Highlights

- Sturgeon head shows high potential as a new source of fish gelatin.
- Gelatin purity was determined by relative gelatin content and gelatin-band intensity.
- Type A gelatin had higher purity and gel strength with higher gelling temperature.
- Type B gelatin had higher emulsifying and forming activities.
- Type A gelatin can be used in biomaterials or drugs and type B in foods or cosmetics.

Purity and properties of gelatins extracted from the head tissue of the hybrid kalamtra sturgeon

Md. Rashidul Islam*[a, b], Tomoharu Yuhi[a], Dawei Meng[a], Takeya Yoshioka[c], Yumi Ogata[c], Kazuhiro Ura[d], Yasuaki Takagi[d]

[a]Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-Cho, Hakodate, Hokkaido 041-8611, Japan

[b]Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

[c]Hokkaido Industrial Technology Center, 379 Kikyo-Cho, Hakodate, Hokkaido 041-0801, Japan

[d]Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-Cho, Hakodate, Hokkaido 041-8611, Japan

*Corresponding author: Md. Rashidul Islam

Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-Cho, Hakodate, Hokkaido 041-8611, Japan

E-mail: mrislam_fbg@hstu.ac.bd

TEL/FAX: +81 138 40 5551

Declaration of Interest: None

Abstract

The head is a major by-product (17.1% of body weight) of sturgeon aquaculture farming and remains unutilized. In this study, the sturgeon head was separated into the skull cartilage and mixed tissue: skin, scales, fins, muscles, bones, gills, and small cartilage pieces. Type A and B gelatins were extracted from the mixed tissue. The type B yield was higher, at 5.8% gelatin dry weight per tissue wet weight. The relative gelatin content in the sample weight, estimated from the sample's hydroxyproline content relative to that of the purified collagen, was higher in type A (60.3%) than in type B (39.7%). Type A gelatin showed higher intensities of the α- and β-bands of gelatin in SDS-PAGE, indicating that gelatin purity was higher in type A. The breaking force-strain curve showed a larger breaking force and lower breaking strain in type A (2.0 N, 12.1%), indicating that this gelatin is stronger than type B (0.7 N, 15.8%). Type B gelatin featured higher emulsion activity and stability and higher foam expansion and stability. In conclusion, type A and B gelatins with distinct proximate composition and functional properties were successfully extracted from sturgeon heads, which could act as a promising source of gelatins for industrial applications.

Abbreviations

BV	Bovine bone gelatin
DF	Dilution factor
DW	Distilled water
EAI	Emulsion activity index
ESI	Emulsion stability index
FBC	Fat-binding capacity
FE	Foaming expansion
FS	Foaming stability
GCp	Relative gelatin content in the protein fraction of the sample
GCs	Relative gelatin content in the sample weight
Hyp	Hydroxyproline
LMM	Low molecular mass
NGC	Non-gelatinous content
WAC	Water-absorbing capacity

1. Introduction

The denatured form of collagen is termed gelatin (Ranasinghe et al., 2020). Hydrolyzed collagen with a molecular mass less than 30 kDa is not considered gelatin because it lacks gel-forming ability (Boran & Regenstein, 2009). Gelatin is commonly extracted from collagen-rich tissues such as skin by hydrothermal extraction. When the raw materials are pretreated with acidic or alkaline solutions, the extracted gelatin is referred to as type A or type B, respectively (Ahmad et al., 2017). These gelatins, generally obtained from mammalian sources, are used in foods, pharmaceuticals, and tissue engineering industries; however, some consumers have raised concerns of zoonotic disease risks (Karim & Bhat, 2009; Ranasinghe et al., 2020). Gelatins derived from aquatic sources are potential alternatives to mammalian gelatins (Gómez-Guillén, Gimenez, Lopez-Caballero, & Montero, 2011) and intensive studies have characterized their properties and functionalities (Ranasinghe et al., 2020; Zhang et al., 2020a, 2020b).

Even if the source tissues are rich in collagen, the extracted gelatin samples can contain traces of non-gelatinous substances such as non-collagenous proteins, lipids, carbohydrates, and ash. Thus, gelatin purity (gelatin content in the sample) is dependent not only on the collagen content of the source tissues but also on the pretreatment and extraction methods. Gelatin purity is a determining factor for the proper applications of aquatic gelatins, as it may significantly affect the properties and functionality of the gelatin sample. One of the gelatin purity indicators, hydroxyproline residue (Hyp), is collagen-specific and is used to measure collagen or gelatin content (Hofman, Hall, Cleaver, & Marshall, 2011; Nelson & Cox, 2005). Although many studies have determined the amino acid composition of aquatic-sourced gelatin samples, only the study by Tümerkan, Cansu, Boran, Regenstein, and Özoğul (2019) addressed the percentage Hyp content of extracted aquatic gelatin relative to the

source tissue (skin), and the relationships between gelatin purity and gelatin properties. Another indicator widely used to assess the purity of type I collagen (Capella-Monsonís, Coentro, Graceffa, Wu, & Zeugolis, 2018) is the intensity of gelatin α-, β-, and γ-bands from the extracted sample in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The disadvantage of this method is that the band intensity highly depends on the staining condition of the SDS-PAGE gel; thus, only the values of samples on the same gel are comparable. Many studies on fish gelatin have shown SDS-PAGE images; however, few studies have quantitatively studied the relationships between the intensities of the bands and gelatin properties and functionality.

In this study, we focused on the head of the sturgeon, as a new source of aquatic gelatin. In 2017, 102 327 tons of sturgeon biomass was produced worldwide on a total of 2 329 commercial aquaculture farms (Bronzi et al., 2019). Because the primary commercial product of sturgeon aquaculture is caviar, large quantities of by-products—sometimes including meat—are generated (Bronzi et al., 2019). To utilize these by-products, methods have been developed to extract gelatin from the sturgeon skin (Hao et al., 2009; Nikoo et al., 2011, 2013, 2014) and collagen from the sturgeon skin, scale, muscle, notochord, cartilage, swim bladder, and digestive tract (Liang et al., 2014; Meng et al., 2019; Wang et., 2014; Zhang et al., 2014). However, there are no reports on the utilization of sturgeon heads for any purpose, including gelatin extraction, although the head tissue is the major by-product, representing approximately 17.1% of total body weight (unpublished observation). This is likely because of low profitability and extraction difficulties arising from the hardness and complexity of the tissue composition.

Kalamtra sturgeon (*Huso dauricus* × *Acipenser schrenckii* × *Acipenser transmontanus*) is a hybrid species developed at the Hokkaido University. Aquaculture production of this species is increasing in Hokkaido, Japan (Islam, Yuhi, Ura, & Takagi,

2020). In this study, we extracted type A and type B gelatins from the head of the kalamtra sturgeon and assessed their properties and functionalities against sample gelatin purity, for potential industrial uses. Sample gelatin purities were expressed as the relative gelatin content per sample weight, calculated from the Hyp content of the sample relative to that of purified collagen, and from the intensity of gelatin bands of the sample in SDS-PAGE.

2. Materials and methods

2.1 Extraction of gelatins

The heads of kalamtra sturgeon were obtained from an aquaculture farm (Bifuka Shinko Kosha, Bifuka, Japan) and stored at -20 °C. The heads (644.4±28.5 g, mean ± SE) were first divided into the skull cartilage and mixed tissue (skin, scales, pectoral fins, muscles, bones, gills, and small cartilage pieces). Gelatin types A and B were extracted from the mixed tissues following our previously optimized conditions (Supplementary Fig. 1). The skull cartilage was stored at -30 °C for future extraction of type II gelatin and chondroitin sulfate. The yield (%) was calculated as the gelatin dry weight per sample wet weight.

2.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE (7.5% gel) was performed as previously described (Meng et al., 2019) after dissolving the gelatin (w/v 0.2%) in distilled water (DW), heating at 50 °C for 15–20 min, and mixing by pipetting. From captured gel images, processed using Image-J software (Version 1.52, National Institutes of Health, Bethesda, MD, USA), the intensities of the gelatin bands (α, β, and γ) and low molecular mass (LMM) bands (<100 kDa) were

quantified. Tricine-SDS-PAGE was performed following the method of Schägger (2006). Briefly, 4% stacking gel, 10% spacer gel, and 15.5% separating gel were prepared using gel buffer (3 M Tris-HCl, 0.3% SDS, pH 8.45), acrylamide, bisacrylamide, glycerol, ammonium persulfate, and TEMED. Samples were dissolved in DW and mixed (v/v 1:1) with the sample buffer (0.5 M Tris-HCl, pH 6.8, with 4% SDS and 20% glycerol) containing 10% β-mercaptoethanol. After boiling for 3 min, the samples were loaded onto the gel (25 μg sample/lane). The protein marker standard (DynaMarker® Protein MultiColor Stable, Low Range, BioDynamics Laboratory Inc., Tokyo, Japan) was used to assess the molecular mass of the samples. A current of 30 volts (14 mA) was applied through the gel for 50–60 min, then 70 volts (24 mA) for 3–3.5 h. Next, the gel was fixed in a mixture of 50% methanol and 10% acetic acid for 30 min, stained with 0.1% Coomassie Brilliant Blue R-250 solution for 45 min, de-stained with 10% acetic acid for 4–5 h, then imaged.

