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Thermal characteristics of gelatin extracted from shaari fish skin

Effects of extraction conditions

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Abstract Gelatin extraction yield increased with the increase of acetic acid concentration and temperature. Gelatin extracted from shaari skin using 0.1 N acid solutions and temperatures of 323 and 353 K gave highest protein content comparable to that of commercial bovine and porcine gelatins. In general, gelatin extracted from shaari gelatin showed lower onset of glass transition temperature than mammalian gelatins. For shaari skin gelatin, the onset of glass transition temperature decreased with the increase of extraction temperature up to 323 K and then remained nearly constant. The decrease in glass transition was more pronounced for gelatin extracted at 0.01 N compared to the 0.1 and 1.0 N samples. Unfolding temperature decreased exponentially with the increase of extraction temperature. The unfolding temperature shifted to lower temperature, and the decrease was more pronounced in the case of higher (1.0 N) concentrated samples. The extraction concentration and temperature did not show significant effect on the onset solids-melting temperature.

Keywords Fish skin gelatin · Glass transition · Solids-melting · Shaari · Amino acid

List of symbols

T_{gi}	Onset temperature of glass transition (K)
T_{gp}	Peak temperature of glass transition (K)
T_{ge}	End temperature of glass transition (K)
T_{mi}	Onset temperature of solids-melting (K)
T_{mm}	Maximum slope temperature of solids-melting (K)
T_{mp}	Peak temperature of solids-melting (K)
T_{me}	End temperature of solids-melting (K)
T_{ui}	Onset unfolding temperature (K)
T_{um}	Maximum slope of unfolding temperature (K)
T_{up}	Peak of unfolding temperature (K)
X_w	Moisture content (g/100 g sample)
ΔC_p	Change of specific heat (J/kg K)
ΔH_u	Enthalpy change for unfolding (kJ/kg)
ΔH_m	Enthalpy change for solids-melting (kJ/kg)

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Introduction

Gelatin is a biopolymer that has very broad applications in the food, pharmaceutical, and photographic industries. In the food industry gelatin is widely used as a gelling agent. Skin and bone from bovine (beef source) and porcine (pork source) have usually been utilized commercially in gelatin production. In recent years, however, fish gelatin has gained importance as the demand for non-bovine and non-porcine gelatin is increasing due to the bovine spongiform encephalopathy (BSE) crisis and for religious and social reasons. Karim and Bhat [1] reviewed different sources of gelatin and found that fish skin could be one of the alternatives for mammalian gelatin. In addition, fish skin is a major by-product of the fish processing industry, causing wastage and pollution. This waste could be used to develop the value-added by-products and it could contribute to solve the problem of waste disposal.

The quality of a gelatin for a particular application depends largely on its structural properties, as well as its physico-chemical properties that are greatly influenced not

only by the species or tissue from which it is extracted, but also by the method of pretreatment and extraction [2]. Collagen, the parental form of gelatin, is abundant in the skins and bones of animals and fish. The extraction of gelatin from collagen involves several steps such as alkali and/or acid pretreatments for collagen hydrolysis, followed by the main extraction in water above 318 K [2, 3]. Two types of gelatin are obtained from selected treatments: acid pretreatment yields Type A gelatin, whereas alkaline pretreatment yields Type B gelatin [4, 5]. Mild acid treatment has been used to disrupt acid-labile cross-links with negligible peptide bond hydrolysis and amino acid degradation [6].

The major challenges in utilizing fish gelatins are the dark color, strong fishy odor, and poor gel and/or film properties [2, 7, 8]. Zhang et al. [9] found significant variations on the molecular weight profile, color, tensile strength, elongation, and water barrier property of the fabricated catfish gelatin film extracted by varying six pretreatment methods considering 0.09 M acetic acid and/or 0.25 M sodium hydroxide at 277 K for 1 h followed by extraction in de-ionized water at 323 K for 3 or 16 h. Yang et al. [10] studied the instrumental textural characteristics and nano-structure of gelatin from cat fish skin as a function of pretreatment with sodium hydroxide (0–1 M for 0–1.5 h) and acetic acid (0.1 M for 0–3 h). They did not observe any specific increasing or decreasing trends in the gel strength and hardness as a function of pretreatment time when acetic acid (0.1 M) was used, whereas increasing sodium hydroxide showed decreasing trends. Zhou and Regenstein [11] optimized the extraction process for gelatin from Pollock skin considering pretreatment temperature, time, and pH; extraction temperature and time; and skin/water ratio. The optimization parameters were gelatin yield, gel strength, and viscosity.

