# Feasibility of collagens obtained from bester sturgeon *Huso huso* × *Acipenser ruthenus* for industrial use

**Xi Zhang[a,b,*], Huiyan Zhang[b], Shigeru Toriumi[c], Kazuhiro Ura[d], Yasuaki Takagi[d]**

[a]Hubei Provincial Engineering Laboratory for Pond Aquaculture, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

[b]Graduate School of Fisheries Sciences, Hokkaido University, 3–1–1 Minato-cho, Hakodate, Hokkaido 041-8611, Japan

[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

Xi Zhang

College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

E-mail: zhangxi@mail.hzau.edu.cn

**Abstract**

To increase the value of bester sturgeon (a hybrid of *Huso huso* × *Acipenser ruthenus*) and promote development of the sturgeon aquaculture industry, this study assessed the feasibility of using collagens from bester sturgeon by-products for industrial applications. We first found that individual stable characteristics in terms of yield, amino acid composition, thermal stability, and fibril-forming ability of bester skin collagen (SC), swim bladder collagen (SBC), and notochord collagen (NC) were comparable with those of the beluga and sterlet, the purebred parental species of bester sturgeon. In addition, seasonal variations in these factors were not evident, except for the fibril-forming ability of SC, which showed significant seasonal differences. In the summer, slower fibril formation with smaller individual variations was detected compared with winter for SC. Overall, the quality of products produced from bester sturgeon collagens was stable for industrial use, but batchwise certification of the fibril-forming ability of SC is essential if the products are to be used in fibril form, as in the case of collagen for tissue engineering scaffolds. Second, we determined that the optimal temperature for extracting SC, SBC, and NC was 4°C, 4°C, and 20°C, respectively. We also found that the high endotoxin content of NC could be reduced by pretreatment with 80% ETOH-0.1 M NaOH and 0.1 M NaOH. This study suggests that industrial use of collagens from bester sturgeon is feasible.

*Keywords*: Sturgeon; Collagen; Biochemical characteristics; Stability; Endotoxin

## 1. Introduction

Sturgeon is a valuable food fish that is renowned for its caviar. Sturgeon aquaculture directed towards caviar production began in the 1980s, and worldwide production increased to 25,000 t by 2008 (Bronzi et al., 2011). However, the culture costs are higher for sturgeon than for other fishes, as a long culture time is required to obtain caviar. For example, the female beluga *Huso huso*, producer of the most expensive caviar, requires 20 years to reach sexual maturity (Ronyai and Varadi, 1995). In contrast, the sterlet *Acipenser ruthenus* requires a shorter time (4–5 years) to reach sexual maturity (Williot et al., 2005), but the value of its caviar is much lower than that of beluga. Therefore, the bester sturgeon, which is a hybrid of beluga and sterlet, has been widely cultured because of its relatively short sexual maturity period (6–9 years) and good caviar quality (Amiri et al., 1996). However, earnings from this industry could be increased further if body parts other than the ovary and meat were used. Our goal is to industrialize collagen obtained from bester sturgeon by-products to increase the value of this fish.

The unique physiological functions and physical and chemical characteristics of collagens have led to their extensive industrial use in food, cosmetics, pharmaceutical, and healthcare product industries (Nagai and Suzuki, 2000; Zhang et al., 2009; Li et al., 2020). In recent years, extraction of collagens or collagen peptides from aquatic animals has been a popular research topic worldwide, both because of the high abundance of fish offal and to avoid zoonosis and religious objection (Singh et al., 2010; Huang et al., 2016; Kumar et al., 2017; Li et al., 2020; Meng et al., 2020). Because 40–55% of the total weight of fish is considered to be inedible (e.g., the head, skin, fins, and bones) (Torres et al., 2007), use of by-products obtained from the seafood processing industry, such as extraction of collagens, can protect the environment and increase the value of fish. Preparation methods, properties, and potential prospects of fish collagen have been extensively studied (Nagai and Suzuki, 2000; Ogawa et al., 2004: Duan et al., 2009; Zhang et al., 2019). Our previous study (Zhang et al., 2014) revealed that

industrial amounts of type I collagen could be extracted from bester skin and swim bladder, whereas type II collagen could be extracted from the notochord. Type II collagen is highly valuable because its market availability is much lower than that of type I collagen.

To industrialize bester collagens, low-cost production of safe and stable products is essential. The thermal stability (denaturation temperature) of collagen molecules is especially crucial because collagen molecules that retain the triple-helical form have different physical and chemical characteristics compared to denatured molecules (gelatin) (Miles et al., 1998). The denaturation temperature is the temperature above which the triple-helical structures of collagen molecules are uncoiled and turn into gelatin (Bigi et al., 2004). Duan et al. (2012) reported that the thermal stability of collagen from carp showed seasonal differences, with higher thermal stability for carp sacrificed in the summer. Although the mechanisms responsible for the seasonal difference are unknown, we assume that the higher water temperature in summer may affect the thermal stability of collagen in carp. However, studies of the seasonal variations and stability of the nature of fish collagen are scarce. Because sturgeon is a freshwater fish, similar to carp, its aquaculture water temperature may vary significantly, as is found in carp aquaculture ponds. Moreover, because bester sturgeon is a hybrid of beluga and sterlet, the characteristics of its collagen may be similar to those of either beluga or sterlet collagen or to a combination of the two, and it may show large individual variations. Thus, determination of individual and seasonal variations in the nature of bester collagen is essential to confirm its feasibility for use in industrial applications.

Another critical factor that must be optimized is the production cost of collagen molecules. To reduce costs, it is crucial to identify the optimum extraction temperature that does not denature the triple-helical form of collagen molecules. A standard laboratory method for extracting collagen molecules while retaining their natural triple-helical form is carried out at 4°C (Ogawa et al., 2004; Duan et al., 2009; Meng et al., 2019; Zhang et al., 2019). In this method, a temperature-controlled facility and/or equipment is required during the extraction and

purification processes, which increases the extraction cost. A higher extraction temperature will also reduce the extraction period. In addition, reduced endotoxin content is vital to ensure product safety. Endotoxins induce strong biological effects even at very low concentrations in the human body. If low-endotoxin collagens become available from bester sturgeon, they could be used in the medical field, as bester collagen has beneficial traits that make it useful as a biomaterial in regenerative medicine (Zhang et al., 2014).

We conducted the present study with the intent to produce bester collagen for use in industry and to promote the development of the sturgeon aquaculture industry. First, we examined individual variations in the biochemical characteristics of collagens obtained from bester, and these variations were compared with those for beluga and sterlet, the purebred parents of the bester sturgeon. In addition, we examined seasonal variations in bester collagens. Next, we analyzed collagen properties at different extraction temperatures to identify an industrially applicable optimal extraction temperature. Finally, we studied the effects of pretreatment on raw materials to obtain low-endotoxin collagen.

## 2. Materials and methods

### *2.1. Extraction of collagens*

Bester sturgeon were obtained from Nanae Fresh-Water Laboratory, Field Science Center for Northern Biosphere, Hokkaido University, Japan. Individuals were obtained in both winter and summer, when the average water temperature of the sturgeon aquaculture ponds was 4°C and 16°C, respectively. Beluga sturgeon were obtained from Fujikin Incorporated, Tsukuba, Japan, and sterlet sturgeon were obtained from Miyazaki Prefectural Fisheries Research Institute, Kobayashi Branch, Kobayashi, Japan. All experimental sturgeons were healthy and intact. In the present study, all procedures were performed in accordance with national and institutional guidelines on animal experimentation and care and were approved by the Institutional Animal Care

and Use Committee (IACUC) of Hokkaido University (approval ID: 22-1). The fish were deeply anesthetized in 2-phenoxyethanol solution and then gutted carefully. Skin, swim bladder, and notochord were dissected from the fish in the laboratory and stored at −30°C until use.