2.3 Amino acid composition and relative gelatin content in the protein fraction of the sample

The amino acid composition was quantified according to Meng et al. (2019), with some modifications. Briefly, the gelatin sample was hydrolyzed in 6 M HCl at 110 ℃ for 24 h and then dried by evaporation. The remaining materials were assessed using an automated amino acid analyzer (L-8900, Hitachi High-Technologies Corporation, Tokyo, Japan) after dissolving in a citric acid buffer solution.

The relative gelatin content in the protein fraction of the sample (GCp) was calculated using the following formula:

$$GCp\ (\%) = [(\text{Hyp of the sample})/(\text{Hyp of the purified collagen})] \times 100$$

where Hyp is the number of hydroxyproline expressed as Hyp residues/1000 amino acid residues. Purified collagen (Supplementary Fig. 2) was extracted from a mixture of the wet

skin of kalamtra and bester sturgeons as described by Meng et al. (2019). Tryptophan (Trp) was not considered for estimating the relative gelatin content in the protein fraction of the sample due to the undetectability of the method. The Trp content is low (0.3%) when estimated from the cDNA sequence of the α1-chain of type I procollagen in Amur sturgeon (Zhang et al., 2016). Hanani (2016) also reported that gelatin lacked Trp. Thus, the Trp content did not affect the calculation of the amino acid composition and GCp.

2.4 Proximate composition

The relative gelatin content in the sample weight (GCs) was obtained using the following formula:

$$GCs\ (\%) = [(\text{Hyp of the sample})/(\text{Hyp of the purified collagen})] \times 100$$

where Hyp is the concentration (nmol Hyp/μg dry sample) of the gelatin sample or purified collagen.

Next, the relative non-gelatinous protein content in the sample (NGCs) was calculated as follows:

$$NGCs\ (\%) = 100\ GCs/GCp - GCs$$

where GCs and GCp are the relative gelatin content (%) in the sample weight and protein fraction of the sample, respectively, obtained as described above. The precise methods used to derive this formula are shown in Supplementary Fig. 3.

Lipid and ash contents were measured according to the methods of Bligh and Dyer (1959) and AOAC (2000, # 920.153), respectively. Finally, the content of other components, mainly carbohydrates, was calculated as the remainder of the sum of gelatin, non-gelatinous protein, lipid, and ash.

181 *2.5 Transparency*

182

183 The absorbance of the gelatin solution (w/v 1%) was measured using a
184 spectrophotometer (UH5300, Hitachi, Tokyo, Japan) as per Zhang et al. (2020a) and Kim et
185 al. (2020). The transparency (%) was calculated using the following formula:

186 $$\text{Transparency (\%)} = 10^{-(\text{Absorbance at 600 nm})} \times 100$$

187

188 *2.6 Rheological properties*

189

190 The gelling and melting temperatures of the samples were measured according to the
191 method of Shakila, Jeevithan, Varatharajakumar, Jeyasekaran, & Sukumar (2012) with slight
192 modifications. Briefly, 2.5 mL of the gelatin solution (w/v 6.67%) was transferred to each test
193 tube (each test was repeated 3 times). The temperatures were lowered or raised at a rate of
194 0.5 °C/min in an incubator (FMU-133I, Fukushima Industries Corp., Osaka, Japan). Bovine
195 bone gelatin (BV; Wako Pure Chemical Industries Ltd., Osaka, Japan) was used as a control.
196 To measure the gel strength, gelatin solution (w/v 6.67%) was poured into a plastic
197 well (2.3 cm diameter × 1.8 cm height) and left undisturbed at 10 °C for 17 h. The breaking
198 force-strain curve of the gel was drawn using a creep meter (RE-3305S, YAMADEN Co.,
199 Ltd., Tokyo, Japan) with a gelatin gel penetration diameter (of the flat-faced cylindrical
200 plunger) of 1.27 cm at 0.5 mm/s crosshead speed. The breaking force at the fracture of the gel
201 was recorded as the gel strength (N). The breaking strain was defined as the percentage of the
202 distance to gel fracture divided by the initial gel height. BV was used as a control.

2.7 Scanning electron microscopy

Gelatin gels (w/v 1.50%, 3.00%, 6.67%) were fixed with 2.5% (v/v) glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) for 1 h at 4 °C and 6 h at room temperature. Gels were washed with phosphate buffer (pH 7.6) and serially dehydrated with 70%, 80%, 90%, and 95% (v/v) ethanol. A scalpel was used to make a 2–3 mm thick cross-section of each gel. Samples were dehydrated with 100% ethanol overnight then twice with 100% *t*-butyl alcohol for 1 h. They were frozen at -30 °C, dried with a freeze-drier (JFD-320, JEOL Ltd., Tokyo, Japan), and coated with gold-platinum using an auto fine coater (JFC-1600, JEOL Ltd.). The surface structure of the sample was observed with a scanning electron microscope (JSM6010LA, JEOL Ltd.) at 8 kV.

2.8 Functional properties

The water-absorbing capacity (WAC) and fat-binding capacity (FBC) of the gelatins were measured following the method of Zhang et al. (2020a) with minor modifications. Briefly, samples were collected in a pre-weighed centrifuge tube, and DW or soybean oil was added to adjust the concentration to 2% (w/v). The tube was kept at room temperature and vortexed 5 times for 5 s at 15-min intervals. Next, the samples were centrifuged at 4 500 ×g for 20 min. The upper phases were discarded, the tubes were drained (tilt angle 45°) on filter paper for 30 min, and the weight of the contents in the tubes was measured. The WAC or FBC of lyophilized gelatins was calculated as follows:

WAC or FBC (%) = [weight of the tube contents after draining (g)/weight of lyophilized gelatin (g)] × 100

The emulsion activity index (EAI) and emulsion stability index (ESI) of the gelatins were measured according to the method of Zhang et al. (2020a) with minor modifications. Briefly, gelatin solutions (w/v 1%) were mixed with soybean oil at a ratio of 4:1 and homogenized (Physcotron, Nition Irika Kikai Seisakusho Co., Ltd., Tokyo, Japan) at 20 000 rpm for 3 min. Next, the gelatin-oil emulsion was diluted 100-fold with 0.1% sodium dodecyl sulfate solution and vortexed for 10 s. Absorbance was measured at 500 nm, and EAI and ESI were calculated using the following formulas:

$$EAI\ (m^2/g) = (2 \times 2.303 \times A_0 \times DF)/(Ø \times C \times 10\,000)$$

$$ESI\ (min) = (A_0 \times \Delta_t)/(A_0 - A_{30})$$

where A_0 is the absorbance of the sample at time zero, DF is the dilution factor, Ø is the oil volumetric fraction, C is the protein concentration (g/mL) before emulsification, Δ_t is the time interval (30 min), and A_{30} is the absorbance after 30 min.

Foaming expansion (FE) and foaming stability (FS) of the gelatin were measured according to Renuka, Ravishankar, Zynudheen, Bindu, & Joseph (2019), with slight modifications. Briefly, 2.5 mL gelatin solution (w/v 1%) was homogenized (Physcotron, Nition Irika Kikai Seisakusho Co., Ltd.) in previously marked test tubes at 20 000 rpm for 2 min. The FE and FS were determined after incubation for 0 and 30 min at room temperature using the following formulas:

$$FE\ (\%) = (V_0 - V)/(V) \times 100$$

$$FS\ (\%) = (V_{30} - V)/(V) \times 100$$

where V is the total volume before homogenization (mL), V_0 is the total volume after homogenization (mL), and V_{30} is the total volume after 30 min (mL).

