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Physicochemical Characterization of Gelatin Extracted from European Perch (*Perca fluviatilis*) and Volga Pikeperch (*Sander volgensis*) Skins

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Abstract

The main purpose of this study was to extract and characterize gelatin from skins of fishes: European perch (*Perca fluviatilis)* and Volga pikeperch (*Sander volgensis)*, which were collected from the Volga-Caspian basin in Russia. The gelatins were prepared using an alkaline pretreatment. The gelatin yield was in the range of 10.2-13.8% on a wet weight basis, and the gel strength exceeded 184 and 193 g for samples from European perch and Volga pikeperch skins, respectively. The pH values of gelatins were 5.49 and 5.73, melting points were 24.5 and 25.5ºC and protein content was 88.6 and 91.0%, respectively. Water-holding and fat-binding capacities of gelatins were 227-235 and 439-453%, respectively. The gelatins were composed of α-chains, β-chains, and γ-chains. The apparent valine content of the two gelatins was significantly higher than those of gelatins from other fish species. The total apparent proportions of imino acids were 192 and 203 residues/1000 residues. The gelatins from European perch and Volga pikeperch skins had relatively high gel strengths, melting points, water-holding and fat-binding capacities as compared with those of other types of fish and could be recommended as potential replacements for mammalian gelatin in the food industry.

Keywords: European perch, *Perca fluviatilis*, Volga pikeperch, *Sander volgensis*, skins, gelatin.

Introduction

Gelatin is a valuable protein, recovered from collagen-containing materials such as skins and bones of fish and animals. Gelatin has a wide range of applications in the food, pharmaceutical, cosmetic, and photographic industries because of its unique physicochemical and technological properties. In particular, gelatin has been used as an emulsifier, foaming agent, colloid stabilizer, fining agent and biodegradable packaging material with foods (Gómez-Guilén, Giménez, López-Caballero, & Montero, 2011); as a matrix for implants, in injectable drug-delivery microspheres, and in intravenous infusions in the pharmaceutical field (Saddler & Horsey, 1987; Pollack, 1990; Rao, 1995); as an application for manufacturing hard and soft capsules, plasma expanders and in wound care (Ahmed, Ptaszek, & Basu, 2016). Additionally, gelatin is recommended for use in foodstuffs to enhance protein levels, especially in body-building food because it is low in calories. Furthermore, gelatin has been used to reduce the carbohydrates in foods formulated for diabetic patients (Phillips & Williams, 2009).

Nowadays, most commercial gelatin preparations are derived from beef bones, pig skins, and pig bones. Recent reports indicate that the annual world output of gelatin is nearly 326,000 tonnes, with gelatin derived from pig skin most prevalent (46%), followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%) (Bhat & Karim, 2009). Fish collagen-containing materials such as bones and skins are major by-products of the fish industry which could be sources of gelatin. These by-products constitute almost 30% of the total weight of the fish (Gómez-Guillén,Turnay, Fernádez-Díaz, Ulmo, Lizarbe, & Montero, 2002). Recently, skin gelatins from several fish species such as cod and hake (Fernádez-Díaz, Montero, & Gómez-Guillén, 2001), Nile perch (Muyonga, Cole, & Duodu, 2004), skate (Cho, Jahncke, Chin, & Eun, 2006), bigeye snapper and brownstripe red snapper (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006), Atlantic salmon (Arnesen & Gildberg, 2007), farmed giant catfish (Jongjareonrak, Rawdkuen, Chaijan, Benjakul, Osako, & Tanaka, 2010), cuttlefish (Balti, Jrihi, Yoshida, Osako, Yamaguchi, & Hara, 2011), skipjack tune, dog shark and rohu (Shyni, Hem, Ninan, Mathew, Joshy, & Lakshmanan, 2014) have been characterized. The results showed that the properties of fish gelatin are different from those of mammalian gelatin. Additionally, some physicochemical properties such as gel strength, melting point, gelling and melting temperatures, and viscosity of gelatins obtained from cold water fishes are different from those of warm water fishes. Furthermore, optimization of process parameters for gelatin extraction from many fish species has been studied (Cho et al., 2004; Silva, Lourenço, & Pena, 2017). However, no information regarding extraction and characteristics of gelatin from European perch and Volga pikeperch skins have been reported.