Samples were washed with cold distilled water (4°C) and cut into small pieces (ca. 0.5 × 0.5 cm). To remove non-collagenous proteins, skin samples were soaked in 0.1 M NaOH for 6 h (with two solution changes) at 4°C at a sample/solution ratio of 1:10 (w/v). The samples then were washed with cold distilled water three times. Subsequently, fat in the skin samples was removed using 99.5% ethanol at a sample/solvent ratio of 1:10 for 24 h (two solution changes). The samples were then washed with cold distilled water three times. To extract collagens, skin and swim bladder samples were continuously stirred in a solution of HCl (pH 2.0) containing 0.5% (w/v) porcine pepsin (EC 3.4.23.1, 1:10,000; Wako Pure Chemical Industries Ltd., Osaka, Japan) at a sample/solvent ratio of 1:10 (w/v) for 2–14 d at 4°C until there was almost no observable debris. The mixtures were then centrifuged at 2,000 × $g$ for 90 min (model 6800, KUBOTA Manufacturing Corporation, Tokyo, Japan) to obtain the supernatants. Notochords were continuously stirred under the same conditions for 3 d at 4°C. The solutions were then centrifuged at 2,000 × $g$ for 90 min to obtain the supernatants, and the residues were re-extracted under the same conditions until there was almost no visible debris. The supernatants were sequentially filtered through membrane filters with pore sizes of 3.0, 0.8, and 0.47 μm (Advantec, Tokyo, Japan). The collagen in the filtrate was precipitated by adding NaCl to a final concentration of 1 M. The precipitate was then collected by centrifugation at 2,000 × $g$ for 90 min and dissolved in HCl solution (pH 2.0). This process was repeated three times to purify the collagen. The purified collagen was dialyzed against 50 volumes of distilled water at 4°C for 24 h with two changes of water. The dialysate was lyophilized using a freeze dryer (FDU-830, Tokyo Rikakiki Co. Ltd., Tokyo, Japan). The percentage dry weight of collagen purified in comparison with the wet weight of the initial tissues was calculated as the collagen yield.

To determine the optimum extraction temperature for bester collagens, collagens extracted at 4, 10, 15, and 20°C were also prepared. In this experiment, skin and swim bladder samples were from winter bester samples. Notochord samples were collected by workers at Bifuka Islands (Bifuka, Hokkaido, Japan) at a commercial sturgeon aquaculture enterprise. The samples were transferred frozen to the laboratory and extracted as described above. The same tissues (skin, swim bladder, and notochord) were divided into four groups and extracted at 4, 10, 15, and 20°C. In addition, to determine a method for reducing the endotoxin content of collagens, samples of notochord were soaked in 0.1 M NaOH or 80% ethanol solution with 0.1 M NaOH for 3 h at 15°C. After the pretreatment, samples were washed with cold distilled water three times. Collagen then was extracted at 15°C as described above.

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

SDS-PAGE was performed largely following Zhang et al. (2014) according to the method of Laemmli (1970). In brief, the lyophilized collagens were dissolved in a solution of HCl (pH 3.0, 1 mg/ml) and mixed at a ratio of 1:1 (v/v) with sample buffer (0.5 M Tris-HCl buffer, pH 6.8, with 4% SDS and 20% glycerol) containing 10% β-mercaptoethanol. The mixed solution was boiled for 2 min. Electrophoresis was performed on a 7.5% running gel. After electrophoresis, the gel was stained for 30 min using 0.1% Coomassie Brilliant Blue R250 solution and then destained in a mixture of 70% ethanol with 20% acetic acid and 10% glycerol until the protein bands became clear. Precision Plus Protein standards (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used to estimate the molecular weight.

*2.3. Amino acid analysis*

Lyophilized collagens were sent to the Instrumental Analysis Division, Equipment Management Center,

Creative Research Institution, Hokkaido University, for amino acid analysis. Samples were hydrolyzed in 6 N HCl at 110°C for 24 h. The hydrolyzates were evaporated, and the remaining materials were dissolved in a citric acid buffer solution and analyzed using an automated amino acid analyzer (JLC-500V, JEOL Ltd., Tokyo, Japan).

*2.4. Denaturation temperature measurement*

The denaturation temperature (Td) of the collagens was measured via circular dichroism (CD) spectroscopy using a JASCO model 725 spectrometer (JASCO, Tokyo, Japan) largely following Zhang et al. (2014) according to the method of Ikoma et al. (2003). In brief, lyophilized collagens were dissolved in HCl solution (pH 3.0) at 1 mg/ml and placed into a quartz cell. CD spectra were measured at 190–250 nm at 10°C at a scan speed of 50 nm/min with an interval of 0.l nm. The rotatory angle at a fixed wavelength of 221 nm was measured at 10–50°C at a rate of 1°C/min to determine the collagen Td.

*2.5. Collagen fibril formation* in vitro

Collagen fibril formation experiments were performed according to the method of Zhang et al. (2014). Lyophilized collagens were dissolved in HCl solution (pH 3.0) at 0.3% (w/v). The collagen solution was then mixed with 0.1 M Na-phosphate buffer (pH 7.4). The ratio of collagen solution to Na-phosphate buffer was 1:2. The mixed solution was placed in a cell and the fibril formation at 22 ± 1°C was monitored by measuring the turbidity change at 320 nm using a model UH5300 spectrophotometer (Hitachi High-technology Corporation, Tokyo, Japan).

*2.6. Scanning electron microscopy (SEM) observation*

The microstructure of collagen fibrils formed *in vitro* was observed using SEM (JSM6010LA, JEOL Ltd.). Collagen fibrils were allowed to form as described above for 24 h at 22 ± 1°C. After fibril formation, sample suspensions were centrifuged at 20,000 × *g* for 20 min (CF15RX II; Hitachi Koki, Tokyo, Japan). The precipitates (collagen fibrils) were fixed with 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.6) for 3 h at room temperature and then rinsed with phosphate buffer. The fibrils were sequentially soaked in 70, 80, 90, 95, and 100% ethanol solutions for 30 min each for dehydration and then in a *t*-butyl alcohol solution for 30 min twice. Finally, collagen fibrils were freeze-dried in *t*-butyl alcohol solution using a freeze dryer (JFD-320, JEOL Ltd.) and coated with gold-platinum using an auto fine coater (JFC-1600, JEOL Ltd.).

### *2.7. Endotoxin measurement*

Lyophilized collagens were suspended in endotoxin-free water at 0.4% (w/v). The solutions were heated at 50°C for 10 min to make gelatin. The samples were mixed with the *Limulus* amebocyte lysate supplied in the QCL-1000™ assay kit (Lonza Japan, Tokyo, Japan) and incubated at 37°C for 10 min. A substrate solution was then added to the samples and they were incubated at 37°C for an additional 6 min. The reactions were stopped with the stopping reagent, and the absorbance of the samples was determined spectrophotometrically at 405 nm using a MTP-810Lab microplate reader (Corona Electric Co. Ltd., Ibaraki, Japan).

### *2.8. Statistical analysis*

Student's *t*-test was conducted to compare values using SPSS 22.0 (IBM, Armonk, NY, USA) between winter and summer samples of bester sturgeon. Differences were considered significant at $P < 0.05$.

## 3. Results

190 *3.1. Characterization of bester, beluga, and sterlet collagens and individual and seasonal variations in the*
191 *characteristics of bester collagens*

192 *3.1.1. Collagen yields*

193 Table 1 summarizes the collagen yields (%, based on the wet weight of the initial samples) extracted at 4°C.
194 In general, the highest yield was observed from swim bladder, whereas notochord showed the lowest yield. The
195 coefficient of variation (CV) for yields, which is defined as the ratio of the standard deviation to the mean, is
196 also shown in Table 1. CV is a normalized measure of the dispersion of a probability distribution or frequency
197 distribution, and it can suggest individual differences to some extent. Although the yields showed significant
198 variation, CVs for the same tissue among the three different sturgeon species were approximately the same,
199 except for the small value observed for skin from beluga. There was no significant difference in the yields of
200 skin collagen (SC) and notochord collagen (NC) between summer and winter besters. However, the yield of
201 swim bladder collagen (SBC) was significantly higher from summer bester than from winter bester ($P < 0.05$).