2.9 Statistical analysis

All experiments were replicated three times. Samples of the three replicates were mixed for SDS-PAGE, Tricine-SDS-PAGE, amino acid, and Hyp content analyses. Where applicable, data are presented as the mean ± standard error (SE). Student's *t*-test or one-way analysis of variance and Tukey-Kramer test were performed using Microsoft Excel add-on statistical software (version 2.12, Social Survey Research Information Co., Ltd., Tokyo, Japan). A p value of < 0.05 was considered to indicate statistical significance.

3. Results and discussion

3.1 Yields of gelatins

The yield of type B was significantly higher (5.8±0.1%) than that of type A (3.6±0.1%) in sturgeon head. The yield of type A gelatin was similar to that of mackerel head (3.3–3.7%; Khiari, Rico, Martin-Diana, & Barry-Ryan, 2011) and higher than that of tiger tooth croaker head (1.7%; Elavarasan et al., 2017), but lower than the gelatin yields obtained from mammalian and fish skin. For example, the gelatin yield from camel skin was 14.1% (Abuibaid, AlSenaani, Hamed, Kittiphattanabawon, & Maqsood, 2020) and 9.4–12.5% from Amur sturgeon skin (Nikoo et al., 2013). The amount of gelatin obtained from approximately 500 g of sturgeon head, excluding the skull cartilage, was calculated to be approximately 18 g for type A and 29 g for type B gelatin. The probable reason for the higher yield in type B gelatin is discussed in Section 3.4.

3.2 SDS-PAGE analysis

Although the same amount of gelatin (10 μg/lane) was loaded onto the gel for SDS-PAGE, the thickness and intensities of the bands corresponding to the α- and β-chains of gelatin were significantly greater in type A gelatin than in type B gelatin (Figs. 1A, B). The intensity of the LMM bands (<100 kDa) was significantly higher for type B gelatin (Fig. 1B). For example, the two dominant LMM bands (close to 37 kDa) in SDS-PAGE (Fig. 1A) and a larger number of LMM bands (<46 kDa) in Tricine-SDS-PAGE (Fig. 1C) were observed for type B gelatin. Peptide chains or LMM bands negatively affect gelatin properties such as gel-forming ability (Nikoo et al., 2013), and gelatin hydrolysates less than 30 kDa are not considered gelatin (Boran & Regenstein, 2009). These data strongly suggest that the type A gelatin purity is higher than that of type B. The thickness of the gelatin bands obtained in this experiment was comparable to those of fish head gelatins reported previously (Elavarasan et al., 2017; Liu, Han, & Guo, 2009). The data of the yield and SDS-PAGE strongly suggest that sturgeon heads can be used as a new material for industrial-scale gelatin manufacturing in food and other industries.

3.3 Amino acid analysis and the relative gelatin content in the protein fraction

Table 1 shows the amino acid compositions and relative gelatin contents of the protein fractions of the samples. The glycine (Gly), proline (Pro), and Hyp contents of type A gelatin were similar to those of purified collagen, and to reported values for Amur sturgeon skin gelatin (Nikoo et al., 2011, 2013). Type B gelatin showed lower Gly, Pro, and Hyp contents. Lower Hyp contents in type B resulted in lower relative gelatin content in the protein fraction, suggesting that the differences in the ratio of gelatinous and non-gelatinous proteins in both gelatins must be the reason for the distinctive amino acid compositions of type A and B gelatins.

3.4 Proximate composition of gelatins

Type A gelatin showed higher relative gelatin content in the sample weight and lower non-gelatinous protein content than type B gelatin (Table 2). Thus, the alkaline pretreatment used to extract type B gelatin may lead to the extraction of more non-gelatinous proteins. Alkaline pretreatment breaks down the amide groups of proteins (Johns & Courts, 1977) without affecting the mother collagen molecules (Liu et al., 2015). Speculatively, the LMM molecules of non-gelatinous proteins, which were produced by alkaline pretreatment, were easily extracted. The LMM molecules likely originated from the dominating collagen-poor muscle tissue in the sturgeon head. Our preliminary study revealed that collagen-poor muscle occupies approximately 26.2% of the sturgeon head weight. The higher relative gelatin content in the sample weight in type A was also supported by the banding pattern in SDS-PAGE, wherein higher intensities of the α- and β-bands of gelatin were observed in type A than in type B (Figs. 1A, B). Collectively, the two gelatin purity indicators—the relative gelatin content in the sample weight and gelatin band intensities in SDS-PAGE—suggest higher gelatin purity in type A than in type B.

The lipid contents of the sturgeon head gelatins were lower than those of tiger tooth croaker head gelatin (3.2%) and similar to the head (0.7%–0.9%) or scale and fin gelatin (0.8%–1.1%) of other fishes (Elavarasan et al., 2017; Khiari et al., 2011; Mirzapour-Kouhdasht, Sabzipour, Taghizadeh, & Moosavi-Nasab, 2019). However, the gelatins in the present study showed higher lipid contents than skin gelatin (0.2%–0.3%) from other fishes (Renuka et al., 2019). Defatting was not performed in this study because hard clustering of the mixed tissue occurred, rendering the samples unsuitable for defatting. This may have resulted from preheating the head for tissue separation (Supplementary Fig. 1).

Ash contents were similar in the two gelatins but higher than in gelatins from the head of mackerel and tiger tooth croaker (Elavarasan et al., 2017; Khiari et al., 2011). Large, thick cranial bones covering and hardening the head may increase the ash content. Except for the higher ash content, the proximate composition of the sturgeon head gelatins was close to that of skin gelatins in other species (Tümerkan et al., 2019). The high ash content may categorize the sturgeon head gelatins as low quality; thus, lowering the ash content is a future challenge to increase the quality of these gelatins.

By multiplying the proximate composition and the yield (Section 3.1), fractional yields could be estimated for gelatin, non-gelatinous proteins, and non-proteinaceous substances (sum of lipid, ash, and others) in type A and type B samples. The respective estimated fractional yields were 2.2%, 0.1%, and 1.3% (total 3.6%) for type A and 2.3%, 1.7%, and 1.8% (total 5.8%) for type B. Thus, a higher fraction of non-gelatinous protein is the primary reason for the high overall yield in type B (Section 3.1 above).

3.5 Transparency of gelatins

The transparencies of BV, type A, and type B gelatin samples were 99.7±0.1%, 67.8±0.4%, and 92.5±0.3%, respectively (Table 2). Contamination of samples with inorganic, proteinaceous, and mucous substances influences transparency (Elavarasan et al., 2017). Therefore, the higher relative content of non-proteinaceous substances may significantly lower the transparency of type A gelatin.

3.6 Rheological properties

Fish gelatin is a widely used biopolymer that commonly has lower rheological properties than mammalian gelatin (Huang et al., 2019). The present samples also showed a lower gelling temperature than BV (Table 3). The gelling temperature of type A gelatin (Table 3) was higher than that of type B gelatin and tiger tooth croaker head gelatin (11 °C; Elavarasan et al., 2017), and similar to that of Amur sturgeon skin gelatin (13–14 °C; Nikko et al., 2011, 2014). The gelling temperature correlated to the levels of the gelatin bands in SDS-PAGE in the skin and bone gelatins of Nile perch (Muyonga, Cole, & Duodu, 2004); thus, the higher intensities of α- and β-bands in type A gelatin (Fig. 1) might strongly increase the gelling temperature of the present samples.

The melting temperatures of type A and type B gelatins were similar, but lower than that of BV (Table 3). The melting temperature of the gelatins of the present study was higher than those of gelatins from Amur sturgeon skin (20.3–22.6 °C; Nikoo et al., 2013, 2014) and tiger tooth croaker head (20.3 °C; Elavarasan et al., 2017). These data suggest that the melting temperature is not influenced by gelatin purity; thus, there are unknown factors affecting the melting temperature of gelatins. The mechanism may be related to the effects of pretreatment on the partial crosslinking of α-chains, which may stabilize the gelatin gel.

The breaking force-strain curve (Fig. 2) showed that the sturgeon head gelatins were softer and more comfortable to deform with a smaller force than BV. The gel strength (N) and breaking strain (%) values were obtained from the breaking force and strain at the moment of gel fracture (Table 3). Type A gelatin showed higher gel strength and lower breaking strain than type B gelatin. When the gel strengths of the head gelatin samples were compared with previous data, measured at 6.67% gel concentration using a method similar to this study, the strengths were higher than those of cod head (0.05–0.2 N) and croaker head

(0.4 N) gelatins (Arnesen & Gildberg, 2006; Elavarasan et al., 2017). Type A gelatin also showed higher gel strength than Amur sturgeon skin gelatin (1.0–1.4 N) (Nikoo et al., 2014). Cold-water fish show lower gelatin gel strength values (1.0 N or lower) than warm-water fish (>2.0 N) (Gómez-Guillén et al., 2011). Based on these data, sturgeon head type A gelatin can be categorized as medium quality with strength close to that of warm-water species, whereas type B gelatin has low quality. Different methods, such as modification and crosslinking of fish gelatins, are now present to overcome the limited industrial applications of fish gelatins due to lower gel strength (Huang et al., 2019).

