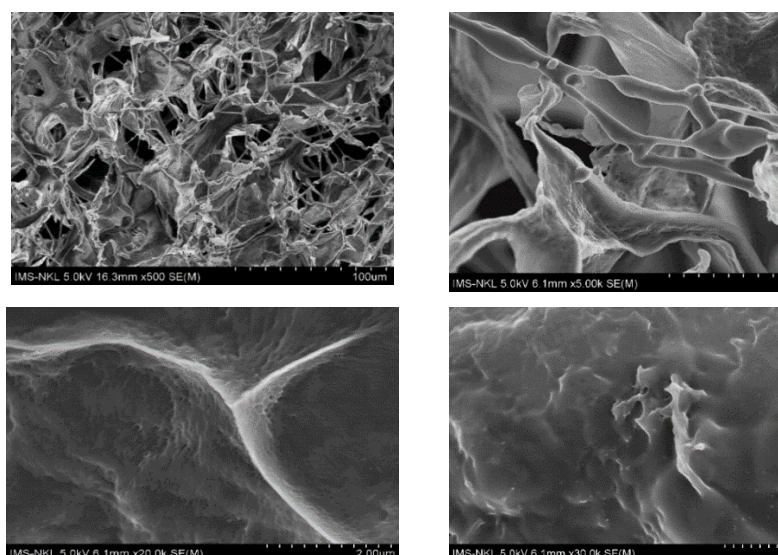


Figure 4. FESEM images of PSC from *C. idella* scales.

3.2.4. Element composition analysis in collagen

The elemental compositions of ASC, PSC, and PrSC were determined using EDX analysis. According to the EDX spectra, all three collagen samples primarily consisted of C, O, and N as their main components, accounting for 97.8–99.7% of the total collagen weight (Table 1). The presence of chlorine elements in ASC and PrSC was attributed to the use of NaCl during collagen precipitation, which was not completely removed during the purification process. However, the minimal amount of chlorine detected (0.08% by weight and 0.03 % by element composition) indicates that the collagen samples were highly purified.

Table 1. Element compositions in ASC, PSC and PrSC.

Element	ASC		PSC		PrSC	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
C	53.04	59.45	45.90	52.10	41.11	47.81
O	9.68	9.31	20.58	20.03	12.26	12.22
N	36.98	31.12	31.96	27.23	44.47	38.82
S	0.22	0.09	1.27	0.54	-	-
Cl	0.08	0.03	-	-	0.08	0.03
Ca	-	-	0.29	0.10	-	-
Mg	-	-	-	-	0.90	0.52
Al	-	-	-	-	0.37	0.19
Si	-	-	-	-	0.81	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00

The presence of sulfur (S) elements in ASC and PSC corresponds to sulfur-containing amino acids in collagen, such as methionine (M) and trace cysteine. In contrast, sulfur was undetectable in PrSC, providing evidence for the cleavage of peptide chains into short fragments, consistent with the SDS-PAGE pattern of PrSC. Additionally, small amounts of calcium, magnesium, aluminum, and silicon were detected in PSC and PrSC.

3.3. Amino acid sequence of collagen

The MS/MS spectrometry analysis of the extracted sample was conducted using Mascot software. The identified proteins were compared to those with known amino acid sequence and precise molecular weight on NCBIprot database. As a result, eight proteins belonging to the skin-scale protein group (collagen) of fish family were identified. These include grass carp (*C. idella*), rainbow trout (*Oncorhynchus mykiss*), grouper (*Epinephelus caninus*) and carp (*Cyprinus carpio*). Among those, three proteins with higher protein score, a greater number of significant matches, and a higher number of significant sequences were listed here: **Protein No.1: collagen type I alpha 1** [*C. idella*] (accession: ADK35755.1, protein score: 231, molecular weight (Mr) (kDa): 138.104, pI: 5.44, amino acid sequence: Figure 5); **Protein No.2: collagen type I alpha 2** [*Oncorhynchus mykiss*] (accession: CO1A2_ONCMY, protein score: 49, molecular weight (Mr) (kDa): 126.908, pI: 9.36); **Protein No.3: collagen type I alpha 1** [*Epinephelus caninus*] (accession: CO1AA_EPICA, protein score: 46, molecular weight (Mr) (kDa): 91.476, pI: 5.61). Grass carp (*C. idella*) exhibited the highest peptide recognition capacity, detecting three peptide fragments of collagen type I alpha 1 (highlighted in red bold in Figure 5), as confirmed by MS/MS spectra (Supporting information). In contrast, *Oncorhynchus mykiss* and *Epinephelus caninus* each recognized a single fragment, identified as collagen type I

alpha 2 and collagen type I alpha 1, respectively. The results supported the conclusion that collagen isolated from grass carp is type I collagen, with the collagen sequence presented above.