Structural attributes of gelatin are related to its thermal characteristics, such as glass transition, freezing point, thermal unfolding, solids-melting, and decomposition. Thermal analysis determines different phases and states of foods as a function of water content and temperature [12, 13]. These characteristics could be visualized clearly in the state diagram when characteristic temperatures are plotted as a function of solids content [14]. D'Cruzand and Bell [15] measured the glass transition, thermal unfolding, and solids-melting of porcine gelatin as a function of water content for high solids (i.e., gelatin containing unfreezable water). Similarly glass transition and solids-melting of mammalian gelatin at limited moisture levels were also reported in the literature [16–20]. Rahman et al. [21] determined the glass transition, unfolding, solids-melting, and decomposition temperature of gelatin from mammalian and fish skin sources at three levels of moisture content. At present there is limited information available in the literature for the thermal characteristics of gelatin from fish skin. There is limited research conducted to determine the effects of temperature and concentration of acid solution used for the extraction step. The objective of this study was to determine the effects of acid concentration and temperature of extraction solution on the physico-chemical characteristics and thermal characteristics (glass transition, unfolding, and solids-melting) of shaari gelatin.

Materials and methods

Raw materials

In the month of August 2008, one batch of emperor (Arabic Shaari) (*Lethrinus microdon*) skin (age $\approx$ 6 months; 60–80 cm in length) was collected from local super market in Muscat. The skin was stored at 233 K until used for the extraction and further analysis.

Sample preparation

Commercial gelatins were bought from Sigma, Saint Louis, MO, USA (porcine powder: catalog number G 2500 and bovine powder: catalog number: G 9382). Frozen skin was thawed at room temperature for about 1 h and then the attached flesh was removed by scratching with a knife and de-scaled. Skin was washed with running tap water and then it was divided into three batches. The first batch was used for gelatin extraction. Second batch of the skin was dried in a convection oven at 353 K for 18 h. The third batch was dried in a desiccator containing silica gel at 293 K. Both dried skin was ground into powder by a hammer mill with sieve size 1.0 mm (Model MF 10 Basic, IKA Works, USA).

Extraction procedure was carried out similarly as previously described by Gómez-Guillén and Montero [22]. Clean and de-scaled skin was washed with running tap water and dipped in sodium chloride solution of 0.5 M for 5 min at 278 K. Glass rod was used manually to stir the skin dipped in sodium chloride solution. Skin was then washed with tap water three times before treating with sodium hydroxide of 0.1 N. The sample in a beaker with sodium hydroxide was placed on a magnetic stirrer for 40 min at room temperature of 293 K. It was then washed three times with distilled water with gentle shake and placed in acetic acid solution for extraction with agitation on a hot plate for 18 h. The concentration and temperature of acetic acid solution were 0.01, 0.1, and 1 N, and 277, 293, 323, and 353 K, respectively. The solution–skin mass ratio for extraction was used as 6:1. Gelatin extracted in the above-mentioned solution was separated by using two-layer filter cloth. The solution was then dried in an oven

initially at 333 K for 24 h and then dried by storing it in a desiccator containing silica gel at 293 K for 2 days. The dried sample was ground in a hammer mill using sieve of 1.0 mm screen (MF 10 basic from IKA Works, Inc, USA). The following abbreviations are used to describe the different types of gelatins and conditions of extraction.

BG	Commercial bovine gelatin
HTHC	Gelatin extracted from shaari skin at 353 K, 1 N
HTLC	Gelatin extracted from shaari skin at 353 K, 0.01 N
HTMC	Gelatin extracted from shaari skin at 353 K, 0.1 N
LTHC	Gelatin extracted from shaari skin at 277 K, 1 N
LTLC	Gelatin extracted from shaari skin at 277 K, 0.01 N
LTMC	Gelatin extracted from shaari skin at 277 K, 0.1 N
MTHC	Gelatin extracted from shaari skin at 293 K, 1 N
MTLC	Gelatin extracted from shaari skin at 293 K, 0.01 N
MTMC	Gelatin extracted from shaari skin at 293 K, 0.1 N
PG	Commercial porcine gelatin
SDO	Shaari skin dried in oven at 343 K
UTHC	Gelatin extracted from shaari skin at 323 K, 1 N
UTLC	Gelatin extracted from shaari skin at 323 K, 0.01 N
UTMC	Gelatin extracted from shaari skin at 323 K, 0.1 N
SDS	Shaari skin dried in silica gel

The first set of powder samples (extracted gelatin, air-dried skin, and silica gel-dried skin) were equilibrated in desiccator maintained at 11.3% relative humidity environment by placing saturated lithium chloride solution (293 K). The equilibration time was around 4 weeks. The equilibrated samples were stored in air tight glass bottle at 253 K. The moisture contents of all equilibrated samples were determined by drying 2 g of samples in a mechanical convection oven for 24 h at 378 K [23].

This equilibration process of the first set of powder samples showed different hygroscopic characteristics with varied moisture content after equilibration. This could pause difficulty to compare the characteristics of gelatin from different sources, thus all equilibrated samples' moisture content was raised to 16.0 g/100 g sample by placing it in a desiccator with water at the bottom until it reached a predetermined weight. The predetermined weight was checked time to time by taking out the sample and recording its weight. These equilibrated samples were considered as second set of powder samples. All samples containing water 16.0 g/100 g sample were stored in air tight glass bottles at 253 K until used for analysis.