Various factors, such as the intensity of gelatin bands, amount of LMM fragments, and amino acid composition (especially Hyp content), determine gel stability (Abuibaid et al., 2020; Casanova et al., 2020; Tümerkan et al., 2019). The LMM fragments make fewer intermolecular junctions, contributing to low gel strength (Intarasirisawat et al., 2007; Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2010). Thus, the higher breaking force of type A gelatin is reasonable as it showed higher purity—that is, higher relative gelatin content in the sample weight (Table 2) and higher intensity of gelatin bands— with lower LMM-fragments in SDS-PAGE (Fig. 1).

The breaking force-strain curve is commonly used in rheological analyses of gels in food science because the factors impact food texture. For example, the breaking force-strain relationships of the processed seafood-meat products *surimi* (Ueki, Matsuoka, Wan, & Watabe, 2018) and *kamaboko* (Mao, Fukuoka, & Sakai, 2006) have been reported. Moreover, the breaking force plays a key role in the field of biomedical materials for the pore architectures and mechanical properties of gelatin-based cellular scaffolds (Fukushima, Ohji, Hyuga, Matsunaga, & Yoshizawa, 2017). The present study is the first to report the breaking force-strain curve of fish gelatins, providing useful information on gelatin quality for use in the food, pharmaceutical, and biomedical industries.

3.7 Structure of gelatins

The structures of the gelatins are shown in Fig. 3. BV and type A gelatin did not show any voids or pores at 6.67% gel concentration (Figs. 3A, B), whereas type B gelatin had small homogeneous voids (Fig. 3C). The voids increased with decreasing sample concentrations, resulting in thinner networks of the gel (Figs. 3D–H). BV showed only small voids at the lowest concentration tested (1.50–3.00%) (Figs. 3D, G); however, type A gelatin showed small voids at 3.00%, and the voids increased and became non-homogeneous in size at 1.50% (Figs. 3E, H). Type B gelatin constituted a gel composed of fibrous networks at 3.00% (Fig. 3F) and did not form a gel at 1.50%. It is likely that rich non-collagenous proteins may inhibit or nullify the intermolecular gelatin junctions in type B gels and result in large voids with fine networks. Abuibaid et al. (2020) showed that camel skin gelatins with lower intensities of gelatin bands produced gels with larger voids and a lower breaking force.

3.8 Functional properties

The WAC of type A gelatin samples was higher than that of type B, and of other fish gelatins reported previously (Renuka et al., 2019; Zhang et al., 2020a; Table 4). However, the WAC of the sturgeon head gelatins was much lower than that of bovine bone gelatin. WAC may relate to several factors such as amino acid composition, especially Hyp content and hydrophilic groups of amino acids (Ninan, Jose, & Aliyamveettil, 2014; Ranasinghe et al., 2020; Shyni et al. 2014). However, the hydrophilic amino acid contents in type A gelatin (375/1000 residues) were lower than those in type B (438/1000 residues). Thus, differences in Hyp content—that is, the relative gelatin content (Tables 1, 2)—may be a reason for the higher WAC in type A than in type B.

A high fat-binding capacity (FBC) is a characteristic feature of type A and B gelatins (Table 4). These values were much higher than those of BV and fish gelatins reported previously (Renuka et al., 2019; Zhang et al., 2020a), except for that of tuna skin gelatin (Tümerkan et al., 2019). Type A and B gelatins would, therefore, be good fat-binding agents for foods or other purposes. The hydrophobic amino acid content (Ala, Gly, Ile, Leu, Met, Phe, Pro, and Val) of protein molecules positively affects the FBC (Renuka et al., 2019). However, the present results suggest that non-gelatinous inclusions such as ash and sugar (Table 2) or other unknown factors may positively affect the FBC, as type A gelatin contains more hydrophobic amino acids (625/1000 residues) compared to type B (562/1000 residues, Table 1).

Gelatin has surface-active and amphiphilic properties and is useful as an oil-in-water emulsifier (Zhang et al., 2020b). The emulsifying factors EAI and ESI were higher in type B than in type A and BV (Table 4), indicating a greater migration ability from water to the interface between oil and water. Several factors—such as molecular mass distribution, amino acid composition, interfacial tension, isoelectric point, and protein aggregation—influence the emulsion activity (Bkhairia, Mhamdi, Jridi, & Nasri, 2016; Ding et al., 2021; Zhang et al., 2020b). The higher emulsion activity of type B gelatin might be due to more LMM fragments (Fig. 1C) or non-collagenous proteins (Tables 1, 2), since the gelatin with more peptide fragments showed higher solubility, surface activity, and creaming stability, and resulted in higher emulsion activity (Ranasinghe et al., 2020; Zhang et al., 2020b). Furthermore, amino acids such as aspartic acid, glutamic acid, leucine, isoleucine, and Trp are potential emulsifiers for oil (Yiase, 2015); these amino acids, except Trp, were higher in type B gelatin (Table 1). In addition, the higher emulsification activity of proteins correlates with higher surface hydrophobicity (Tan, Karim, Uthumporn, & Ghazali, 2020). Therefore, the higher emulsifying activity of type B gelatin suggests that alkali-pretreatment might increase the

surface activity and hydrophobicity of gelatin more than acid pretreatment. The EAI of type B gelatin was higher than that of previously reported fish gelatins (Alfaro, Balbinot, Weber, Tonial, & Machado-Lunkes, 2015) or camel gelatins (Abuibaid et al., 2020), and close to that of frog skin gelatin, but lower than that of tuna and chicken skin gelatins (Tümerkan et al., 2019). The ESI of type B gelatin was even higher than that of frog, tuna, chicken, and camel skin gelatins (Abuibaid et al., 2020; Tümerkan et al., 2019).

The foaming activities, FE and FS, of type B gelatin were similar to those of BV and higher than those of type A (Table 4). These data suggest that the migration of type B gelatin led to the adsorption of more air at the newly made gas-liquid surface—rapidly reorganized the protein molecules—and also led to a stronger interaction with hydrophobic residues (Alfaro et al., 2015; Gómez-Guillén et al., 2011). The foaming properties of the sturgeon head gelatins were higher than those of tiger tooth croaker and pink perch skin and bone gelatins, but lower than those of giant catfish skin (Jongjareonraka et al., 2010; Koli et al., 2012) and camel gelatin (Abuibaid et al., 2020).

4. Conclusion

The present study clearly showed the high potential of sturgeon head as a new source of fish gelatin. Using the two indicators of gelatin purity—the relative gelatin content based on the Hyp content of the sample weight and the intensities of gelatin bands in SDS-PAGE—we found that gelatin purity was positively related to rheological properties, but negatively related to functional properties, except for WAC. Type A gelatin had a lower yield, higher purity, higher gel strength, and higher gelling temperature; whereas type B gelatin had a higher yield along with higher emulsifying factors and foam-forming activities. This is the first study to evaluate the relationships between gelatin purity and the properties and

functionalities of fish gelatin. Therefore, the parameters used in this study would be a useful basis for future gelatin studies. This study suggests that type A gelatin is suitable for use in biomaterials and pharmaceuticals because of its higher purity and rheological properties, whereas type B gelatin is suitable for use in foods or cosmetics based on its yield and functional properties.

Acknowledgments

This study was partly supported by a Grant-in-Aid for Scientific Research (B) 18H02273 from the Japan Society for the Promotion of Science. We thank the Editage group (www.editage.jp) for English language review.

References

Abuibaid, A., AlSenaani, A., Hamed, F., Kittiphattanabawon, P., & Maqsood, S. (2020). Microstructural, rheological, gel-forming and interfacial properties of camel skin gelatin. Food Structure, 26, 100156. doi:10.1016/j.foostr.2020.100156.

Ahmad, T., Ismail, A., Ahmad, S. A., Khalil, K. A., Kumar, Y., Adeyemi, K. D., & Sazili, A. Q. (2017). Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: A review. Food Hydrocolloids, 63, 85–96. doi:10.1016/j.foodhyd.2016.08.007.