1	MFSFVDIRLA	LLLSATVLLA	RGQGEDDRTG	GSCTLDGQVY	NDRDVWKPEP
51	CQICVDSGT	VMCDEVICED	TTDCPNPVI	HDECCPVCPD	DDFQEPSVEG
101	PRGTPGEKGD	RGPAGPPGND	GIPGQPLPG	PPGPPGPPGL	GGNFSPQMSG
151	GFDEKSGGAM	AVPGPMGPMG	PRGPPGPPGT	PGPQGFTGPP	GEPGEAGAPG
201	PMGPRGAAGP	PGKNGEDGES	GKPGRPGERG	PPGPQGARGF	PGTPGLPGIK
251	GHRGFSGLDG	AKGDTGPSGP	KGEAGAPGEN	GTPGAMGPRG	LPGERGRAGP
301	PGAAGARGND	GAAGAAGPPG	PTGPAGPPGF	PGGPGAKGEV	GPQGARGAEG
351	PQGARGEAGN	PGPAGPAGPA	GNNGADGAAG	PKGSPGTPGI	AGAPGFPGPR
401	GPPGPGSAG	APGPKGNTGE	VGAPGAKGEA	GAKGEAGAQQ	VQGPFGPPGE
451	EGKRGARGE	GAAGGRGPPG	ERGAPGARGF	PGADGSAGPK	GAPGERGGPG
501	VVGPKGATGE	PGRNGEPGMP	GSKGMTGSPG	SPGPDGKTGP	SGTPGQDGRP
551	GPPGPVGARG	QPGVMGFPGP	KGAAGEAGKP	GERGVMGAVG	ATGAPGKDGD
601	VGAPGAPGPA	GPAGERGEQG	PAGPPGFQGL	PGPQGATGEP	GKSSEQGVPG
651	EAGAPGPAGS	RGDRGFPGER	GAPGPAGPAG	ARGSPGSAGN	DGAKGDAGAP
701	GAPGAQGGPG	LQGMPGERGA	AGLPGLKGDR	GDQGAAGTDG	APGKDGIRGM
751	TGPIGPPGPA	GAPGDKGETG	APGLVGPTGA	RGPPGERGET	GAPGPAGFAG
801	PPGADGLPGA	KGEAGDNGAK	GDAGSPGPAG	ATGAPGPQGP	VGATGPKGAR
851	GAAAGPPGATG	FPGAAGRVP	PGPAGNAGPP	GPPGPGGKEG	QKGNRGETGP
901	AGRTGEVGAP	GPPGAPGEKG	TPGAEPTGP	AGIPGPQGIA	GQRGIVGLPG
951	QRGERGFPL	PGSPGEPGKQ	GPSGSPGERG	PPGPMGPPGL	AGPPGEPGRE
1001	GTPGNEGSAG	RDGAPGPKGD	RGETGAAGTP	GAPGPPGAPG	PVGPAGKTGD
1051	RGESGPAGPA	GAAGPAGPRG	PAGPAGARGD	KGETGEAGER	GMKGHRGFTG
1101	MQGPPGPPGP	SGEPGPAGAS	GPAGPRGPAG	SSGPAGKDGM	SGLPGPIGPP
1151	GPRGRNGEIG	PAGPPGAPGP	PGPPGSPGGG	FDIGFIAQPQ	EKAPDPFRHF
1201	RADDANVMRD	RDLEVDTTLK	SLSQQIESIM	SPDGTKKNPA	RTCRDLKMCH
1251	PDWKSGEYWI	DPDQGCNQDA	IKVYCNMETG	ETCVYPTST	IPKKNWYTSK
1301	NIKEKKHVWF	GEAMTDGFQF	EYGESESKAE	DVNIQLTFLR	LMSTEASQNI
1351	TYHCKNSIAY	MDQASGNLKK	ALLQGSNEI	EIRAEGNSRF	TYSVTEDGCT
1401	SHTGAWGKTV	IDYKTTKTSR	LPIIDIAPMD	VGAPNQEFGI	EVGPVCFL

Figure 5. Amino acid sequence of collagen type I alpha 1 extracted from grass carp (*C. idella*) scales.

As shown in Figure 5, the GAA motif, consisting of glycine (G) and alanine (A), plays a critical role in maintaining the stability of the triple-helical structure in collagen type I. Glycine, being the smallest amino acid, facilitates tight packing of the helix, while alanine contributes to its flexibility and robustness through hydrophobic interactions. These amino acids collectively ensure the mechanical strength, flexibility, and resilience of collagen. Furthermore, sequence analysis identified cell-adhesion-promoting motifs such as arginine (R)-glycine (G)-aspartic acid (D) (RGD) sequences, which are recognized for facilitating cell attachment and proliferation. Collagen from *C. idella* contains potential cell-adhesive tripeptide motifs (such as RGD/GDR sequences); this sequence may help support cell attachment and growth, making it valuable for applications such as tissue engineering, wound healing, and biomaterial design [41, 43]. Based on the results, collagen was successfully extracted from the grass carp (*C. idella*) scales, with MS/MS spectrometry confirming the presence of type I alpha 1 collagen.

4. CONCLUSIONS

Type I collagen was successfully extracted from grass carp (*C. idella*) scales under optimized conditions, notably at room temperature (20–25 °C) instead of the conventional 4 °C, simplifying the process and reducing energy costs. The approach yielded acid-solubilized (ASC), pepsin-solubilized (PSC), and protease-solubilized collagen (PrSC). Pepsin demonstrated superior efficiency in preserving collagen integrity, while the application of protease highlighted its potential in producing hydrolyzed collagen for functional uses. Advanced techniques, including FTIR, FESEM, and SDS-PAGE, confirmed the molecular structure and purity of the extracted collagen. Furthermore, LC-MS/MS analysis coupled with Mascot database searching confirmed that collagen isolated from *C. idella* scales was type I alpha 1 collagen, with the amino acid sequence of grass carp grown in Viet Nam being reported for the first time. The utilization of fish scales, a by-product often overlooked, provides an eco-friendly and resource-efficient alternative to conventional collagen sources.

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CRedit authorship contribution statement. Nguyen Thi Kim An: Conceptualization, Methodology, Writing-Reviewing and Editing, Project administration. Dam Xuan Thang: Methodology, Investigation. Nguyen Ngoc Thanh: Methodology, Investigation. Nguyen Thi Huong: Analyzation, Supervision. Le Thi Bich Thao: Methodology, Analyzation.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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