Yield of extracted solids

The yield of solids extracted was estimated from the total solids in the fresh skin before extraction and the solids dissolved in the extracted solution. It was expressed as g/100 g solids in fresh skin.

Chemical composition and pH

Moisture, crude protein, crude lipid, and ash contents of the extracted gelatin derived from shaari skin were determined in triplicate using AOAC methods [23]. All values were calculated as a g/100 g sample.

Amino acid analysis

The protein samples and individual amino acids were hydrolyzed, derivatized, and determined by procedure as described in the Pico-Tag Operator's Manual [24]. Protein samples (50–100 mg) were placed in screw-capped tubes and mixed with 5 ml of 6 N HCl (with 1% phenol v/v). The mixture was incubated at 383 K for 24 h in an oven for hydrolysis. After cooling, samples were diluted (1:10) with Milli-Q water and filtered through Waterman filter paper No. 4.

Twenty-five microliter of the protein hydrolysate solution (or amino acid standard) was dried in speed vacuum system at 303 K for 10 min. Samples and standards were then re-dissolved in 20 μL of a methanol–water–triethylamine (TEA) (2:2:1) solution and re-dried for 15 min. To each sample, 20 μL of a methanol–water–triethylamine-phenylisothiocyanate (PITC) solution was added, and then the tubes were vortexed and allowed to stand at room temperature for 20 min and dried again. Samples were re-reconstituted in 200 μL of Pico-Tag sample with water for free amino acid analysis.

The HPLC analysis was carried on an Agilent 1100 system, equipped with a Pico-Tag amino acid analysis column (150 mm × 3.9 mm i.d., 4 μm) and a Diode Array detector Agilent DAD 1100 (Agilent, USA). The column was mounted in a column compartment and operated at 311 K. The injection volume was 10 μL and the detection wavelength was set at 254 nm. The procedure of data collection and data integration were performed using Chemstation software from Agilent run on a P700 Compaq personal computer. A binary gradient solvent system was used for elution.

Differential scanning calorimetry (DSC)

Unfolding, glass transition, solids-melting, and decomposition temperatures of gelatin samples at different moisture content were measured by differential scanning calorimetry

(DSC Q10, TA Instruments, New Castle, DE, USA). Mechanical refrigerated cooling system was used to cool the sample up to 183 K. The instrument was calibrated for heat flow and temperature using distilled water (m.p. 273 K; ΔH_m 334 J/g), and indium (m.p. 429.5 K; ΔH_m 28.5 J/g). Aluminum pan of 30 mL, which could be sealed with lid, was used in all experiments with an empty sealed pan as reference. Nitrogen at a flow rate of 50 mL/min was used as a carrier gas.

Samples of 10 mg placed in a sealed aluminum pan were cooled to 183 K at 5 K/min, and kept for 10 min. It was then scanned from 183 to 623 or 523 K at a rate of 10 K/min. The glass transition (shift in the curve line), thermal unfolding (endothermic peak after glass transition), solids-melting (endothermic peak after unfolding), and decomposition (exothermic and endothermic peaks) characteristics were determined from the curve. Glass transition was analyzed for the onset, mid, and end points from the shift in the curve line. Thermal unfolding and melting peaks were characterized from initial, maximum slope and peak and enthalpy from the area of the endothermic peak. Average and standard deviation of 5–6 replicates were obtained for each experiment.

Results and discussion

Table 1 shows chemical composition, and amino acid profile of extracted fish skin and commercial sources gelatin when equilibrated at 11.3% relative humidity. The extraction yield of gelatin from raw skin varied from 1.2 to 32.1 g/100 g dry solids in the skin. Yields of extraction were 26.4 for grouper skin [25], 57.8 g/100 g dry solids for tuna [21], 13.0 g/100 g dry solids for young and adult Nile perch [26], 7.8 g/100 g dry solids for tilapia [27], 8.3 sole, 7.4, 7.2, and 6.5 g gelatin/100 g clean skin for, sole, megrin, cod, and hake, respectively [28]. The yield of extraction varied with the extraction conditions as well as the pretreatments used. However, none of the works did study the effect of concentration and temperature on the extraction process.