Fisheries in the Caspian region are an important part of the fishing industry in Russia. Astrakhan province has a significant role in the fishing industry of the Astrakhan region with about 80% of the regional catch. The aquaculture production of European perch (*Perca fluviatilis)* and Volga pikeperch *(Sander volgensis)* has increased steadily and has become economically significant. Many of these fish are filleted providing a source of skins and bones. Therefore, the aim of this study was to prepare and characterize the gelatin from skins of European perch and Volga pikeperch.

Material and Methods

Materials and Chemicals

The materials used for gelatin production were fresh fish skins of the two species obtained after machine filleting in the Astrakhan region. The fishes were collected in the lower section of the Volga River, which belongs to the Astrakhan region, in the summer of 2015. The average temperature during this season in Astrakhan is about 17-32ºC while the highest temperature may be 40ºC. The fresh skins were obtained from the Astrakhan Fishery, Co., Ltd., Astrakhan, kept in a cool chamber with temperatures between 2 and 8ºC and transported to the Department of Food Biotechnology and Technology of Foodstuffs, Astrakhan State Technical University within 2 h. Samples of skins were washed with distilled water ≤ 20ºC, placed in polyethylene bags and then stored at -18ºC until the analysis was done (the storage time was 6 months or less).

Sodium dodecyl sulfate (SDS), bisacrylamide (N,N´-methylenebisacrylamide), and acrylamide for electrophoresis were from Sigma-Aldrich (St. Louis, MO, USA). Citric acid monohydrate (granular) was from Fisher Scientific (Loughborough, UK). All other chemicals and reagents used were of analytical grade.

Preparation of Gelatin from Fish Skins

Frozen skins were thawed at 4-8ºC for 18-24 h and then thoroughly washed with warm water (about 35ºC) for 20-25 min to remove superfluous materials and reduce the fat content. Skin samples were cut into 1 × 1 cm pieces using a scissor. Preparation of gelatin was carried out according to the method of Gómez-Guillén and Montero (2001) with a minor modification. Before gelatin extraction, the skins were soaked in 0.1 M NaOH at ambient temperature (25-27ºC) for 3 h with a skin/solution ratio 1:10 (*w/v*). The alkaline solution was changed every 1 h to remove non-collagenous protein and pigments. Alkaline-pretreated skins were washed with tap water until the pH of wash water was 7.2-7.5 using a pH meter (Model S400 SevenExcellence™, Schwerzenbach, Switzerland), then the skins were washed with distilled water and pressed using the spin cycle of a washing machine (Electrolux W 3600 H, Astrakhan, Russia) to remove water.

Gelatin extraction was carried out 2 times with distilled water with gentle stirring: First at 60ºC for 3.0-3.5 h with a water/skin ratio 2:1, then at 55ºC for 2.0-2.5 h with a water/skin ratio 1:1. A 2% (*w/v*) citric acid solution was used to adjust the pH of the liquid phase to 4.3-4.5 for both extractions. The resulting extracts were centrifuged (Frontier FC 5706, Ohaus, Parsippany, NJ, USA) at 4000 g for 15-20 min. The supernatants obtained were evaporated in a vacuum concentrator (Rotavapor R-210 with Vacuum Controller-850, Vacuum Pump V-700, Heating Bath B-491, Buchi, Flawil, Switzerland) at 55-60°C and 70-72 mbar until the concentration of solids was 15-20%. The solid concentration of the gelatinous broths was measured using a refractometer (Model IRF-454 B2M, Niki MLT, Povolgye, Russia). Before drying, the concentrated gelatin was left at 2-6°C for 12 h to gelatinize. The strong gels were cut into plates with a thickness of ~0.3 cm using a knife and dried for 18-24 h in the drying apparatus (Memmert UF110, Schwabach, Germany**)** with forced air circulation at 18-22°C and relative humidity 55-60%. The gelatin granules were packed in plastic containers and kept in a refrigerator at 8-12ºC before analysis for a maximum of 12 months.

Gelatin Yield and Proximate Composition

The yield of gelatin was calculated on basis of the weight of fresh skins:

$$\% \text{ Yield (wet weight basis)} = \frac{\text{Dry weight of gelatin}}{\text{Wet weight of skin}} \times 100$$

Moisture, ash, fat and protein contents of skins and gelatins were determined according to standard methods 934.01, 942.05, 991.36 and 954.01, respectively (AOAC, 2000). A conversion factor of 5.55 was used for calculating protein from total nitrogen (Wangtueai & Noomhorm, 2009).