Alfaro, A. T., Balbinot, E., Weber, C. I., Tonial, I. B., & Machado-Lunkes, A. (2015). Fish gelatin: characteristics, functional properties, applications and future potentials. Food Engineering Reviews, 7, 33–44. doi:10.1007/s12393-014-9096-5.

AOAC. (2000). Official methods of analysis. (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

Arnesen, J. A., & Gildberg, A. (2006). Extraction of muscle proteins and gelatine from cod head. Process Biochemistry, 41, 697–700. doi:10.1016/j.procbio.2005.09.001.

Bkhairia, I., Mhamdi, S., Jridi, M., & Nasri, M. (2016). New acidic proteases from *Liza aurata* viscera: Characterization and application in gelatin production. International Journal of Biological Macromolecules, 92, 533–542. doi:10.1016/j.ijbiomac.2016.07.063.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911–917. doi:10.1139/y59-099.

Boran, G., & Regenstein, J. M. (2009). Optimization of gelatin extraction from silver carp skin. Journal of Food Science, 74, 432–441. doi:10.1111/j.1750-3841.2009.01328.x.

Bronzi, P., Chebanov, M., Michaels, J. T., Wei, Q., Rosenthal, H., & Gessner, J. (2019). Sturgeon meat and caviar production: Global update 2017. Journal of Applied Ichthyology, 35, 257–266. doi:10.1111/jai.13870.

Capella-Monsonís, H., Coentro, J. Q., Graceffa, V., Wu, Z., & Zeugolis, D. I. (2018). An experimental toolbox for characterization of mammalian collagen type i in biological specimens. Nature Protocols, 13, 507–529. doi:10.1038/nprot.2017.117.

Casanova, F., Mohammadifar, M. A., Jahromi, M., Petersen, H. O., Sloth, J. J., Eybye, K. L., Kobbelgaard, S., Jakobsen, G., & Jessen, F. (2020). Physico-chemical, structural and techno-functional properties of gelatin from saithe (*Pollachius virens*) skin. International Journal of Biological Macromolecules, 156, 918–927. doi:10.1016/j.ijbiomac.2020.04.047.

520 Ding, M., Liu, L., Zhang, T., Tao, N., Wang, X., & Zhong, J. (2021). Effect of interfacial
521 layer number on the storage stability and *in vitro* digestion of fish oil-loaded
522 multilayer emulsions consisting of gelatin particle and polysaccharides. Food
523 Chemistry, 336, 127686. doi:10.1016/j.foodchem.2020.127686.
524 Elavarasan, K., Kumar, A., Uchoi, D., Tejpal, C. S., Ninan, G., & Zynudheen, A. A. (2017).
525 Extraction and characterization of gelatin from the head waste of tiger tooth croaker
526 (*Otolithes ruber*). Waste and Biomass Valorization, 8, 851–858. doi:10.1007/s12649-
527 016-9639-5.
528 Fukushima, M., Ohji, T., Hyuga, H., Matsunaga, C., & Yoshizawa, Y. (2017). Effect of
529 gelatin gel strength on microstructures and mechanical properties of cellular ceramics
530 created by gelation freezing route. Journal of Materials Research, 32, 3286–3293.
531 doi:10.1557/jmr.2017.94.
532 Gómez-Guillén, M. C., Gimenez, B., Lopez-Caballero, M. E., & Montero, M. P. (2011).
533 Functional and bioactive properties of collagen and gelatin from alternative sources: A
534 review. Food Hydrocolloids, 25, 1813–1827. doi:10.1016/j.foodhyd.2011.02.007.
535 Hanani, Z. A. N. (2016). Gelatin. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.),
536 Encyclopedia of food and health (pp. 191–195). Oxford: Academic Press.
537 doi:10.1016/B978-0-12-384947-2.00347-0.
538 Hao, S., Li, L., Yang, X., Cen, J., Shi, H., Bo, Q., & He, J. (2009). The characteristics of
539 gelatin extracted from sturgeon (*Acipenser baeri*) skin using various pretreatments.
540 Food Chemistry, 115, 124–128. doi:10.1016/j.foodchem.2008.11.080.
541 Hofman, K., Hall, B., Cleaver, H., & Marshall, S. (2011). High-throughput quantification of
542 hydroxyproline for determination of collagen. Analytical Biochemistry, 417, 289–
543 291. doi:10.1016/j.ab.2011.06.019.
544 Huang, T., Tu, Z.-c., Shangguan, X., Sha, X., Wang, H., Zhang, L., & Bansal, N. (2019). Fish

545 gelatin modifications: A comprehensive review. Trends in Food Science and
546 Technology. 86, 260–269. doi:10.1016/j.tifs.2019.02.048.
547 Intarasirisawat, R., Benjakul, S., Visessanguan, W., Prodpran, T., Tanaka, M., & Howell, N.
548 K. (2007). Autolysis study of bigeye snapper (*Priacanthus macracanthus*) skin and its
549 effect on gelatin. Food Hydrocolloids, 21, 537–544.
550 doi:10.1016/j.foodhyd.2006.05.012.
551 Islam, M. R., Yuhi, T., Ura, K., & Takagi, Y. (2020). Optimization of extraction of gelatin
552 from the head of kalamtra sturgeon (*Huso dauricus* × *Acipenser schrenckii* ×
553 *Acipenser transmontanus*). Applied Sciences, 10, 6660. doi:10.3390/app10196660.
554 Johns, P., & Courts, A. (1977). Relationship between collagen and gelatin. In A.G. Ward, &
555 A. Courts (Eds.), The science and technology of gelatin (pp. 138–168). New York:
556 Academic Press.
557 Jongjareonraka, A., Rawdkuenb, S., Chaijanic, M., Benjakul, S., Osakoe, K., & Tanaka, M.
558 (2010). Chemical compositions and characterization of skin gelatin from farmed giant
559 catfish (*Pangasianodon gigas*). LWT - Food Science and Technology, 43, 161–165.
560 doi:10.1016/j.lwt.2009.06.012.
561 Karim, A. A., & Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an
562 alternative to mammalian gelatins. Food Hydrocolloids, 23, 563–576.
563 doi:10.1016/j.foodhyd.2008.07.002.
564 Khiari, Z., Rico, D., Martin-Diana, A. B., & Barry-Ryan, C. (2011). The extraction of
565 gelatine from mackerel (*Scomber scombrus*) heads with the use of different organic
566 acids. Journal of Fisheries Sciences.com, 5, 52–63. doi:10.3153/jfscom.2011007.
567 Kim, T. K., Ham, Y. K., Shin, D. M., Kim, H. W., Jang, H. W., Kim, Y. B., & Choi, Y. S.
568 (2020). Extraction of crude gelatin from duck skin: effects of heating methods on
569 gelatin yield. Poultry Science, 99, 590–596. doi:10.3382/ps/pez519.

570 Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2010). Comparative
571 study on characteristics of gelatin from the skins of brownbanded bamboo shark and
572 blacktip shark as affected by extraction conditions. Food Hydrocolloids, 24, 164–171.
573 doi:10.1016/j.foodhyd.2009.09.001.
574 Koli, M. J., Basu, S., Nayak, B. B., Patange, B. S., Pagarkar, U. A., & Gudipati, V. (2012).
575 Functional characteristics of gelatin extracted from skin and bone of tiger-toothed
576 croaker (*Otolithes ruber*) and pink perch (*Nemipterus japonicus*). Food and
577 Bioproducts Processing, 90, 555–562. doi:10.1016/j.fbp.2011.08.001.
578 Liang, Q., Wang, L., Sun, W., Wang, Z., Xu, J., & Ma, H. (2014). Isolation and
579 characterization of collagen from the cartilage of Amur sturgeon (*Acipenser*
580 *schrenckii*). Process Biochemistry, 49, 318–323. doi:10.1016/j.procbio.2013.12.003.
581 Liu, D., Wei, G., Li, T., Hu, J., Lu, N., Regenstein, J. M., & Zhou, P. (2015). Effects of
582 alkaline pretreatments and acid extraction conditions on the acid-soluble collagen
583 from grass carp (*Ctenopharyngodon idella*) skin. Food Chemistry, 172, 836–843.
584 doi:10.1016/j.foodchem.2014.09.147.
585 Liu, H. Y., Han, J., & Guo, S. D. (2009). Characteristics of the gelatin extracted from channel
586 catfish (*Ictalurus punctatus*) head bones. LWT - Food Science and Technology, 42,
587 540–544. doi:10.1016/j.lwt.2008.07.013.
588 Mao, W., Fukuoka, M., & Sakai, N. (2006). Gel strength of kamaboko gels produced by
589 microwave heating. Food Science and Technology Research, 12, 241–246.
590 doi:10.3136/fstr.12.241.
591 Meng, D., Tanaka, H., Kobayashi, T., Hatayama, H., Zhang, X., Ura, K., Yunoki, S., &
592 Takagi, Y. (2019). The effect of alkaline pretreatment on the biochemical
593 characteristics and fibril-forming abilities of types I and II collagen extracted from

bester sturgeon by-products. International Journal of Biological Macromolecules, 131, 572–580. doi:10.1016/j.ijbiomac.2019.03.091.