Results of this study showed that in general, gelatin extraction yield increased with the increase of acetic acid concentration and extraction temperature. Yield reflects the amount of gelatin that could be extracted from the collagen and is dependent on the solubility of collagen. Very low collagen solubility, even after heat-denaturing at 353 K or even 363 K has been reported for some marine fish species

Table 1 Yield, chemical composition, and amino acid of gelatin from different sources (at water activity 0.113)

Sample	Chemical composition					Amino acid composition			
	Moisture[a]	Protein[a]	Lipid[a]	Ash[a]	Yield[b]	Amino acid[a]	Porcine	Bovine	Fish skin
LTLC	10.40f (0.27)	64.67f (2.52)	4.23b (0.59)	15.47a (4.42)	1.6	Alanine	11.32a (1.00)	11.64ab (1.60)	13.30b (1.98)
MTLC	11.80d (0.46)	82.00bcde (2.00)	3.73b (0.25)	3.59cde (0.02)	1.2	Arginine	6.56a (0.04)	5.29ab (0.77)	5.31b (0.33)
UTLC	8.20i (0.03)	87.00abc (2.65)	2.27 cd (0.85)	3.84cde (0.04)	23.9	Aspartic acid	2.83a (0.94)	3.35a (0.40)	3.59a (0.62)
HTLC	8.90h (0.03)	82.33bcde (2.08)	2.20d (0.87)	4.60 cd (0.05)	22.7	Cysteine	0.04a (0.02)	0.06a (0.00)	0.37a (0.61)
LTMC	7.60j (0.08)	80.67cde (2.08)	4.03b (0.15)	8.12b (0.28)	9.5	Glutamic acid	5.41a (1.29)	6.24a (0.06)	6.12a (0.46)
MTMC	6.40l (0.12)	85.33abcd (2.08)	2.93d (0.32)	4.88bcd (0.33)	18.2	Glycine	33.87b (0.84)	33.68b (0.17)	36.39a (0.20)
UTMC	6.60kl (0.07)	89.00a (2.00)	2.50d (0.20)	3.84cde (0.07)	23.1	Histidine	0.57a (0.07)	0.55a (0.10)	0.58a (0.08)
HTMC	7.00k (0.06)	87.67ab (2.52)	2.63d (0.35)	4.02cde (0.24)	32.1	Hydroxyproline	10.47a (0.58)	10.41a (0.31)	7.46b (0.33)
LTHC	11.70d (0.09)	81.33bcde (2.31)	3.67bc (0.38)	4.96bc (0.05)	19.0	Isoleucine	1.75a (0.31)	1.31ab (0.54)	0.67b (0.09)
MTHC	15.90a (0.11)	77.00cde (2.64)	1.97e (0.50)	5.55bc (0.50)	19.4	Leucine	0.99a (0.37)	0.67a (0.95)	1.36a (1.79)
UTHC	14.30b (0.14)	79.67de (1.53)	2.00e (0.56)	4.27cde (0.04)	24.2	Lysine	4.54a (0.43)	5.49a (0.37)	4.10a (1.37)
HTHC	13.60c (0.03)	81.33bcde (1.53)	1.43e (0.93)	5.59bc (0.12)	28.6	Methionine	0.56a (0.19)	0.26a (0.32)	0.25a (0.42)
SDS	11.10e (0.25)	80.00de (3.00)	5.97a (4.23)	0.92e (0.85)		Phenylalanine	0.68a (0.40)	0.70a (0.43)	1.18a (0.95)
SDO	7.70ij (0.04)	81.00bcde (1.53)	8.30a (0.17)	2.64cde (0.18)		Proline	14.46a (1.69)	14.11a (0.98)	9.27b (0.37)
BG	9.70g (0.02)	86.67abc (0.58)	1.10e (0.2)	4.20de (0.26)		Serine	3.44a (0.52)	3.56a (0.09)	3.49a (0.26)
PG	10.00fg (0.23)	89.00a (1.00)	1.13e (0.12)	1.44de (0.39)		Threonine	–	–	1.98 (0.17)
						Tyrosine	0.73a (0.14)	0.48a (0.14)	0.61a (0.18)
						Valine	1.77a (0.29)	1.61a (0.30)	1.58a (0.15)
						Imino acids (Pro + Hyp)	24.93	24.52	16.73

Note: Values in a column followed by the same letter are not significantly different ($p < 0.05$). Values in the parentheses are standard deviations

[a] Composition (g/100 g sample)

[b] g/100 g dry solids of skin

and other invertebrates [28, 29]. Peterson and Yates [30] noticed that an appropriate digestion of the raw collagen with proteases should be necessary to improve the final yield of a highly cross-linked collagen. The conversion of collagen to gelatin involves the breakage of hydrogen bonds by raising the temperature [31]; this destabilizes the triple helix by means of a helix-to-coil transition, leading to conversion into soluble gelatin [32]. Denaturation can be defined as a transition from the triple helix to a randomly coiled form due to many factors such as heat, acid, alcohol, and other agents [33]. The denaturation temperature for mammalian collagen is in the range 308–313 K [34]. Although the denaturation temperature for cold water fish collagen is much lower, the extraction temperatures for mammalian gelatins have been adopted for the extraction of cold water fish gelatins [26, 35, 36]. In this study acetic acid at various concentrations coupled with temperatures in the range 277–353 K was used in order to convert collagen into gelatin. Results showed clearly that the higher the temperature used the higher the yield of gelatin obtained, for all acetic acid concentrations used.