Physical Measurement of Gelatin: pH, Gel Strength and Melting Point

The pH values of gelatins were determined as follows: 1% (*w/v*) solutions of gelatins were prepared in distilled water at 55-60ºC with constant mechanical stirring for 30 min, cooled to room temperature (25ºC) and measured using the pH meter.

The gelatin gel strength was determined according to the method of Gómez-Guillén et al. (2002). Gelatin samples were heated with distilled water (6.67% *w/w*) at 60ºC with constant mechanical stirring (IKA® RW20, Selangor, Malaysia) for 30 min. The gelatin solutions were poured into small bottles (50 × 80 mm, flat bottom, KlinLab, Moscow, Russia), then kept at 6ºC overnight for gel maturation. The gel strength (g) was determined using the Texture Analyzer (Model TA.XT, Stable Microsystems, Surrey, UK), equipped with a load cell of 5 kg, using a 12.5 mm diameter flat-bottomed Teflon plunger pressed 4 mm into the gelatin gels at a speed of 1 mm/s.

The determination of melting point was done according to the method described by Choi and Regenstein (2000): gelatin solutions 6.67% (*w/w*) were prepared with warm distilled water, and a 5 ml aliquot of each sample was transferred to a small glass tube (12 × 75 mm). The samples were degassed in a desiccator for 5 min. The tubes were then covered with Parafilm®M (Pechiney Plastic Packaging Co., Chicago, IL, USA) and heated in a water bath (LOIP LB-140, Loip, Saint Petersburg, Russia) at 60ºC for 20 min, then cooled immediately in ice chilled water and matured at 10ºC for 18 h. Five drops of a mixture of 75% chloroform and 25% reddish-brown dye (Food color "Red Burgundy", NizhegorodChemProduct, Nizhny Novgorod, Russia) were placed on the surface of the gel. The gel samples were put in the water bath at 15ºC and the bath was heated at the rate of 0.2ºC/min. The temperature at which the dye droplets began to move freely down the gel was taken as the melting point.

Determination of Water-Holding and Fat-Binding Capacities

Water-holding and fat-binding capacities (WHC and FBC) were measured using the methods reported in Cho et al. (2004). For measuring WHC, gelatin samples (1 g) were placed in centrifuge tubes and weighed (tubes with gelatin), 50 ml distilled water was added and held at ambient temperature for 1 h. The gelatin solutions were mixed with a Vortex mixer (Model Genius 3, IKA, Selangor, Malaysia) for 5 s every 15 min, and then centrifuged at 500 g for 15 min. The upper phase was removed, and the centrifuge tube was drained on a filter paper. The WHC was calculated as the weight of the content of the tube after draining divided by the weight of the dried gelatin and expressed as the % weight of dried gelatin.

FBC was determined similarly. Instead of 50 ml water, 10 ml sunflower oil (Donskoy Yantar, Rostov, Russia) was used (Cho et al., 2004).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was done according to Laemmli (1970). For polyacrylamide gels, 5% stacking gel and 6% separating gel were used. Gelatin solutions (10 mg/mL) and tracking dye mixtures containing 0.5 M Tris-HCl buffer (pH 6.8), 5% 2-mercaptoethanol, 20% glycerol, and 0.1% bromophenol blue were heated at 100°C for 5 min. After heating and cooling, the solutions were injected into the wells in the stacking gel, and electrophoresis was done at 15 mA/gel with a Mini-Protean 3 (Bio-Rad Laboratories, Hercules, CA, USA). Calf skin collagen (Sigma-Aldrich) was used as the marker protein. The calf skin collagen was composed of α1, α2 and β-chains with molecular weights of 95, 100 and 200 kDa, respectively.