202 *3.1.2. SDS-PAGE*

203 Figure 1 shows typical SDS-PAGE patterns for SCs, SBCs, and NCs from beluga, sterlet, and bester in
204 winter and summer. Both SCs and SBCs from all sturgeon species used in this study had band patterns indicative
205 of type I collagen; two bands (ca. 120 and 100 kDa) were the major constituents. The density of the 120-kDa
206 band was higher than that of the 100-kDa band for the same species. The molecular weight of these bands and
207 the band pattern indicated that the 120-kDa bands were α1-chains and the 100-kDa bands were α2-chains. In
208 addition, α3-chains may have been present in the 120-kDa bands for SCs and SBCs because SBCs from white
209 sturgeon, bester sturgeon, and many other teleost fish contain α3-chains (Kimura, 1992). However, we could not
210 whether α3-chains were present in our samples because of the migration similarity of α3- and α1-chains on
211 SDS-PAGE (Kimura, 1992). In contrast to SCs and SBCs, NCs from all specimens showed band patterns

212 characteristic of type II collagen, with one band (α1-chains, ca. 130 kDa) as the major constituent (Fig. 1). There
213 were no observable individual differences in the band patterns, including the molecular weights of the major
214 bands, in any of the species and tissues or in bester collagens from different seasons.

215 *3.1.3. Amino acid composition*

216 The amino acid compositions of collagens from beluga, sterlet, winter bester, and summer bester were
217 analyzed and the mean and standard deviation were obtained (Supplementary Tables 1–3). The amino acid
218 compositions of collagens from the same tissue of all sturgeon were similar, and the compositions for bester
219 sturgeon were similar to those for the parental species.

220 Table 2 shows the content of imino acids and the degree of hydroxylation of proline and lysine residues in
221 collagens, both of which are closely related to the stability of collagen molecules. For the same tissue, similar
222 values were observed for all collagens from all sturgeon species, and the values for bester sturgeon were similar
223 to those for the parental species. When the values between winter and summer bester were compared, no
224 significant differences were observed, except for the degree of hydroxylation of proline residues between winter
225 NC and summer NC ($P < 0.05$).

226 *3.1.4. Thermal stability*

227 Table 3 lists the Td of SC, SBC, and NC from beluga, sterlet, winter bester, and summer bester sturgeon. In
228 all examined species, NC showed the highest Td, followed by SBC and then SC. These results are the same as
229 those previously reported for bester collagens (Zhang et al., 2014). Interspecies differences in Td for the same
230 tissue were small. The CV data showed that individual Td differences in bester sturgeon were similar to those in
231 purebred beluga and sterlet. No significant seasonal differences in the Td values for bester SC, SBC, or NC were
232 detected.

233 *3.1.5. Process of collagen fibril formation* in vitro

Fibril formation was monitored as the change in turbidity at 320 nm (Fig. 2). In all examined species, SBC (type I collagen) showed a more rapid increase in turbidity and a shorter time to attain the maximum turbidity compared to SC (type I collagen). These data confirm the results reported by Zhang et al. (2014).

Compared with SBC, SC showed larger species differences in the turbidity change: the turbidity of beluga SC increased most rapidly, followed by that of bester and then sterlet SC. In addition, SC from bester sampled in summer showed a slower increase in turbidity compared with that from winter bester. When the standard deviations were compared, SCs from all species showed individual diversity in turbidity changes compared with the other collagens. The diversity for winter bester sturgeon was especially large. In contrast, NCs (type II collagen) from all species showed no visible absorbance changes under the same conditions in this experiment.

*3.1.6. SEM observation of collagen fibrils*

Figure 3 shows typical SEM images of collagen fibrils after 24 h of incubation. SBC fibrils were generally thicker than SC fibrils. Collagen fibrils from the same tissue from all sturgeon were similar in the degree of fibril thickness. The differences between winter and summer bester were also small. In all examined species, SEM images showing fusion of several fibrils to form a thicker fibril were sometimes observed in SC and SBC. NCs partially formed thin fibrils in all sturgeon.

*3.2. Optimal extraction temperature of bester collagens for industrial production*

Table 4 summarizes the time needed for extraction and the yields of bester collagens extracted at 4°C, 10°C, 15°C, and 20°C. In general, the extraction time decreased with an increase in extraction temperature. For SC, SBC, and NC, the extraction time decreased from 7 d, 4 d, and 14 d at 4°C to 2 d, 2 d, and 1 d at 20°C, respectively. The SC yield of 10% at 4°C remained constant until 10°C, but decreased to 8% at 15°C. No SC was obtained at 20°C. The SBC yield remained at 15% when the extraction temperature was increased from 4°C

to 15°C. However, no SBC was obtained at 20°C. The NC yield increased from 4.67% to 8.81% when the extraction temperature was increased from 4°C to 20°C.

Figure 4 shows the SDS-PAGE patterns for bester collagens extracted at different temperatures. When the extraction temperature was increased from 4°C to ≥10°C for SC and SBC, bands corresponding to lower molecular weights became clearer along with decreases in the density of α-chain bands. However, no smaller peptides or decreases in the density of the α-chain band were observed for NC, even when extracted at 20°C.

Table 4 shows Td values for the collagens. The Td for SC extracted at 15°C was lower than for SC that extracted at 4°C and 10°C. The Td values for SBC extracted at 4°C and 10°C were similar. NC was not denatured even when extracted at 20°C, and Td values were similar to those for NC extracted at 4°C.

*3.3. Reduction of the endotoxin content of bester collagens*

The effects of extraction temperature on the endotoxin content in purified collagens are shown in Table 4. The endotoxin content of SC and SBC was low (close to the detection limit) for all extraction temperatures. The endotoxin content of NC extracted at 15°C and 20°C was much higher compared with NC extracted at 4°C or 10°C. To decrease the endotoxin content of NC, pretreatments with 0.1 M NaOH (alkaline pretreatment) or an 80% ethanol solution with 0.1 M NaOH (alkaline-ethanol pretreatment) were studied. All NCs were extracted at 15°C after the pretreatments, and the yields and endotoxin content are summarized in Table 5. The pretreatments had slight effects on the NC yields but did not affect the Td. Compared with the high endotoxin content of NC extracted without pretreatments (610.0 ± 150.0 EU/mg, mean ± standard deviation (SD) of three measurements), the endotoxin content of NC extracted with the two pretreatments was much lower (10.0 ± 0.0 EU/mg after alkaline treatment; 8.4 ± 2.4 EU/mg after alkaline-ethanol treatment). Thus, both treatments were sufficient in reducing the endotoxin content of NC. CD spectra of NCs after alkaline or alkaline-ethanol pretreatment did not

differ. Therefore, both alkaline and alkaline-ethanol pretreatments did not affect the nature of NC.

## 4. **Discussion**

The results of this study suggest the strong possibility of industrial use of collagens from bester sturgeon. The individual variations for bester SC, SBC, and NC in terms of yield, amino acid composition, and Td were comparable to those for beluga and sterlet. Moreover, seasonal variations in these factors for bester collagens were small. SDS-PAGE analyses also showed small individual differences in band patterns for bester SC, SBC, and NC, suggesting that the molecular weight and composition of α-chains were similar in all individuals. Such small individual differences in the hybrids may occur because their parental species, beluga and sterlet, have collagens with a similar nature. Indeed, differences in yield, amino acid composition, Td, and SDS-PAGE patterns for beluga and sterlet collagens were small. These results strongly suggest that bester collagens possess individual and seasonally stable characteristics, pointing to potential stability in the quality of their collagen products for use in industrial applications. To date, sturgeon collagen has not been industrialized and has not been commercially available, so it is a new prospective collagen source for industrial application.

Type I collagen resources for biomedical uses currently on the market include porcine skin, bovine skin, and tilapia skin and scales. The price of bioactive type I collagen can reach $1/mg (e.g., type I collagen from porcine skin, bovine skin, and tilapia skin, Nippi, Inc., Tokyo, Japan; type I collagen from tilapia scales, Taki Chemical Co., Ltd, Kakogawa, Japan). Based on results of our study, 6 g of bioactive type I collagen from skin and 4 g from swim bladder could be obtained from one bester sturgeon (length = 0.76 m, 2.00 kg) (Zhang et al., 2014). Therefore, if sturgeon collagen is industrially produced, its estimated revenue would be considerable and much higher than its production cost. Compared with porcine and bovine collagen, sturgeon collagen has the advantage of high abundance of the precursor material (i.e., fish offal) and it avoids the problems of zoonosis

and religious objection (Meng et al., 2019; Zhang et al., 2019; Li et al., 2020). In addition, compared with tilapia, more collagen can be extracted from one fish owing to the larger size of sturgeon. However, sturgeon collagen has the disadvantage of a low denaturation temperature compared with the collagen resources listed above. We found that the Td of bester sturgeon type I collagen fibrils was lower than 37°C, which is a temperature commonly used for cell culture, but it could be increased by introducing chemical crosslinks using genipin (Moroi et al., 2019; Meng et al., 2020). In addition, although SBC collagen is rare in the market, SBC fibril coatings have great potential as a tool for tissue engineering research (Moroi et al., 2019).