The protein contents of PG and BG were 89.0 and 86.7 g/100 g sample, while protein content for shaari fish varied from 64.7 to 89.0 g/100 g sample depending on the conditions of extraction. The highest protein contents were obtained with samples extracted using 0.1 N acid solutions and temperatures of 323 and 353 K (UTMC and HTMC, respectively). Fish gelatin extracted using a 0.01 N acid solution and 277 K (LTLC) had the lowest protein content, this probably due to the incomplete conversion of collagen into gelatin as it is also reflected by the low yield obtained. Conversion of collagen into gelatin involved the breakage of hydrogen bonds by raising the temperature [31]. Fat content was in general higher in fish gelatins than in mammalian gelatins. Ash content was significantly ($p < 0.05$) lower in porcine gelatin as compared to bovine and fish gelatins which exhibited comparable values. It was, however, considerably high in shaari skin gelatin extracted using 0.01 N acid solution and 277 K (LTLC).

Table 1 shows the amino acid profile of gelatin extracted from shaari fish as compared with commercial mammalian gelatins. Significant differences in the content of Ala, Gly, Hyp, Ile, Pro, and Thr between shaari gelatin and the two mammalian gelatins can be observed ($p < 0.05$). Shaari gelatin contained significantly higher content of the amino acids alanine, glycine, and threonine, whereas it contained significantly lower amount of hydroxyproline, isoleucine, and proline as compared to bovine and porcine gelatins ($p < 0.05$). The arginine content of fish gelatin was similar to that of porcine gelatin. All gelatins used in this study contained glycine more than 30 residues (36.4, 33.8, and 33.9 for shaari, porcine, and bovine gelatins, respectively), which is the most dominant amino acid in gelatin [37–41]. Threonine content was high in shaari gelatin but was not detected in bovine and porcine gelatins. Similarly, Jamilah et al. [27] reported that red and black tilapia have very high contents of glycine and threonine. Shaari gelatin had also lower content of isoleucine as well as of the imino amino acids proline, and hydroxyproline than its counterpart bovine and porcine gelatins. Johnston-Banks [42] reported that the imino acids proline and hydroxyproline impart considerable rigidity to the collagen structure and that the relatively limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of the gelatin. It has been reported that the stability of the triple-helical structures in denatured gelatins is dependent on the total imino acid content, as regions rich in proline and hydroxyproline are likely involved in the formation of nucleation zones [43]. Hydroxyproline is believed to play a singular role in the stabilization of the triple-helical structures due to its hydrogen bonding ability through its hydroxyl group [43–45]. The imino acids (Pro and Hyp) content of shaari gelatin (16.73 g/100 g sample) was in the range reported for other fish species mainly Sole (17.4), Megrim, (17.5) Cod (15.6), Hake (17.3), and Squid gelatins (17.5) [28]. In contrast to the other fish species shaari gelatin showed a higher content of alanine. This amino acid, together with Pro and Hyp, is found in non-polar regions where sequences of the type Gly-Pro-Y predominate [43]. Thus, in general, a gelatin preparation with high proline, hydroxyproline, and alanine content shows better viscoelastic properties than others with a low content in these amino acids [28, 39].

The hygroscopic characteristics of a biological material could be assessed by equilibrating the sample in a specific relative humidity environment [21]. It is expected that more hygroscopic material contains more equilibrium moisture content compared to the less hygroscopic material. Medium acid concentration (0.1 N) produced less hygroscopic gelatin (equilibrium moisture: 6.4–7.6 g/100 g sample) compared to the gelatin extracted in high (1.0 N) (equilibrium moisture: 11.7–15.9 g/100 g sample) and low (0.01 N) (equilibrium moisture: 8.2–11.8 g/100 g sample) acid concentration solution (Table 2). This indicated that concentration of 0.1 N acetic acid created more polar sites for water binding as compared to the 1.0 and 0.01 N solutions. This could be due to the fact that high concentration completely breaks the structure, and low concentration affects the structure less.

Thermal behavior of fish skin was studied by differential scanning calorimetric (DSC) analysis. DSC provides a sensitive means of understanding the thermal denaturation events when collagen is heated [46]. The DSC curve for skin dried in oven and then equilibrated at water content 16.0 g/100 g sample shows a shift in the curve line

Table 2 Thermal characteristics of gelatin from different sources after equilibration at 11.3% relative humidity