Determination of Amino Acid Composition

The amino acid analysis was done by Ms. Kostyanaya M.I. at the Research Center for Animal Feeds and Food ANO NTC "Kombikorm" (Voronezh, Russia). All samples of gelatin were hydrolyzed using 6 M HCl at 110ºC for 24 h in the absence of oxygen (Morimura, Nagata, Uemura, Fahmi, Shigematsu, & Kida, 2002). The amino acid composition of gelatin hydrolysates was analyzed using an automated amino acid analyzer (Model HITACHI 835-50 Amino Acid Analyser, Tokyo, Japan). The approximate amounts of the amino acid residues were determined by summing up the peak areas of the 20 peaks (using the software with the equipment) (denominator) and dividing that into the peak area of the individual peaks. This methodology has a number of limitations and thus, the results should only be seen as an estimate of the actual amino acid composition. Further work doing more careful amino acid analysis would be needed to obtain more reliable data (Regenstein et al., 1984).

Statistical Analysis

All experiments were carried out in triplicate. Mean values with standard deviations (SD) are reported. Statistical analyses were done using Statistical Package for Social Science, SPSS Version 17.0 for Windows (SPSS Inc., Chicago, IL, USA), and the value of $P < 0.05$ was used to indicate significant differences. Mean comparisons were done using a T-test.

Results and Discussions

Proximate Composition of Fish Skins

The proximate composition of European perch and Volga pikeperch skins is shown in Table 1. The protein content of raw materials provides a suggestion of the potential gelatin yield. The Volga pikeperch skin had significantly more protein than the European perch ($P < 0.05$), similar to kumakuma skins (31%) (Silva et al., 2017), which is higher than other fish species such as Nile perch (20-22%) (Muyonga et al., 2004), rohu (19%), tuna (21%) (Shyni et al., 2014), blue whiting (18%) (Khiari, Rico, Martin-Diana, & Barry-Ryan, 2015), Tunisia cuttlefish (14%) (Balti et al., 2011), and India cuttlefish (16%) (Ninan, Zynudheen, John, Binsi, & Joshy, 2015). Additionally, the ash and fat contents of these fish skins were lower than those of other fish species such as Nile perch (5.0-6.8% fat and 3.7-6.0% ash) (Muyonga et al., 2004), skipjack tuna (18% fat and 4.4% ash), rohu (2.9% fat and 2.0% ash) (Shyni et al., 2014), cobia (7.4% fat and 2.6% ash), and croaker (3.9% fat and 1.9% ash) (Silva, Bandeira, & Pinto, 2014). The skins' low lipid and ash contents suggested that lipid-removal and demineralization prior to collagen extraction should not be necessary.

Yield of Gelatins

The yields of gelatin were significantly different ($P < 0.05$). These values were much higher than those for gelatin from red tilapia (7.8%), black tilapia (5.4%) (Jamilah & Harvinder, 2002), cuttlefish (2.2-7.8%) (Balti et al., 2011), cultured Amur sturgeon (9.4-12.5%) (Nikoo, Benjakul, Ocen, Yang, Xu, Zhang, & Xu, 2013) and blue whiting (1.5-2.4%) (Khiari et al., 2015). However, the yields were lower than for shark (17%) and tuna skin gelatins (20%) (Shyni et al., 2014). The differences in gelatin yields may depend on collagen extraction parameters and the gelatin content of the raw materials (Jongjareonrak et al., 2010; Koli, Basu, Nayak, Kannuchamy, & Gudipati, 2011) along with the amount of impurity in the preparations that has generally not been accounted for. As the two gelatins were prepared using the same methodology, it is presumed that the different yields were due to differences in the initial gelatin in the skins and the reaction of the skins to the preparation procedures.

Proximate Composition of Gelatins

The proximate composition of gelatins from European perch and Volga pikeperch skins is shown in Table 2. The gelatins were mostly protein and the low ash and fat suggested that the decision not use an extra step was justified. According to Russian National Standards (GOST 11293-89), the maximum ash content of edible gelatin is 2% which was easily met by both gelatins. These were similar to the protein in gelatins from young Nile perch skin and bone was 89 and 83%, respectively (Muyonga et al., 2004), from bigeye snapper 88% (Jongjareonrak et al., 2006), from cuttlefish 91% (Balti et al., 2011), from triggerfish 90%, from skipjack tuna 88%, from dog shark 90% and from rohu 89% (Shyni et al., 2014). The above values were much higher than those from sin croaker

gelatin 69%, from shortfin scad 69% (Cheow, Norizah, Kyaw, & Howell, 2007) and from kumakuma gelatin 73% (Silva et al., 2017). These variations are probably most due to differences in the method of preparation (Gómez-Guillén et al., 2011).