To date, type II collagen is rare in the market, and bioactive type II collagen is very expensive. For example, the price of bioactive type II collagen extracted from bovine and chicken cartilage is higher than $10/mg (Sigma-Aldrich Co., Tokyo, Japan). Our results indicate that 400 mg of bioactive type II collagen from notochord could be obtained from one bester sturgeon (length = 0.76 m, 2.00 kg) (Zhang et al., 2014). We also were able to achieve a five-fold increase in the yield of notochord type II collagen (Meng et al., 2019). Moreover, compared with cartilage, the process for extracting type II collagen from notochord is simple and convenient (Zhang et al., 2014, 2019; Meng et al., 2019). Therefore, sturgeon notochord is a promising resource for industrial production of type II collagen.

Collagen molecules can self-assemble into fibrils in a solution with appropriate pH and salt concentration. It is now generally accepted that all of the information needed for fibril formation is contained in the collagen molecules themselves (Helseth and Veis, 1981; Bareil et al., 2010). The fibril-forming ability of bester SBC showed small individual variations that were comparable to those of beluga and sterlet SBC. The seasonal differences were also small. In contrast, the fibril-forming ability of bester SC showed greater individual variations, especially for SC sampled in winter. These data suggest that batchwise certification of the fibril-forming ability of SC would be essential if the products were to be used in fibril form, such as collagen for

cell culture. The reason why SC showed greater individual variations than the other collagens is unknown, as the factors responsible for the speed of collagen fibrillogenesis are not clear. Further studies need to be carried out in the future.

The optimal temperature for extracting bester collagens was also determined in the present study. Although a higher extraction temperature reduced the time for tissue digestion, no SC or SBC was obtained at 20°C. In addition, the SDS-PAGE patterns changed for SC and SBC extracted at ≥10°C: the bands corresponding to lower molecular weights were increased, there were more low-molecular-weight bands present in addition to the major α-chain bands, and decreases in the density of α-chain bands occurred. These data suggest that the higher extraction temperature may have slightly and partially uncoiled the triple-helical structure of each SC and SBC molecule, and then the molecules were partially digested by pepsin. Such partial digestion might not disturb the gross triple-helical structure of the molecule and could result in precipitation of partly-digested collagen molecules after addition of NaCl during the purification procedure. The denaturation procedure for SDS-PAGE then resulted in the formation of peptides (bands with a lower molecular weight in SDS-PAGE). By contrast, no adverse effects of extraction at 20°C were observed for NC. According to these data, the optimal temperature for extracting SC, SBC, and NC was 4°C, 4°C, and 20°C, respectively. These observations also indicate that the optimal extraction temperatures for SC, SBC, and NC are much lower than their Td values and even lower than the temperatures at which rapid a decrease in the CD spectra at 221 nm begins (SC, ca. 23°C; SBC, ca. 27°C; NC, ca. 32°C). Td was defined as the temperature with the fastest decrease in the CD spectrum at 221 nm when the sample was heated at from 10°C to 50°C at a rate of 1°C/min. Although this condition is commonly used to determine Td values for collagen molecules, the present results suggest that the heating condition for Td measurement was too rapid to estimate the optimal temperature for collagen extraction. In addition, an increase in extraction temperature may amplify any bacterial contamination and accelerate the decomposition and

denaturation of collagen during the extraction process.

The endotoxin content of SC and SBC was sufficiently low in this study. Because endotoxins originate from bacteria, SC should contain a certain amount of endotoxins because the skin is in direct contact with the environmental water, which always contains bacteria. Thus, the present results suggest that the alkaline pretreatment used to reduce the contamination of non-collagenous proteins in the skin samples may have reduced the endotoxin content. In fact, Niwa et al. (1969) reported that endotoxins are broken down in alkaline as well as in ethanolic alkaline solutions. In contrast, the swim bladder in the body should be free from bacterial contamination. Thus, the lower values obtained for SBC suggest that contamination by bacteria during the dissection, extraction, and purification processes was minimal. However, extraction of NC at 15°C or higher increased the endotoxin content, but it was reduced by pretreatment with 0.1 M NaOH. We propose that the endotoxin levels detected were low enough for the threshold applied for intravenously injectable drugs. The endotoxin limit for most injectable drugs has been set as 5 endotoxin units (EU) per kg of body weight per hour because the pyrogenic threshold for humans is 5 EU/kg of body weight (Akel, 2015). Use of endotoxin-free solutions and sterile environments during collagen extraction and purification may further decrease endotoxin levels. The notochord in the body should be free from bacteria. However, the source of the notochord in this study differed from that of the skin and swim bladder. The endotoxin content significantly increased during extractions at 15°C and 20°C because the notochord samples were contaminated with bacteria during the dissection process at Bifuka Island, and proliferation of bacteria occurred during extractions at 15°C and 20°C. Considering the extraction cost during industrial production, pretreatment with 0.1 M NaOH (alkaline pretreatment) is recommended to extract NC with low endotoxin content.

## 5. **Conclusions**

Collagens from skin, swim bladder, and notochord of bester sturgeon had individual and seasonally stable characteristics in terms of yield, amino acid composition, and thermal stability. These results show that bester sturgeon collagen products are stable and usable in industrial applications. The optimal temperature for NC extraction could be increased to 20°C, which would greatly reduce the production cost compared with the usual 4°C extraction temperature. In addition, the high endotoxin content of NC could be reduced by pretreatment with low-cost NaOH. This study illustrates the feasibility of industrial use of collagens from the by-products of bester sturgeon to further enhance the value of this fish.

**Declaration of competing interest**

The authors declare no conflicts of interest. The work has not been published previously.

**Acknowledgment**

This study was partly supported by the Regional Innovation Strategy Promotion Program, MEXT, Japan, and the National Natural Science Foundation of China (project number: 31902390). The authors thank Professor Shinji Adachi, Hokkaido University, for his help in collecting sturgeon samples and International Science Editing for editing this manuscript.

383 **References**


384 Amiri, M.B., Maebayashi, M., Hara, A., Adachi, S., Yamauchi, K., 1996. Ovarian development and serum sex
385 steroid and vitellogenin profiles in the female cultured sturgeon hybrid, the bester. J. Fish Biol. 48,
386 1164–1178. https://doi.org/10.1111/j.1095-8649.1996.tb01812.x.

387 Akel, S., 2015. Cell banking: process development and cell preservation, in Atala, A., Allickson, J.G. (Eds.),
388 Translational Regenerative Medicine. Elsevier Inc., PP. 21-37.
389 https://doi.org/10.1016/B978-0-12-410396-2.00003-7.

390 Bareil, P.R., Gauvin, R., Berthod, F.B., 2010. Collagen-based biomaterials for tissue engineering applications.
391 Materials 3, 1863–1887. https://doi.org/10.3390/ma3031863.

392 Bigi, A., Panzavolta, S., Rubini, K., 2004. Relationship between triple-helix content and mechanical properties
393 of gelatin films. Biomaterials 25, 5675–5680. https://doi.org/10.1016/j.biomaterials.2004.01.033.

394 Bronzi, P., Rosenthal, H., Gessner, J. 2011. Global sturgeon aquaculture production: An overview. J. Appl.
395 Ichthyol. 27, 169–175. https://doi.org/10.1111/j.1439-0426.2011.01757.x.

396 Duan, R., Zhang, J., Du, X., Yao, X., Konno, K. 2009. Properties of collagen from skin, scale and bone of carp
397 (*Cyprinus carpio*). Food Chem., 112 (3), 702–706. https://doi.org/10.1016/j.foodchem.2008.06.020.