	Glass transition					Thermal unfolding			
	X_w	T_{gi}/K	T_{gp}/K	T_{ge}/K	ΔC_P/J/kg K	T_{ui}/K	T_{um}/K	T_{up}/K	ΔH_u/kJ/kg
LTLC	10.4	307.4e (3.5)	314.3ef (2.7)	320.5ef (2.6)	60cde	329.2gh (3.1)	332.9gh (3.4)	337.1fg (2.8)	2.2cd (1.3)
MTLC	11.8	284.9bcd (4.9)	310.9cd (1.0)	316.7cd (2.5)	540e	391.9gh (2.1)	396.0gh (2.1)	404.8fg (1.3)	9.5d (2.3)
UTLC	8.2	328.9hi (0.9)	332.5f (0.3)	333.2f (0.1)	468de	335.3c (0.3)	336.1c (0.2)	338.3c (0.3)	0.8bcd (0.1)
HTLC	8.9	329.0bcd (2.7)	333.5cd (2.6)	334.2cd (2.4)	765bcde	336.9gh (2.3)	338.1gh (2.4)	340.7fg (2.6)	1.5d (0.4)
LTMC	7.6	322.5d (2.2)	327.2de (0.8)	328.2de (0.5)	935abc	331.2gh (0.3)	332.9gh (0.5)	336.3fg (0.5)	3.7cd (0.8)
MTMC	6.4	327.3cd (2.3)	333.6c (3.4)	336.7c (2.7)	617cde	344.1fg (6.1)	346.0g (8.9)	350.5f (10.3)	0.7d (0.3)
UTMC	6.6	326.8bcd (3.5)	331.7cd (2.9)	333.4cd (2.7)	589cde	337.7gh (3.3)	339.2gh (2.9)	341.1fg (3.1)	0.2d (0.1)
HTMC	7.0	326.2bcd (0.8)	329.3cd (0.9)	329.7cd (0.8)	630bcde	331.0gh (0.4)	333.2gh (0.3)	336.8fg (0.3)	6.1cd (1.9)
LTHC	11.7	304.5ef (9.4)	312.3f (9.5)	317.5f (2.9)	688bcde	323.8h (1.9)	328.9h (2.7)	330.0g (4.0)	2.7cd (0.8)
MTHC	15.9	292.4gh (4.1)	300.4g (7.5)	307.7g (7.1)	612cde	443.3b (43.5)	450.0b (36.7)	456.0b (30.8)	15.5abc (8.0)
UTHC	14.3	281.1i (2.0)	289.1g (4.5)	306.0g (5.8)	725abcde	465.7a (6.1)	469.5a (4.6)	477.0a (5.0)	24.7a (19.3)
HTHC	13.6	295.1fgh (1.8)	306.3fg (0.5)	312.5fg (1.2)	594cde	-	-	-	
SDS	11.1	297.3fg (2.2)	307.5fg (0.4)	313.4fg (1.3)	536de	372.1d (4.0)	374.8de (3.4)	380.6de (2.1)	18.9ab (10.2)
SDO	7.7	335.2bc (0.9)	341.5b (0.2)	345.5b (0.8)	863abcd	359.7ef (0.5)	364.3ef (0.5)	369.4e (0.3)	0.9d (0.1)
BG	9.7	346.8a (8.1)	356.3a (6.2)	360.6a (5.9)	1100ab	383.6cd (6.3)	384.9cd (5.5)	388.4d (5.2)	1.3d (0.5)
PG	10.0	335.9b (7.7)	344.0b (4.4)	345.7b (4.5)	1062a	346.6fg (0.4)	347.8fg (0.3)	350.4f (0.4)	1.4cd (0.3)

Note: Values in a column followed by the same letter are not significantly different ($p < 0.05$). Values in the parentheses are standard deviations

(marked as A, onset glass transition, T_{gi}) before the solids-melting endotherm (marked as S, onset glass transition, T_{mi}) (Fig. 1a). Similar curve was observed for the skin dried over silica gel at room temperature with an addition of small peak in the solids-melting endotherm (Fig. 1b). The small peak B could be considered as the denaturation temperature.

Commercial porcine gelatin showed glass transition as a shift in the curve line (marked as A), unfolding as one small endothermic peak (marked as B), and solids-melting as another endothermic peak (marked as S) (Fig. 1c). Similar trend was also observed for the commercial bovine gelatin. Figure 1d shows the DSC curve for fish skin gelatin extracted in 1 N acetic acid at 277 K (LTHC). This figure shows glass transition as a shift in the curve line (marked as A), a small peak as unfolding (marked as B, in some cases difficult to see over the full scale), another shift in the curve line before the solids-melting endotherm