Mirzapour-Kouhdasht, A., Sabzipour, F., Taghizadeh, M. S., & Moosavi-Nasab, M. (2019). Physicochemical, rheological, and molecular characterization of colloidal gelatin produced from common carp by-products using microwave and ultrasound-assisted extraction. Journal of Texture Studies, 50, 416–425. doi:10.1111/jtxs.12408.

Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloids, 18, 581–592. doi:10.1016/j.foodhyd.2003.08.009.

Nelson, L., & Cox, M. (2005). Lehninger: Principles of biochemistry. (4th ed.). New York: W. H. Freeman and Company.

Nikoo, M., Benjakul, S., Bashari, M., Alekhorshied, M., Cissouma, A. I., Yang, N., & Xu, X. (2014). Physicochemical properties of skin gelatin from farmed Amur sturgeon (*Acipenser schrenckii*) as influenced by acid pretreatment. Food Bioscience, 5, 19–26. doi:10.1016/j.fbio.2013.10.004.

Nikoo, M., Benjakul, S., Ocen, D., Yang, N., Xu, B., Zhang, L., & Xu, X. (2013). Physical and chemical properties of gelatin from the skin of cultured Amur sturgeon (*Acipenser schrenckii*). Journal of Applied Ichthyology, 29, 943–950. doi:10.1111/jai.12238.

Nikoo, M., Xu, X., Benjakul, S., Xu, G., Ramirez-Suarez, J. C., Ehsani, A, Kasankala, L. M., Duan, X., & Abbas, S. (2011). Characterization of gelatin from the skin of farmed Amur sturgeon *Acipenser schrenckii*. International Aquatic Research, 3, 135–145.

Ninan, G., Jose, J., & Aliyamveettil, Z. A. (2014). A comparative study on the physical, chemical and functional properties of carp skin and mammalian gelatins. Journal of Food Science and Technology, 51, 2085–2091. doi:10.1007/s13197-012-0681-4.

Ranasinghe, R. A. S. N., Wijesekara, W. L. I., Perera, P. R. D., Senanayake, S. A., Pathmalal,

M. M., & Marapana, R. A. U. J. (2020). Functional and bioactive properties of gelatin extracted from aquatic bioresources–A review. Food Reviews International, 1–44. doi:10.1080/87559129.2020.1747486.

Renuka, V., Ravishankar, C. N. R., Zynudheen, A. A., Bindu, J., & Joseph, T. C. (2019). Characterization of gelatin obtained from unicorn leatherjacket (*Aluterus monoceros*) and reef cod (*Epinephelus diacanthus*) skins. LWT - Food Science and Technology, 116, 108586. doi:10.1016/j.lwt.2019.108586.

Schägger, H. (2006). Tricine-SDS-PAGE. Nature Protocols, 1, 16–22. doi:10.1038/nprot.2006.4.

Shakila, R. J., Jeevithan, E., Varatharajakumar, A., Jeyasekaran, G., & Sukumar, D. (2012). Functional characterization of gelatin extracted from bones of red snapper and grouper in comparison with mammalian gelatin. LWT - Food Science and Technology, 48, 30–36. doi:10.1016/j.lwt.2012.03.007.

Shyni, K., Hema, G. S., Ninan, G., Mathew, S., Joshy, C. G., & Lakshmanan, P. T. (2014). Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). Food Hydrocolloids, 39, 68–76. doi:10.1016/j.foodhyd.2013.12.008.

Tan, C. C., Karim, A. A., Uthumporn, U., & Ghazali, F. C. (2020). Effect extraction temperature on the emulsifying properties of gelatin from black tilapia (*Oreochromis mossambicus*) skin. Food Hydrocolloids, 108, 106024. doi:10.1016/j.foodhyd.2020.106024.

Tümerkan, E. T. A., Cansu, Ü., Boran, G., Regenstein, J. M., & Özoğul, F. (2019). Physiochemical and functional properties of gelatin obtained from tuna, frog and chicken skins. Food Chemistry, 287, 273–279. doi:10.1016/j.foodchem.2019.02.088.

Ueki, N., Matsuoka, Y., Wan, J., & Watabe, S. (2018). The effects of endogenous proteases

within abdominal muscle parts on the rheological properties of thermally induced gels from white croaker (*Pennahia argentata*). Food Chemistry, 268, 498–503. doi:10.1016/j.foodchem.2018.06.080.

Wang, L., Liang, Q., Chen, T., Wang, Z., Xu, J., & Ma, H. (2014). Characterization of collagen from the skin of Amur sturgeon (*Acipenser schrenckii*). Food Hydrocolloids, 38, 104–109. doi:10.1016/j.foodhyd.2013.12.002.

Yiase, S. G. (2015). Amino acids as potential emulsifiers in stabilizing oil/water emulsions. International Journal of Innovation and Scientific Research, 15, 409–414.

Zhang, T., Sun, R., Ding, M., Li, L., Tao, N., Wang, X., & Zhong, J. (2020a). Commercial cold-water fish skin gelatin and bovine bone gelatin: Structural, functional, and emulsion stability differences. LWT - Food Science and Technology, 125, 109207. doi:10.1016/j.lwt.2020.109207.

Zhang, T., Xu, J., Zhang, Y., Wang, X., Lorenzo, J. M., & Zhong, J. (2020b). Gelatins as emulsifiers for oil-in-water emulsions: Extraction, chemical composition, molecular structure, and molecular modification. Trends in Food Science and Technology, 106, 113–131. doi:10.1016/j.tifs.2020.10.005.

Zhang, X., Azuma, N., Hagihara, S., Adachi, S., Ura, K., & Takagi, Y. (2016). Characterization of type I and II procollagen α1chain in Amur sturgeon (*Acipenser schrenckii*) and comparison of their gene expression. Gene, 579, 8–16. doi:10.1016/j.gene.2015.12.038.

Zhang, X., Ookawa, M., Tan, Y., Ura, K., Adachi, S., & Takagi, Y. (2014). Biochemical characterisation and assessment of fibril-forming ability of collagens extracted from bester sturgeon *Huso huso* × *Acipenser ruthenus*. Food Chemistry, 160, 305–312. doi:10.1016/j.foodchem.2014.03.075.

Figure captions

Fig. 1.

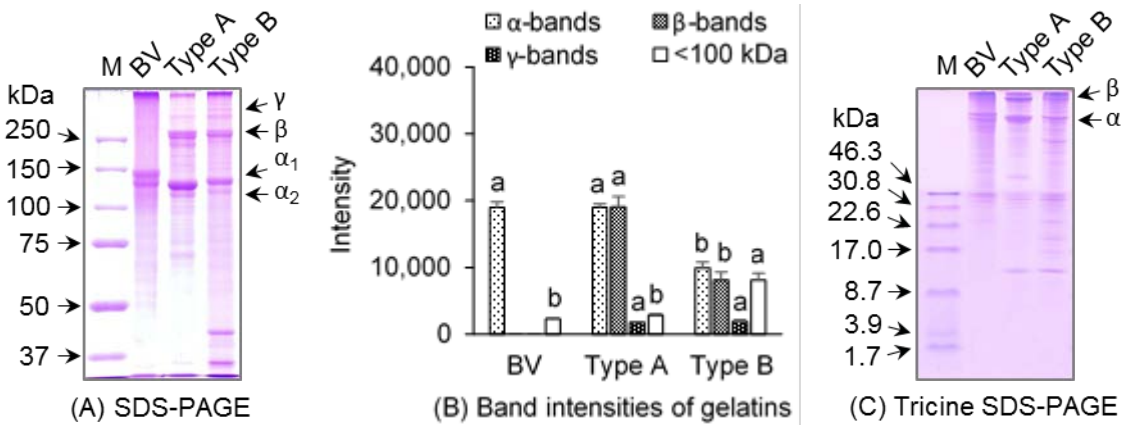
7.5% SDS-PAGE banding patterns (A), the intensity of α-, β-, and γ-bands and low molecular mass bands (<100 kDa) in SDS-PAGE (B), and 15.5% Tricine SDS-PAGE banding patterns (C) of bovine bone gelatin (BV) and type A and type B gelatins extracted from the head of kalamtra sturgeon. M, molecular marker. All gelatins were loaded as 10 μg/lane. Positions of α-, β-, and γ-bands were shown by arrows. Different letters in each band show significant difference ($p < 0.05$), in B.