The pH Value of Gelatins

The pH values of 1% (*w/v*) gelatin solutions were a bit higher than for other fish gelatin preparations. A wide variation of the pH values have been observed: shark (4.3), rohu (4.2), tuna (4.3) (Shyni et al., 2014), cuttlefish (5.5-6.1) (Ninan et al., 2015), red tilapia (3.1), black tilapia (3.9) (Jamilah & Harvinder, 2002), catfish (3.7), common carp (3.6) (Mostafa, Shaltout, Abdallah, & Osheba, 2015), panga catfish (5.8), Asian redtail catfish (5.9), Nile tilapia (5.7) and striped snakehead (5.8) (Ratnasari, Yuwono, Nusyam, & Widjanarko, 2013). The differences in the pH values probably reflect the differences in pretreatments (including the neutralization step) used during the extraction involving both alkaline and acid treatments (Shyni et al., 2014). In this study extraction was carried out in the pH range of 4.3-4.5.

The Gel Strength and Melting Point of Gelatins

Typically, gelatins from fish have lower gel strengths than those of mammals (Gilsenan & Ross-Murphy, 2000; Jellouli, Baltim, Bougated, Hmidet, Barkia, & Nasri, 2011). As shown in Table 2, the gel strength after overnight maturation at 6ºC was higher ($P < 0.05$) for Volga pikeperch. Both values were higher than those of gelatins from many fish species previously reported although differences in method may have a major impact: red tilapia (128 g), black tilapia (181 g) (Jamilah & Harvinder, 2002), cod (71 g), Atlantic salmon (108 g) (Arsenen & Gilberg, 2007), sin croaker (125 g), shortfin scad (177 g) (Cheow et al., 2007), cuttlefish (181 g) (Balti et al., 2011), grey triggerfish (168 g) (Jellouli et al., 2011), rohu (124 g), tuna (177 g) (Shyni et al., 2014), tuna fin (126 g) (Aewsiri, Benjakul, Visessanguan, & Tanaka, 2008) and common carp (185 g) (Mostafa et al., 2015). However, both gel strengths were lower than from shark skins (206 g) (Shyni et al., 2014), rohu skins (258 g) and mrigal skins (343 g) (Madhamuthanalli & Bangalore, 2014). The differences in gel strength, if real, between species were possibly due to difference in amino acid content, especially proline and hydroxyproline (Gómez-Guillén et al., 2011). Other factors that could affect the gel strength are the molecular weight distribution and the type of extraction treatments (Balti et al., 2011).

Like gel strength, the melting point is also an indicator of gelatin quality. The lower melting point ($P < 0.05$) was observed with European perch. These values were similar to those of gelatins from red tilapia (22.5ºC) (Jamilah & Harvinder, 2002), shark (25.8ºC), tuna (24.2ºC) (Shyni et al., 2014), pacu (23.7-24.8ºC) (Sahoo, Dhanapal, Reddy, Balasubramanian, & Sravani, 2015). The melting points observed in this study are higher than those reported for cold water fishes such as hake (14ºC), sole (19.4ºC), mergim (18.8ºC), grass carp (19.5ºC) (Gómez-Guillén et al., 2002), cole skin (8-10ºC) (Gudmundsson & Hafsteinsson, 1997). The difference in melting point could be related to fish species, amino acid composition, and fishing season along with the effect of the preparation.

The Water Holding and Fat-Binding Capacities of Gelatins

Water-holding and fat-binding capacities can be used to begin to examine gelatin's applicability in food product formulation. As shown in Table 2, the WHC values were similar to that of gelatin from shark skin (256%) and higher than those reported for rohu (163%) and tuna (214%) (Shyni et al., 2014).

Fat-binding capacities were higher than those of gelatins from shark (347%) and rohu (360%) (Shyni et al., 2014). The degree of exposure of the hydrophobic residues and the relatively high estimates of tyrosine, leucine, valine and isoleucine, which were similar for both gelatins, probably helped give the high FBC (Cho et al., 2004) (Table 2). The relatively high water-holding and fat-binding capacities indicated that these gelatins have a potential to be used by the food industry.