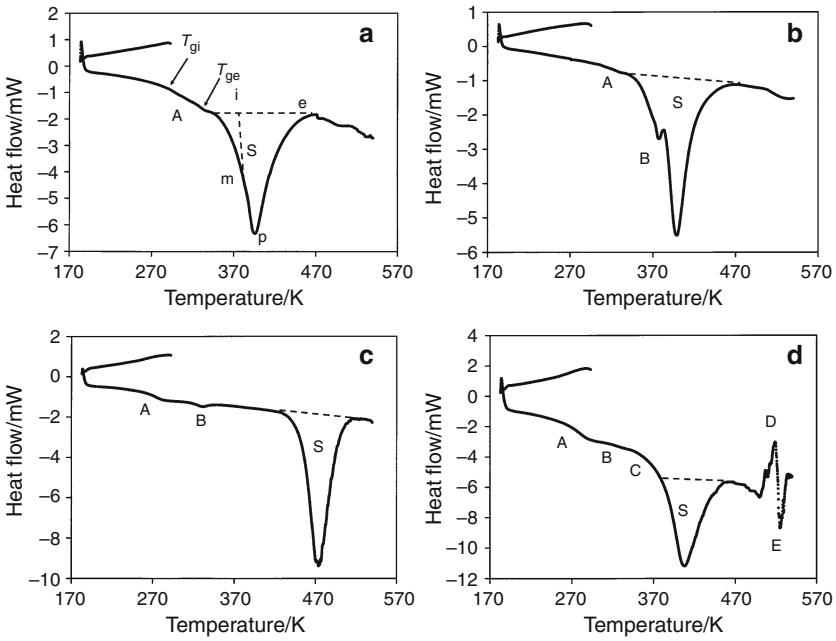

Fig. 1 **a** DSC curve for air-dried shaari skin (*A* glass transition, *S* solids-melting, T_{gi} onset glass transition, T_{ge} end of glass transition; *i, m, p, e* onset, maximum slope, peak and end of solids-melting); **b** DSC curve for silica gel-dried shaari skin (*A* glass transition, *B* unfolding, *S* solids-melting); **C**: DSC curve for porcine gelatin (*A* glass transition, *B* unfolding, *S* solids-melting); **d** DSC curve for gelatin extracted from shaari skin at 1 N concentration and 277 K (*A* glass transition, *B* unfolding, *C* shift before solids-melting, *S* solids-melting, *D* and *E* decomposition)

(marked as C), solids-melting as an endothermic peak (marked as S), and decomposition as exothermic–endothermic peak (marked as D and E) (Fig. 1d). The shift before solids-melting (marked as C) was observed for 277, 293, and 323 K, whereas 353 K did not show the second shift before solids-melting. Two shifts in the curve line (marked as A and C) indicated two types of amorphous regions existed in the extracted gelatin at or below 323 K.

The gelatin extracted in 0.1 N at 353 K (HTMC) also showed similar curve without second shift in curve line before solids-melting endothermic peak (Fig. 2a). Similar absence of the shift was also observed for 323, and 293 K, whereas the second shift in the curve line was observed at 277 K. At all temperatures, low concentration of 0.01 N showed similar curve without the appearance of second shift before the solids-melting. In addition, curve of gelatin extracted in 0.01 N and 293 or 277 K (MTLC and LTLC, respectively) showed larger clear endothermic peak as unfolding (marked as B), glass transition shift (marked as A) was merged with unfolding endothermic peak, and another the endothermic peak (marked as G) after the unfolding and before solids-melting (marked as S) (Fig. 2b). This indicated that less structural changes occurred in the extracted gelatin using low concentration and low temperature.

Table 2 showed the glass transition and thermal unfolding of fish skin, extracted gelatin from fish skin and commercial gelatin samples equilibrated at 11.3% relative humidity. Table 3 shows melting characteristics of these samples. It was difficult to compare the data since different samples contained different level of moisture due to difference in hygroscopic characteristics. For this reason all samples were equilibrated in a desiccator with water at the bottom up to moisture content 16.0 g/100 g sample.

Tables 4 and 5 show glass transition, unfolding and solids-melting characteristics of fish skin, extracted and commercial gelatin samples at moisture content 16.0 g/100 g sample. The onset glass transition temperatures of gelatin from shaari skin decreased from 308.7 to 260.3 K, respectively (Table 4). Rahman et al. [21] pointed that wide variations in gelatin molecules from different sources indicated the complexity of the hydration and plasticization causing differences in the glass transition. Bell and Touma [16] also explained that the wide variation of the glass transition values in the literature may be due to different types of gelatin transformed during different extraction method and characteristics of animal skins based on species, age, and sex. The T_{gi} was observed at 262.6 K when extraction concentration and temperature were 0.1 N and 323 K (UTMC). Rahman et al. [21] observed the onset glass transition temperature of extracted gelatin from tuna skin at 254.0 K (moisture content: 18.1 g/100 g sample) at similar extraction concentration and temperature (0.1 N and 323 K, UTMC). The lower value for tuna could be due to the higher moisture content as well as the difference in composition of gelatin extracted from tuna skin. Similar to this study, they also reported higher T_{gi} for bovine (307 K at moisture content 15.4 g/100 g sample) and porcine (308 K at moisture content 16.1 g/100 g sample) than gelatin from tuna skin. It was possible to develop gelatin from shaari skin with comparable onset glass transition temperature of mammalian gelatin (bovine and porcine) by selecting appropriate concentration (0.01 N acetic acid at 277 (LTLC) or 293 K (MTLC)) (Table 4). It was mentioned in the literature that gelatin from fish skin showed lower glass transition, lower gelling point, and gel strength than