Fig. 2.

Typical breaking force-breaking strain curves of bovine bone gelatin (BV), and type A and type B gelatins extracted from the head of kalamtra sturgeon. The arrows indicate the breaking points of the gels where the breaking forces and breaking strains of gelatins were obtained.

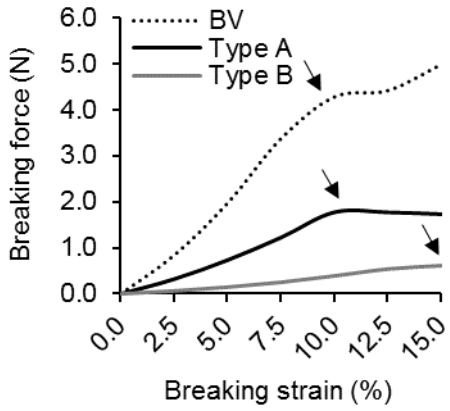
Fig. 3.

Scanning electron microscopy of bovine bone gelatin (BV; A, D, G), and type A (B, E, H) and type B (C, F) gelatins extracted from the head of kalamtra sturgeon. A–C, 6.67% gelatin (w/v); D–F, 3.00% gelatin; G and H, 1.50% gelatin. Scale bars, 10 μm.


M BV Type A Type B
kDa
250
150
100
75
50
37
γ
β
α1
α2
(A) SDS-PAGE
α-bands
β-bands
γ-bands
<100 kDa
Intensity
40,000
30,000
20,000
10,000
0
BV
Type A
Type B
(B) Band intensities of gelatins
M BV Type A Type B
kDa
46.3
30.8
22.6
17.0
8.7
3.9
1.7
β
α
(C) Tricine SDS-PAGE

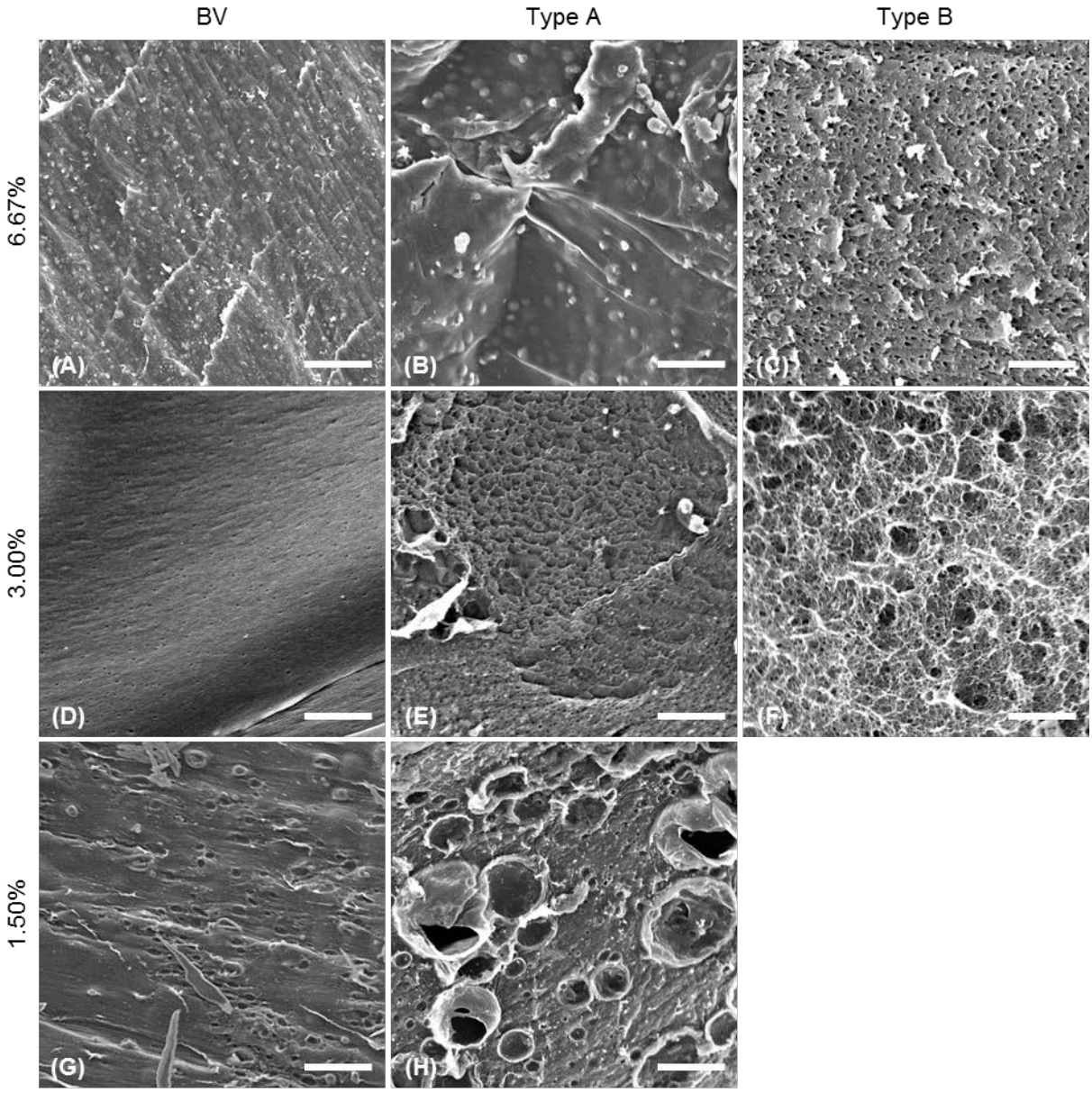
721

722 **Fig. 1.**


BV
Type A
Type B
Breaking force (N)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Breaking strain (%)

723

724 **Fig. 2.**



725

726 **Fig. 3.**

Table 1

Amino acid composition (residues/1000 residues) and gelatin content (% in the protein fraction) of purified collagen extracted from the sturgeon skin and type A and type B gelatins extracted from the head of kalamtra sturgeon.

Amino acids	Amino acid composition (residues/1000 residues)		
	Purified collagen	Type A	Type B
Ala	115	114	101
Arg	50	49	47
Asp	50	53	75
Cys	0	1	2
Glu	71	78	101
Gly	346	322	227
His	5	10	14
Hylys	9	9	6
Hyp	65	61	37
Ile	13	14	29
Leu	19	24	48
Lys	24	32	52
Met	10	11	19
Phe	13	17	25
Pro	116	104	79
Ser	51	51	53
Thr	24	26	36
Tyr	2	5	15
Val	16	19	34
Gelatin content*	100.0	93.9	56.9

*Relative gelatin content (%) in the protein fraction of sample. Values of each sample were obtained from the mixed samples of three replicates.

Table 2

Proximate composition (%) of type A and type B gelatins extracted from the head of kalamtra sturgeon.

Components	Proximate composition (%)	
	Type A	Type B
Gelatin[†]	60.3	39.7
Non-gelatinous proteins[†]	4.0	30.0
Lipid	2.4±0.1	1.1±0.2[*]
Ash	19.6±1.2	17.3±1.2
Others	13.6±1.1	12.0±1.2
Total	100.0	100.0

All values are expressed as percent in the sample weight. [†]Values were obtained from the mixed samples of three replicates. Other values are the mean ± standard error of three replicates. *Significantly different from the value in type A gelatin ($p < 0.05$).

Table 3

Rheological properties of bovine bone gelatin (BV) and type A and type B gelatins extracted from the head of kalamtra sturgeon.

Rheological properties	Gelatins		
	BV	Type A	Type B
Gelling temperature (°C)	22.4±0.1[a]	13.1±0.1[b]	8.2±0.2[c]
Melting temperature (°C)	29.3±0.1[a]	23.4±0.1[b]	23.1±0.1[b]
Gel strength (N)	4.4±0.1[a]	2.0±0.0[b]	0.7±0.0[c]
Breaking strain (%)	11.1±0.8[b]	12.1±0.4[b]	15.8±1.2[a]

All values are the mean ± standard error of three replicates. Different letters in the superscript at the row show significant difference ($p < 0.05$).