Molecular Weight Distribution of Gelatins

According to Sims, Bailey, and Field (1997), gelatin is composed of α-chains, β-chains (covalently liked α-chain dimers), and higher molecular weight polymers including γ components (α-chain trimmers) and some lower molecular weight fragments. The SDS-PAGE patterns of the gelatins are shown in Figure 1. The calf skin collagen was used as the maker for the molecular weight of the gelatin. Three major protein bands were found in all the gelatins, suggesting α1, α2 and β-chains. These are similar to the patterns founds with other fish gelatins. The α2 chain of gelatins from European perch and Volga pikeperch skins was less clear than that of calf skin collagen. In addition, the proteins with high molecular weight including the γ component and some fragments between α2 and β-chains were observed. Shyni et al. (2014) also reported the presence of peptides with molecular weights below 100 kDa in gelatins from shark, rohu and tuna skins. This may be explained in part as the degradation of α, β and/or γ components during gelatin preparation although it may also represent impurities.

Estimated Amino Acid Composition of Gelatins

The estimated amino acid composition of gelatin from European perch and Volga pikeperch skins is shown in Table 3. Tryptophan cannot be detected after acid hydrolysis and cysteine was not detected in either gelatin. Glycine, the most abundant amino acid found in gelatin, accounted for ~23% of the total amino acids, which suggests potential non-gelatin impurities. The percentage of glycine was lower than those of gelatins from kumakuma (25%) (Silva et al., 2017), grey triggerfish (29%) (Jellouli et al., 2011) and carp (33%) (Duan, Zhang, Xing, Konno, & Xu, 2011), but higher than those of gelatins from cornet fish (21%) (Nazeer & Deepthi, 2013), lizardfish scale (18%) (Wangtueai & Noomhorm, 2009) and jellyfish (19%) (Cho, Ahn, Koo, & Kim, 2014).

The proportion of essential amino acids of two gelatins were similar to those of cornet fish gelatin (22%) (Nazeer & Deepthi, 2013), kumakuma gelatin (25%) (Silva et al., 2017), and higher than those of gelatins from carp (13%) (Duan et al., 2011), cuttlefish (14%) (Balti et al., 2011), grey triggerfish (17%), cobia (13%) and croaker (15%) (Silva et al., 2014). The higher content of essential amino acids was mainly due to the higher content of valine while the others were similar to the values of other fish. The valine content of gelatins were much higher than those from grey triggerfish (3%) (Jellouli et al., 2011), cuttlefish (2%) (Balti et al., 2011), carp (2%) (Duan et al., 2011), cobia (2%), croaker (2%) (Silva et al., 2014) and kumakuma (2%) (Silva et al., 2017).

It is known that the thermal stability of collagen is affected by the content of the imino acids, and the proportion of these amino acids is related to the habitat temperature of the fish (Kimura, Zhu, Matsui, Shijoh, & Takamizawa, 1988). The proportion of imino acids of gelatins were higher than those of gelatins from cuttlefish (18%) (Balti et al., 2011), rohu (18%), tuna (18%) (Shyni et al., 2014), croaker (19%) (Silva et al., 2014), tilapia (17%) (Wu, Tsai, Chen, & Sung, 2014) and carp (19%) (Duan et al., 2011); similar to those in gelatin from seabass (20%) (Sinthusamran, Benjakul, & Kishimura, 2014), corbia (21%) (Silva et al., 2014) and shark (20%) (Shyni et al., 2014) and lower than the 36% for tuna fin gelatin (Aewsiri et al., 2008). The hydroxyproline content was lower

than the values of gelatin from shark (10%), tuna (10%) (Shyni et al., 2014) and cobia (9%) (Silva et al., 2014), and higher than those of gelatins from rohu (7%) (Shyni et al., 2014), tilapia (5%), tuna (7%) (Aewrisi et al., 2008), seabass (8%) (Sae-leaw, Benjakul, Nora, & Kideki, 2016) and carp (7%) (Duan et al., 2011). In general, collagen extracted from fish living in cold water has a lower imino acid content than that from fish living in a warm environment (Bae, Osatomi, Yoshida, Osako, Yamaguchi, & Hara, 2008).

The higher temperature in Astrakhan is thought to be the reason for the higher content of imino acids in gelatin from European perch and Volga pikeperch skins in comparison with some other fish species.

Conclusions

The gelatins from the two fish skins had relatively high contents of imino acids, gel strength, melting point, water-holding and fat-binding capacities in comparison with those of some other fish species. The gelatins met the standards for edible gelatin according to the Russian National Standards (GOST 1193-89). Based on the chemical composition and physicochemical characterization, gelatins from European perch and Volga pikeperch skins may be usable by the food industry as a source of gelatin.