398 Duan, R., Zhang, J., Li, J., Zhong, X., Konno, K., Wena, H. 2012. The effect of the subunit composition on the
399 thermostability of collagens from the scales of freshwater fish. Food Chem., 135 (1), 127–132.
400 https://doi.org/10.1016/j.foodchem.2012.04.112.

401 Guitton, J.D., Le Pape, A., Sizaret, P.Y., Muh, J.P. 1981. Effects of in vitro N-glucosylation of type I collagen
402 fibrillogenesis. Biosci. Rep. 1 (12), 945–954. https://doi.org/10.1007/BF01114964.

403 Helseth, D.L., Veis, A. 1981. Collagen self-assembly *in vitro*: Differentiating specific telopeptide-dependent
404 interactions using selective enzyme modifications and addition of free amino telopeptide. J. Biol. Chem.

256 (14), 7118–7128. http://www.jbc.org/content/256/14/7118.short.

Huang, C.Y., Kuo, J.M., Wu, S.J., Tsai, H.T., 2016. Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion-hydro-extraction process. Food Chem. 190, 997–1006. https://doi.org/10.1016/j.foodchem.2015.06.066.

Ikoma, T., Kobayashi, H., Tanaka, J., Walsh, D., Mann, S. 2003. Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticus*. Int. J. Biol. Macromol. 32 (3), 199-204. https://doi.org/10.1016/S0141-8130(03)00054-0.

Kimura, S. 1992. Wide distribution of the skin type I collagen a3 chain in bony fish. Comp. Biochem. Phys. B 120, 255–260. https://doi.org/10.1016/0305-0491(92)90119-C.

Kumar, P.G., Nidheesh, T., Govindaraju, K., Jyoti, Suresh, P.V., 2017. Enzymatic extraction and characterisation of a thermostable collagen from swim bladder of rohu (*Labeo rohita*). J. Sci. Food Agric. 97, 1451–1458. https://doi.org/10.1002/jsfa.7884.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (5259), 680-685. https://www.nature.com/articles/227680a0.

Li, P., Lu, W., Chan, Y., Ko, W., Jung, C., Huynh, D.L.T., Ji, Y. 2020. Extraction and characterization of collagen from sea cucumber (*Holothuria cinerascens*) and its potential application in moisturizing cosmetics. Aquaculture 515, 73450. https://doi.org/10.1016/j.aquaculture.2019.734590.

Meng, D., Tanaka, H., Kobayashi, T., Hatayama,H., Zhang, X., Ura, K., Yunoki, S., Takagi, Y. 2019. The effect of alkaline pretreatment on the biochemical characteristics and fibrilforming abilities of types I and II collagen extracted from bester sturgeon byproducts. Int. J. Biol. Macromol. 131, 572–580.https://doi.org/10.1016/j.ijbiomac.2019.03.091.

Meng, D., Li, W., Ura, K., Takagi, Y. 2020. Effects of phosphate ion concentration on in-vitro fibrillogenesis of

427 sturgeon type I collagen. Int. J. Biol. Macromol. 148, 182-191.
428 https://doi.org/10.1016/j.ijbiomac.2020.01.128.
429 Moroi, S., Miura, T., Tamura,T., Zhang, X., Ura, K., Takagi, Y. 2019. Self-assembled collagen fibrils from the
430 swim bladder of Bester sturgeon enable alignment of MC3T3-E1 cells and enhance osteogenic
431 differentiation. Mater. Sci. Eng. C 109925. https://doi.org/10.1016/j.msec.2019.109925.
432 Miles, C.A., Knott, L., Sumner, I.G., Bailey, A.J. 1998. Differences between the thermal stabilities of the three
433 triple-helical domains of type IX collagen. J. Mol. Biol. 277 (1), 135–144.
434 https://doi.org/10.1006/jmbi.1997.1603.
435 Nagai, T., Suzuki, N. 2000. Isolation of collagen from fish waste material-skin, bone and fins. Food Chem. 68,
436 277–281. https://doi.org/10.1016/S0308-8146(99)00188-0.
437 Niwa, M., Milner, K.C., Ribi, E., Rudbach, J.A. 1969. Alteration of physical, chemical and biological properties
438 of endotoxin by treatment with mild alkali. J. Bacteriol. 97(3), 1069–1077.
439 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC249816.
440 Ogawa, M., Moody, M.W., Portier, R.J., Bell, J., Schexnayder, M.A., Losso, J.N. 2004. Biochemical properties
441 of bone and scale collagens isolated from the subtropical fish black drum (*pogonia cromis*) and
442 sheepshead seabream (*Archosargus probatocephalus*). Food Chem. 88, 495–501.
443 https://doi.org/10.1016/j.foodchem.2004.02.006.
444 Ronyai, A., Varadi, L. 1995. The sturgeons In: Reproduction of Aquatic Animals: Fishes, (eds. by Nash CE &
445 Novotny AJ), World Animal Sciences C8 (pp. 95–108). Amsterdam: Elsevier.
446 Singh, P., Benjakul, S.,Maqsood, S., Kishimura, H. 2010. Isolation and characterisation of collagen extracted
447 from the skin of striped catfish (*Pangasianodon hypophthalmus*), Food Chem. 124 (1), 97-105.
448 https://doi.org/10.1016/j.foodchem.2010.05.111.Torres, J. A., Chen, Y. C., Rodrigo-Garcia, J., Jaczynski, J.

(2007). Recovery of by-products from seafood processing streams. In F. Shahidi (Ed.), Maximising the value of marine by-products (pp. 65–90). Boca Raton, USA: CRC Press.

Williot, P., Brun, T. R., Rouault, T., Pelard, M., Mercier, D., Ludwig, A. 2005. Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. Aquaculture, 246, 263–273. https://doi.org/10.1016/j.aquaculture.2005.02.048.

Zhang, M., Liu, W., Li, G. 2009. Isolation and characterization of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*). Food Chem. 115, 826–831. https://doi.org/10.1016/j.foodchem.2009.01.006.

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

Zhang, X., Adachi, S., Ura, K., Takagi, Y. 2019. Properties of collagen extracted from Amur sturgeon *Acipenser schrenckii* and assessment of collagen fibrils *in vitro*. Int. J. Biol. Macromol. 137, 809-820. https://doi.org/10.1016/j.ijbiomac.2019.07.021.

464 **Figure Captions**

465 **Fig. 1.** SDS-PAGE of collagens from the skin, swim bladder, and notochord of beluga, sterlet, winter, and
466 summer bester sturgeon. M, high molecular weight marker; SC, skin collagen; SBC, swim bladder collagen; NC,
467 notochord collagen.

468

469 **Fig. 2.** Process of collagen fibril formation *in vitro* of the skin (SC, blue line), swim bladder (SBC, red line) and
470 notochord (NC, green line) of beluga, sterlet, winter and summer bester sturgeon. Means and standard deviations
471 of the absorbance are shown.

472

473 **Fig. 3.** Scanning electron micrographs of collagen fibrils of skin (a, d, g, j), swim bladder (b, e, h, k) and
474 notochord (c, f, i, l) of beluga (a-c), sterlet (d-f), winter bester (g-i), summer bester (j-l) sturgeon. Collagen
475 solution (0.3% w/v) was mixed with 0.1 M Na-phosphate buffer (pH 7.4) at a ratio of 1:2 (v/v), and fibrils were
476 formed at 22 ± 1°C for 24 h.

477

478 **Fig. 4.** SDS-PAGE of bester sturgeon collagens from the skin, swim bladder, and notochord extracted at 4°C,
479 10°C, 15°C, and 20°C. M, high molecular weight marker; SC, skin collagen; SBC, swim bladder collagen; NC,
480 notochord collagen.
481

**Table 1**

Yields (%, based on the wet weight of initial samples) of collagens purified from the skin (SC), swim bladder (SBC) and notochord (NC) of beluga, sterlet, and winter and summer bester sturgeon

| | SC | SBC | NC |
|---|---|---|---|
| Beluga (n=4) | 9.04±1.49 (0.16) | 11.47±1.15 (0.10) | 4.99±0.46 (0.09) |
| Sterlet (n=4) | 3.98±1.73 (0.44) | 14.74±2.21 (0.15) | 3.03±0.86 (0.29) |
| Winter bester (n=8) | 7.44±2.50 (0.34) | 13.34±1.94 (0.15) | 4.71±0.63 (0.13) |
| Summer bester (n=8) | 9.56±3.23 (0.34) | 16.92±2.09 (0.12)* | 4.53±0.10 (0.22) |

Values are expressed as means ± SD with coefficient of variations in parentheses.