693 **Table 4**

694 Functional properties of bovine bone gelatin (BV) and type A and type B gelatins extracted

695 from the head of kalamtra sturgeon.

Functionalities	Gelatins		
	BV	Type A	Type B
WAC (%)	1123.6±82.1[a]	560.0±24.9[b]	2.1±0.2[c]
FBC (%)	153.4±6.3[b]	2283.4±69.6[a]	2199.7±45.2[a]
EAI (m^2/g)	5.4±0.1[b]	6.3±0.5[b]	45.02±0.3[a]
ESI (min)	53.2±2.6[b]	59.8±1.4[b]	423.4±44.2[a]
FE (%)	34.5±0.3[a]	27.8±1.1[b]	34.8±0.4[a]
FS (%)	27.5±1.0[a]	9.0±0.7[b]	29.8±0.4[a]

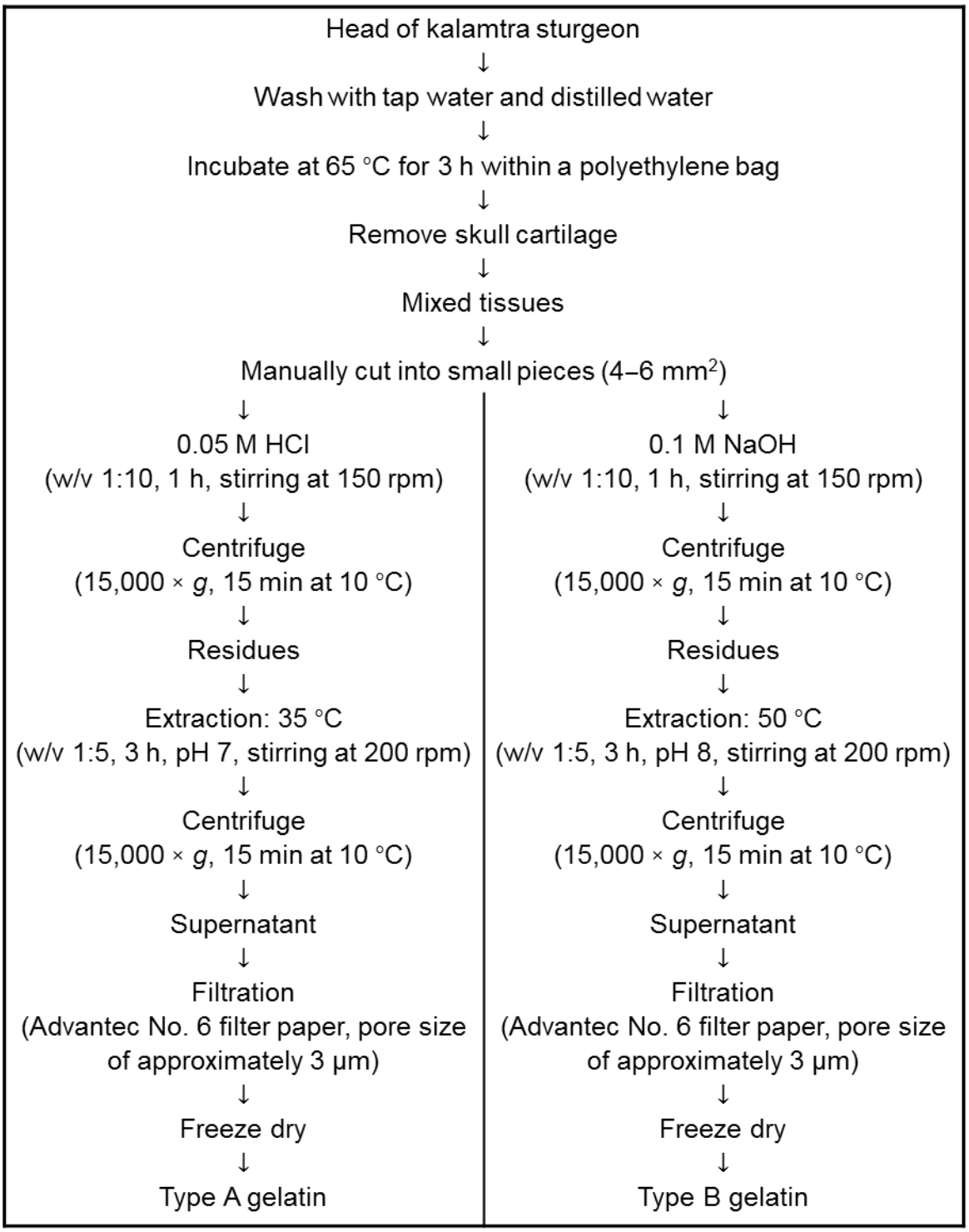
696

697 WAC, water absorbing capacity; FBC, fat binding capacity; EAI, emulsion activity index;

698 ESI, emulsion stability index; FE, foam expansion; and FS, foam stability. All values are the

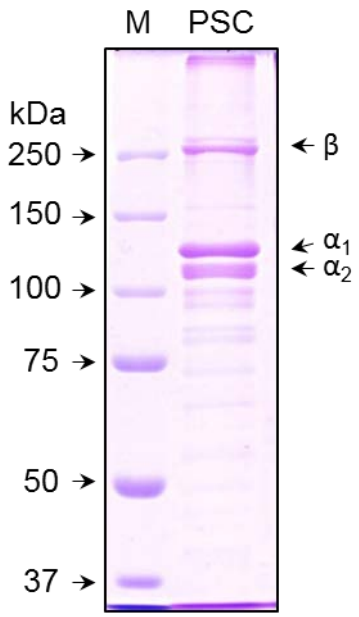
699 mean ± standard error of three replicates. Different letters in the superscript at the row show

700 significant difference ($p < 0.05$).



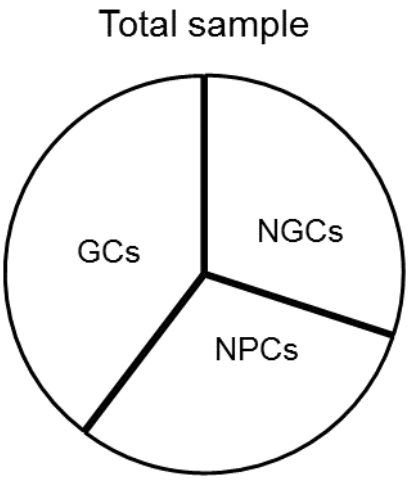
Supplementary Fig. 1.

Protocols used for the extraction of type A and type B gelatins from the mixed tissue of the kalamtra sturgeon head.



Supplementary Fig. 2.

SDS-PAGE (7.5% gel) patterns of purified skin collagen (5 μg/lane) using a mixture of kalamtra and bester sturgeon skin. M, molecular maker; PSC, purified skin collagen. Positions of type I collagen α_1-, α_2-, and β-bands were shown by arrows.



Here,
Sample is composed of gelatin, non-gelatinous proteins and non-proteinaceous substances; thus,
GCs + NGCs + NPCs = 100 (%)

Protein fraction in the sample is GCs + NGCs.

We can write as,
→ GCp (%) = GCs / (GCs + NGCs) x 100
→ NGCs(%) = 100 GCs/GCp – GCs

Where,
GCp, Relative gelatin content in the protein fraction of the sample (%)
GCs, Relative gelatin content in the sample (%)
NGCs, Relative non-gelatinous protein content in the sample (%)
NPCs, Non-proteinaceous content [lipid, ash, carbohydrate, etc.] in the sample (%)

In this study, GCp and GCs was measured from the Hyp content using the following equations;

GCp (%) = [(Hyp of the sample) / (Hyp of the purified collagen)] × 100,
where Hyp is the number of hydroxyproline expressed as Hyp residues/1,000 amino acid residues. Since amino acids come from protein fraction (gelatin and non-gelatinous protein) of the sample, and Hyp is the gelatin- and collagen-specific amino acid, the percentage of Hyp number in the sample to that in the purified collagen shows the relative gelatin content in the protein fraction of the sample.

GCs (%) = [(Hyp of the sample) / (Hyp of the purified collagen)] × 100,
where Hyp is the concentration (nmol Hyp/μg dry sample) of the gelatin sample or the purified collagen. In this case, Hyp concentration is amount of Hyp in the total sample (or collagen) weight. Thus, the percentage of Hyp in the sample to that in the purified collagen shows the relative gelatin content in the sample.

Supplementary Fig. 3.

Equation to calculate the non-gelatinous protein contents based on both relative gelatin content in the total protein fraction and in the total sample weight.