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Table 1. Proximate composition of European perch and Volga pikeperch skin

Composition (g/100 g wet weight)	European perch skin	Volga pikeperch skin
Moisture	73 ± 1^{a}	66 ± 1^{b}
Protein	24 ± 2^{a}	31.4 ± 0.2^{b}
Fat	1.6 ± 0.05^{a}	1.1 ± 0.04^{b}
Ash	1.6 ± 0.2^{a}	1.9 ± 0.2^{a}

Results are mean values of three replicates ± SD

[a, b] Different superscripts in the same row show significant differences between the samples ($P < 0.05$).

Table 2. Proximate composition and physicochemical characterization of fish skin gelatin

Properties	Sample of gelatins		
	European perch	Volga pikeperch	RNS* for edible gelatin
Protein, g/100g	88.6 ± 0.4[a]	91.0 ± 0.4[b]	NR
Moisture, g100g	10.8 ± 0.2[a]	8.2 ± 0.2[b]	Not higher than 16.0
Fat, g/100g	0.2 ± 0.01[a]	0.2 ± 0.01[b]	NR
Ash, g/100g	0.4 ± 0.03[a]	0.6 ± 0.02[b]	Not higher than 2.0
pH, 1% (*w/v*) solution	5.49 ± 0.03[a]	5.73 ± 0.02[b]	5.0-7.0
Melting point, ºC	24.5 ± 0.1[a]	25.5 ± 0.2[b]	27-32ºC[A]
Gel strength, g	184 ± 2[a]	193 ± 2[b]	700-1300N[B]
WHC, %	227 ± 3[a]	235 ± 4[a]	NR
FBC, %	453 ± 4[a]	439 ± 4[b]	NR

Results are mean values of three replicates ± SD

[a,b] Different superscripts in the same row show significant differences between the samples ($P < 0.05$)

* RNS: Russian National Standard for edible gelatin (GOST 11293-89)

NR: Not required

[A, B] Values for gel solution at 10% (*w/v*)

Table 3. Estimated amino acid composition of European perch and Volga pikeperch skin gelatins

Amino acid	Number of residues/1000	
	European perch skin gelatin	Volga pikeperch skin gelatin
Hydroxyproline	83	89
Aspartic acid	65	69
Threonine[e]	27	25
Serine	35	31
Glutamic acid	98	101
Proline	109	114
Glycine	227	230
Alanine	100	93
Cysteine	ND	ND
Valine[e]	96	99
Methionine[e]	8	6
Isoleucine[e]	12	10
Tryptophan[e]	ND	ND
Leucine[e]	25	27
Tyrosine	7	5
Phenylalanine[e]	19	18
Hydroxylysine	11	9
Histidine	5	6
Arginine	27	24
Lysine[e]	46	44
Total	1000	1000
TEAA	233	229
TIA	192	203

The amino acid composition was done in triplicate and data correspond to mean values. Standard deviations were in all cases lower than 3%. TEAA: total essential amino acids. TIA: total imino acids (proline and hydroxyproline). [e]Essential amino acid. ND: not detected.

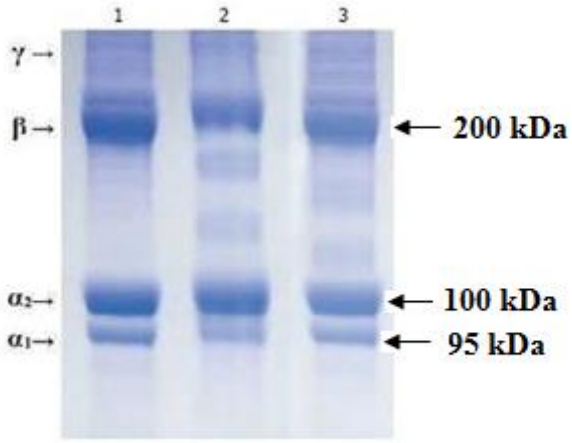

Figure 1. SDS-PAGE pattern of gelatin extracted from European perch and Volga pikeperch skins: lane 1: the protein maker (calf skin collagen); lane 2: European perch skin gelatin; and lane 3: Volga pikeperch skin gelatin.