*Significantly different from winter bester group ($P < 0.05$)

**Table 2**

Comparison of numbers of imino acid, proline, hydroxyproline, lysine and hydroxylysine residues, and hydroxylation degree of proline and lysine residues of collagens from skin (SC), swim bladder (SBC) and notochord (NC) of beluga, sterlet, and winter and summer bester sturgeon (expressed as residues/1,000 total amino acid residues)

| | | Beluga | Sterlet | Winter bester | Summer bester |
|---|---|---|---|---|---|
| SC | Imino acids | 177±2 | 180±3 | 183±8 | 173±2 |
| | Pro | 111±4 | 114±3 | 115±3 | 109±8 |
| | HyPro | 66±5 | 66±1 | 67±5 | 64±8 |
| | Hydroxylation (%) | 37±2 | 37±1 | 37±1 | 37±4 |
| | Lys | 23±1 | 23±1 | 23±1 | 23±1 |
| | Hylys | 8±1 | 7±1 | 8±1 | 8±1 |
| | Hydroxylation (%) | 25±3 | 24±2 | 25±2 | 26±2 |
| SBC | Imino acids | 176±2 | 174±4 | 177±9 | 169±4 |
| | Pro | 100±3 | 92±3 | 98±5 | 95±2 |
| | HyPro | 76±1 | 82±2 | 79±4 | 74±2 |
| | Hydroxylation (%) | 43±1 | 47±1 | 45±4 | 44±0 |
| | Lys | 22±1 | 19±1 | 21±1 | 21±1 |
| | Hylys | 10±1 | 12±0 | 10±1 | 10±1 |
| | Hydroxylation (%) | 31±2 | 39±1 | 31±2 | 31±3 |
| NC | Imino acids | 188±2 | 187±4 | 188±12 | 184±5 |
| | Pro | 116±3 | 116±3 | 117±5 | 118±4 |
| | HyPro | 72±1 | 71±2 | 71±7 | 66±2 |
| | Hydroxylation (%) | 38±1 | 38±1 | 38±1 | 36±4* |
| | Lys | 15±0 | 14±1 | 14±1 | 14±1 |
| | Hylys | 21±3 | 23±1 | 22±2 | 21±1 |
| | Hydroxylation (%) | 59±2 | 62±1 | 61±2 | 59±1 |

Values are expressed as means ± SD.

*Significantly different from winter bester group ($P < 0.05$). Hylys, hydroxylysine; HyPro, hydroxyproline

**Table 3**

The denaturation temperature (Td) of beluga, sterlet, and winter and summer bester sturgeon

| Td (ºC) | SC | SBC | NC |
|---|---|---|---|
| Beluga | 29.6±0.3 (0.008) | 32.1±0.5 (0.015) | 34.9±0.3 (0.007) |
| Sterlet | 30.0±0.8 (0.026) | 31.9±0.2 (0.007) | 34.9±0.2 (0.006) |
| Winter bester | 30.3±0.5 (0.002) | 32.3±0.2 (0.007) | 34.9±0.5 (0.001) |
| Summer bester | 29.9±0.4 (0.015) | 32.0±0.2 (0.008) | 34.7±0.4 (0.011) |

Means ± SD with coefficient of variations in parentheses

**Table 4**

The extraction time, yields (%, based on the wet weight of initial samples), Td and endotoxin contents of collagens purified from the skin (SC), swim bladder (SBC) and notochord (NC) of bester sturgeon at the extracted temperatures of 4°C, 10°C, 15°C, and 20ºC

| | | 4ºC | 10ºC | 15ºC | 20ºC |
|---|---|---|---|---|---|
| Extraction time (day) | SC | 7 | 3 | 3 | 2 |
| | SBC | 4 | 3 | 3 | 2 |
| | NC | 14 | 7 | 4 | 1 |
| Yields (%) | SC | 10.1 | 10.3 | 8.24 | - |
| | SBC | 15.3 | 15.6 | 15.4 | - |
| | NC | 4.7 | 5.6 | 6.3 | 6.8 |
| Td (ºC) | SC | 30.0 | 30.0 | 26.0 | - |
| | SBC | 32.5 | 31.5 | 32.0 | - |
| | NC | 35.0 | 34.5 | 33.0 | 33.0 |
| Endotoxin (EU/mg) | SC | 0.6 | 0.1 | 0.1 | - |
| | SBC | 0.1 | 0.1 | 0.1 | - |
| | NC | 9.8 | 5.6 | 130 | 150 |

-, No collagen was obtained after salting-out.

512 **Table 5**

513 Yields (%, based on the wet weight of initial samples), the Td and the endotoxin contents of collagens purified
514 from the notochord (NC) of bester sturgeon at 15ºC with the pretreatment of 0.1 M NaOH and the pretreatment
515 of 0.1 M NaOH in 80 % ethanol solution

| | Without treatment | 0.1 M NaOH | 80% ETOH-0.1 M NaOH |
|---|---|---|---|
| Yields (%) | 4.4 | 3.1 | 5.6 |
| Td (°C) | 33.0 | 33.0 | 33.0 |
| Endotoxin (EU/mg) | 610.0±150.0 | 10.0±0.0 | 8.4±2.4 |

516 Means ± SD of three measurements

**Fig. 1.**

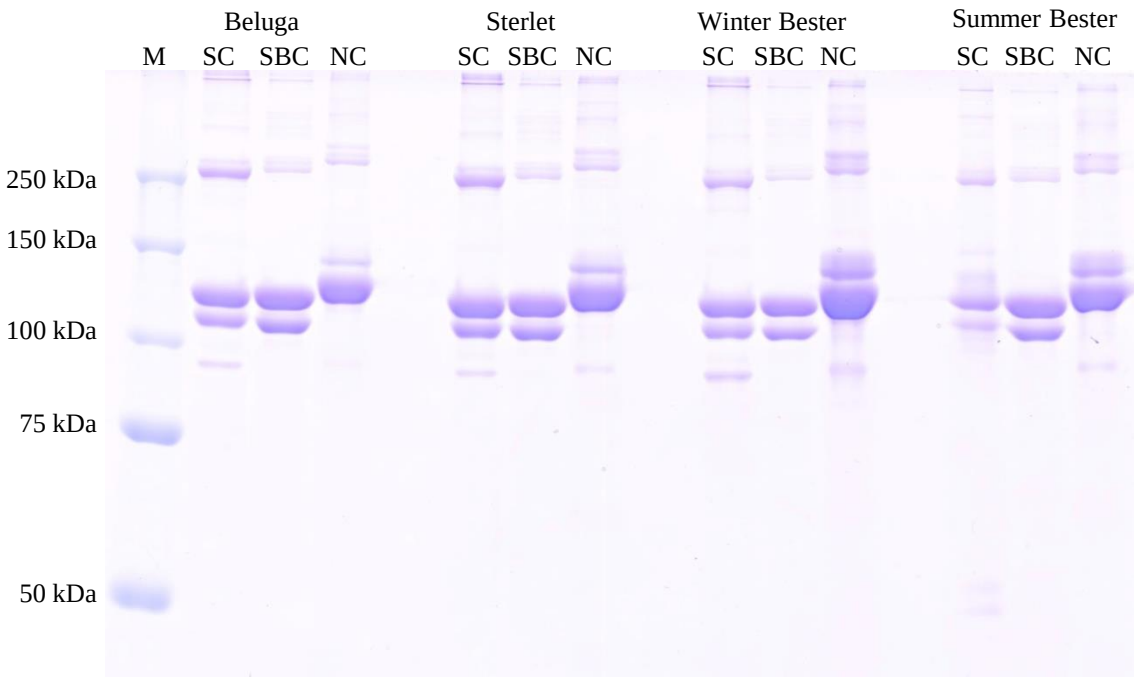

Beluga
Sterlet
Winter Bester
Summer Bester
M
SC
SBC
NC
SC
SBC
NC
SC
SBC
NC
SC
SBC
NC
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa

**Fig. 2.**

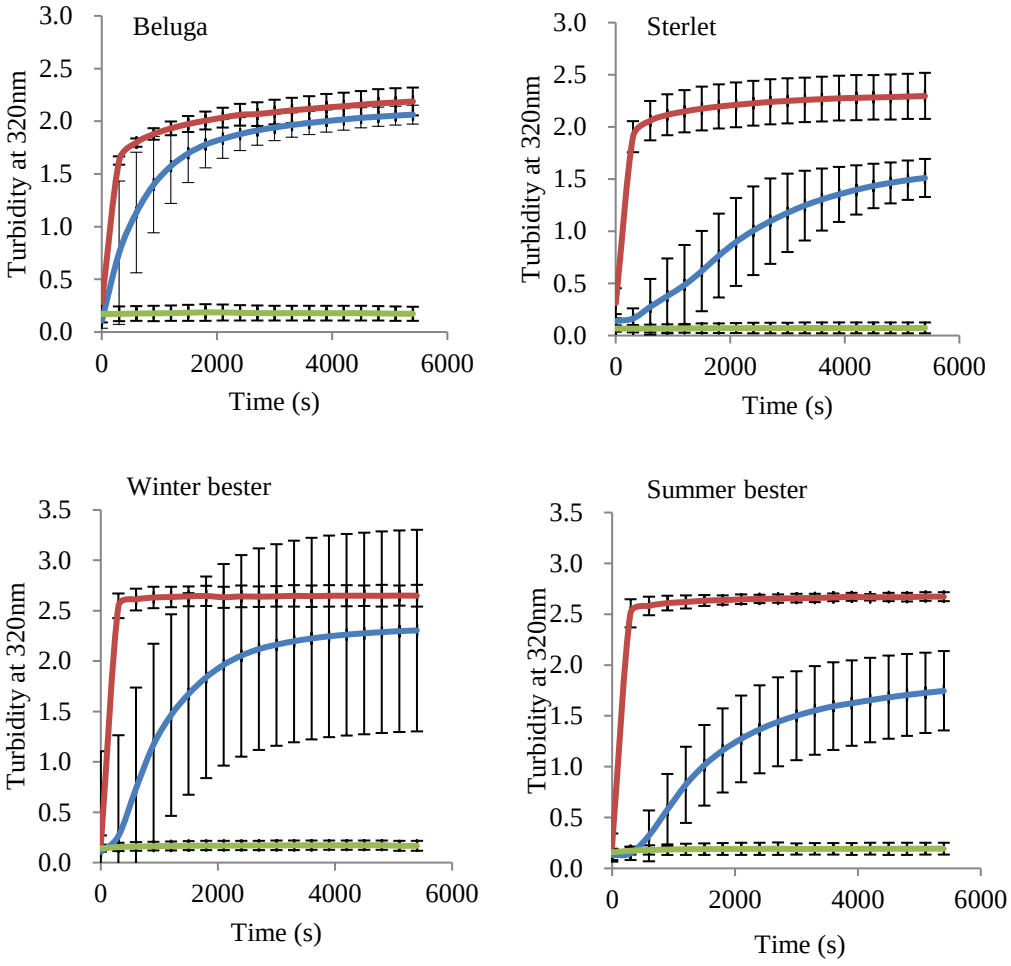

Beluga
Sterlet
Winter bester
Summer bester
Turbidity at 320nm
Time (s)
0
2000
4000
6000
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

**Fig. 3.**

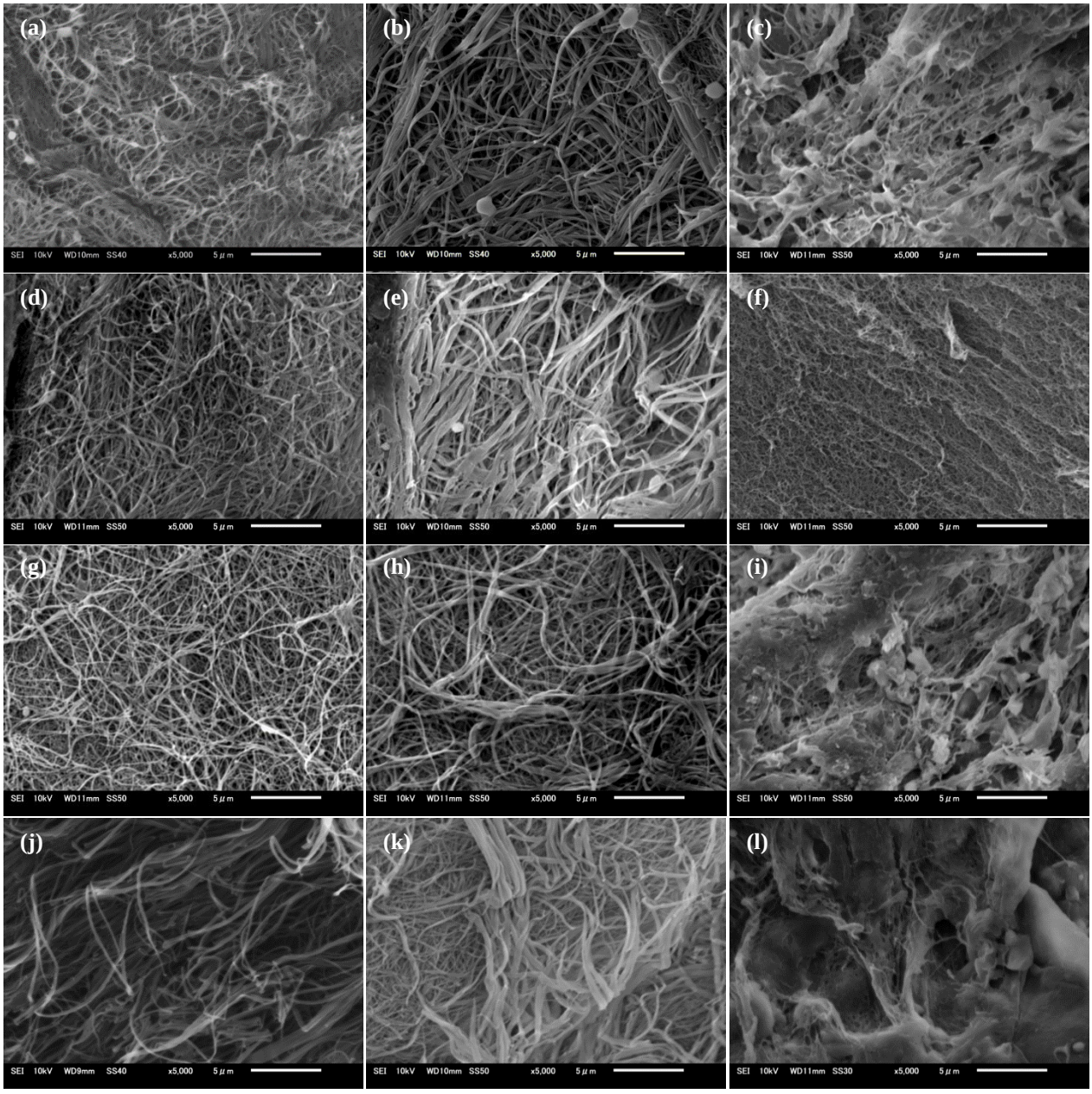
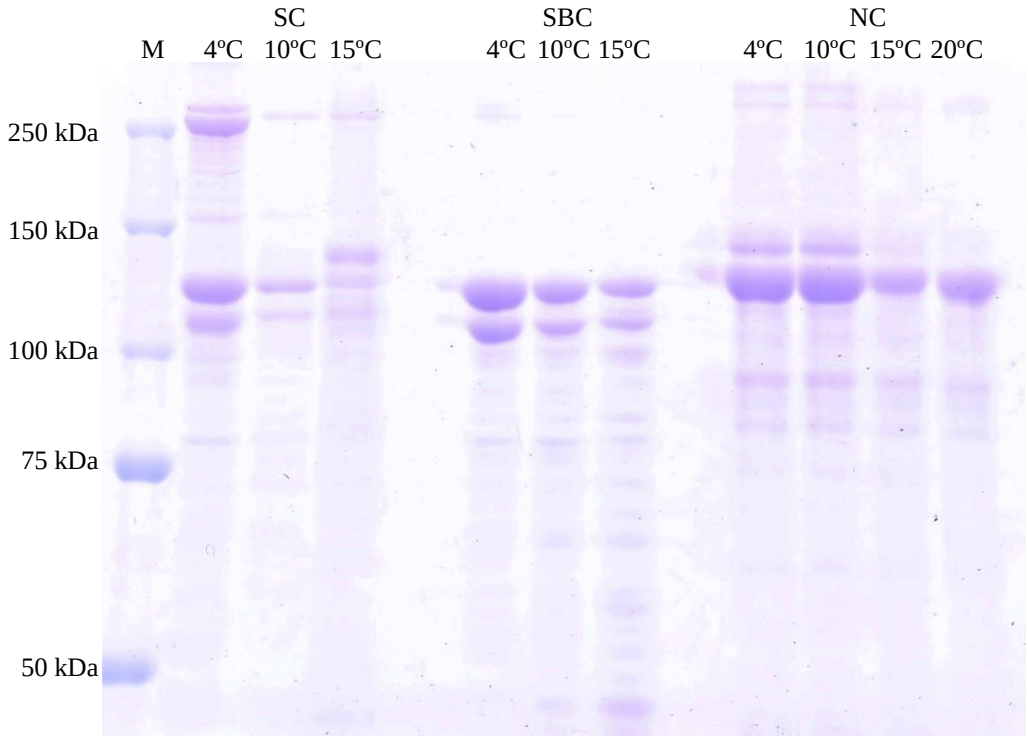

(a)
SEI 10kV WD10mm SS40 x5,000 5 μm
(b)
SEI 10kV WD10mm SS40 x5,000 5 μm
(c)
SEI 10kV WD11mm SS50 x5,000 5 μm
(d)
SEI 10kV WD11mm SS50 x5,000 5 μm
(e)
SEI 10kV WD10mm SS50 x5,000 5 μm
(f)
SEI 10kV WD11mm SS50 x5,000 5 μm
(g)
SEI 10kV WD11mm SS50 x5,000 5 μm
(h)
SEI 10kV WD11mm SS50 x5,000 5 μm
(i)
SEI 10kV WD11mm SS50 x5,000 5 μm
(j)
SEI 10kV WD9mm SS40 x5,000 5 μm
(k)
SEI 10kV WD10mm SS50 x5,000 5 μm
(l)
SEI 10kV WD11mm SS30 x5,000 5 μm

**Fig. 4.**


SC
SBC
NC
M
4ºC
10ºC
15ºC
4ºC
10ºC
15ºC
4ºC
10ºC
15ºC
20ºC
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa

**Supplementary Table 1**

Amino acid composition of collagens from the skin (SC) of beluga, sterlet, and winter and summer bester sturgeons (expressed as residues/1000 total amino acid residues).

| Amino acids | Beluga | Sterlet | Winter bester | Summer bester |
|---|---|---|---|---|
| Asp | 47±2 | 47±1 | 46±0 | 46±2 |
| Thr | 24±1 | 23±0 | 23±0 | 24±1 |
| Ser | 42±0 | 44±1 | 42±1 | 42±0 |
| Glu | 68±1 | 68±1 | 68±0 | 68±1 |
| Gly | 334±6 | 328±3 | 325±4 | 331±1 |
| Ala | 111±1 | 114±3 | 113±2 | 118±3 |
| Val | 17±1 | 17±1 | 17±0 | 16±1 |
| Met | 9±0 | 9±1 | 9±0 | 9±1 |
| Ile | 11±0 | 12±1 | 11±0 | 11±1 |
| Leu | 18±0 | 18±1 | 17±0 | 17±1 |
| Tyr | 2±1 | 2±1 | 2±0 | 2±0 |
| Phe | 12±1 | 12±1 | 12±0 | 12±0 |
| Hylys | 8±1 | 7±1 | 8±1 | 8±1 |
| Lys | 23±1 | 23±1 | 23±1 | 23±1 |
| His | 4±1 | 4±0 | 4±0 | 3±1 |
| Arg | 50±1 | 48±2 | 50±0 | 49±2 |
| HyPro | 66±5 | 66±1 | 67±5 | 64±8 |
| Pro | 110±4 | 114±3 | 115±4 | 109±8 |
| Imino acids | 177±2 | 180±3 | 183±8 | 173±2 |

Values are expressed as means ± SD.

**Supplementary Table 2**

Amino acid composition of collagens from the swim bladder (SBC) of beluga, sterlet, and winter and summer sturgeons (expressed as residues/1000 total amino acid residue.

| Amino acids | Beluga | Sterlet | Winter bester | Summer bester |
|---|---|---|---|---|
| Asp | 44±1 | 44±0 | 44±1 | 44±1 |
| Thr | 25±0 | 25±0 | 25±0 | 25±1 |
| Ser | 42±1 | 43±0 | 42±1 | 42±1 |
| Glu | 69±1 | 70±1 | 69±0 | 69±0 |
| Gly | 332±6 | 327±3 | 325±4 | 329±2 |
| Ala | 114±1 | 116±1 | 117±1 | 119±5 |
| Val | 17±0 | 17±0 | 17±0 | 17±1 |
| Met | 9±0 | 9±1 | 9±0 | 9±0 |
| Ile | 12±1 | 12±1 | 11±0 | 12±1 |
| Leu | 17±1 | 18±0 | 17±0 | 18±0 |
| Tyr | 2±0 | 2±1 | 2±0 | 2±0 |
| Phe | 12±0 | 12±1 | 11±0 | 12±0 |
| Hylys | 10±1 | 12±0 | 10±1 | 10±1 |
| Lys | 22±1 | 19±1 | 21±1 | 21±1 |
| His | 5±0 | 4±1 | 4±0 | 4±0 |
| Arg | 50±1 | 50±1 | 50±1 | 50±1 |
| HyPro | 76±1 | 82±2 | 79±4 | 74±2 |
| Pro | 100±3 | 92±3 | 98±5 | 95±2 |
| Imino acids | 176±2 | 173±4 | 176±9 | 169±4 |

Values are expressed as means ± SD.

**Supplementary Table 3**

Amino acid composition of collagens from the notochord (NC) of of beluga, sterlet, and winter and summer bester sturgeons (expressed as residues/1000 total amino acid residues).

| Amino acids | Beluga | Sterlet | Winter bester | Summer bester |
|---|---|---|---|---|
| Asp | 47±1 | 47±1 | 47±1 | 47±1 |
| Thr | 20±1 | 21±1 | 20±0 | 20±1 |
| Ser | 32±1 | 32±1 | 31±0 | 31±1 |
| Glu | 88±1 | 88±0 | 87±1 | 88±1 |
| Gly | 318±7 | 313±2 | 312±5 | 315±2 |
| Ala | 87±3 | 87±2 | 90±3 | 90±5 |
| Val | 17±1 | 18±1 | 17±1 | 17±1 |
| Met | 7±0 | 7±0 | 7±0 | 7±0 |
| Ile | 12±1 | 13±1 | 11±1 | 12±1 |
| Leu | 29±2 | 30±0 | 29±2 | 30±2 |
| Tyr | 3±0 | 3±0 | 2±0 | 3±0 |
| Phe | 12±0 | 13±1 | 12±0 | 12±0 |
| Hylys | 21±3 | 23±1 | 22±2 | 21±1 |
| Lys | 15±0 | 14±1 | 14±1 | 14±1 |
| His | 6±1 | 6±0 | 5±0 | 6±1 |
| Arg | 48±2 | 46±1 | 47±1 | 48±0 |
| HyPro | 72±1 | 71±2 | 71±7 | 66±2 |
| Pro | 115±3 | 116±3 | 117±5 | 118±4 |
| Imino acids | 188±3 | 187±4 | 187±12 | 184±5 |

Values are expressed as means ± SD.