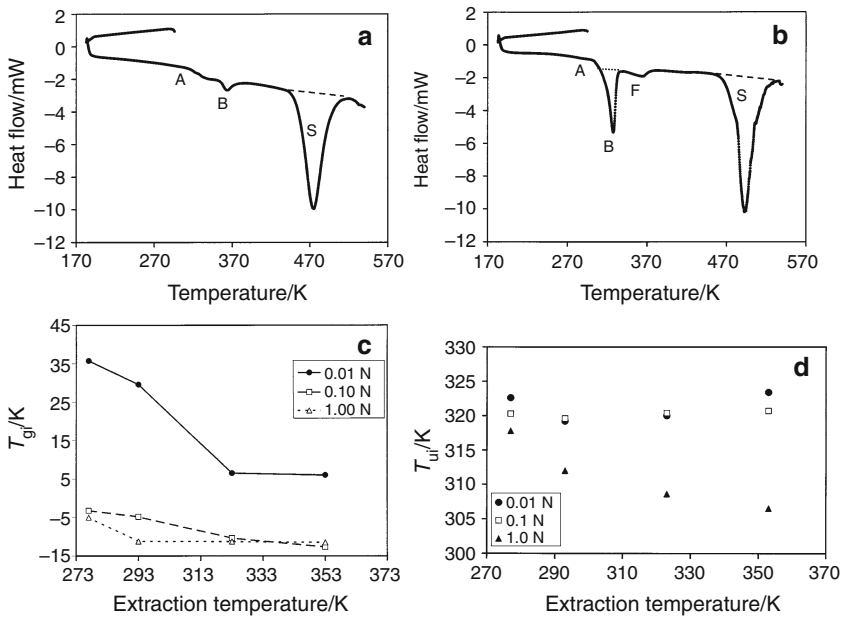

Fig. 2 **a** DSC curve for gelatin extracted from shaari skin at 0.1 N concentration and 353 K (*A* glass transition, *B* unfolding, *S* solids-melting); **b** DSC curve for gelatin extracted from shaari skin at 0.01 N concentration and 293 K (*A* glass transition, *B* unfolding, *F* endothermic peak, *S* solids-melting); **c** Onset glass transition temperature as a function of extraction temperature for different acid concentration; **d** Onset unfolding temperature as a function of extraction temperature for different acid concentration

Table 3 Thermal characteristics of gelatin from different sources after equilibration at 11.3% relative humidity

	Melting of solids				
	T_{mi}/K	T_{mm}/K	T_{mp}/K	T_{me}/K	ΔH_m/kJ/kg
LTLC	403.7bcdef (47.8)	419.6bcd (48.4)	456.3abcd (33.7)	499.2abc (19.0)	144.8abc (36.2)
MTLC	437.5abc (31.0)	440.7abc (35.1)	461.2abcd (34.6)	510.9abc (12.1)	49.3cde (5.0)
UTLC	447.9abcd (6.4)	461.9abcd (4.8)	446.1abcd (57.5)	513.6abc (3.5)	91.2e (16.4)
HTLC	403.3bcdef (42.9)	416.7bcd (38.2)	439.9abcd (31.4)	504.9abc (13.1)	125.7bcd (28.7)
LTMC	362.1def (13.9)	383.4cd (13.8)	417.4bcd (0.1)	476.9abcd (0.8)	70.9de (1.8)
MTMC	378.5def (23.8)	401.6bcd (39.8)	439.6abcd (35.8)	485.5abcd (26.8)	74.1e (48.0)
UTMC	430.0abcdef (36.9)	444.8abcd (37.4)	466.3abcd (36.7)	521.8ab (6.1)	136.9abc (33.2)
HTMC	456.5ab (1.7)	466.2abc (3.1)	480.6abc (3.3)	527.2a (10.2)	153.3ab (24.8)
LTHC	410.6abcde (22.7)	456.0abcd (54.2)	465.4abcd (40.8)	448.0d (89.7)	140.9abc (46.8)
MTHC	431.7abcd (52.6)	439.3abcd (54.9)	463.7a (49.0)	517.9ab (17.2)	180.8a (25.1)
UTHC	494.4a (16.1)	501.2a (14.8)	503.1a (14.0)	525.6ab (6.6)	145.7abc (42.8)
HTHC	383.0cdef (10.5)	394.8bcd (12.0)	419.7bcd (1.0)	462.6cd (1.8)	43.3e (6.2)
SDS	433.1abcde (42.9)	443.0abcd (36.1)	457.4abcd (35.5)	499.6abc (21.7)	143.7abc (12.3)
SDO	452.4abc (10.3)	472.6ab (11.9)	483.0ab (2.1)	521.0ab (1.8)	131.9abcd (30.2)
BG	376.3ef (19.0)	379.6d (18.8)	410.9d (15.9)	471.7abcd (11.0)	117.3abc (32.1)
PG	374.7f (36.8)	388.8d (49.0)	420.0cd (39.7)	480.7bcd (23.0)	161.6ab (13.3)

Note: Values in a column followed by the same letter are not significantly different ($p < 0.05$). Values in the parentheses are standard deviations

Table 4 Thermal characteristics of gelatin from different sources containing water at 16.0 g/100 sample

	Glass transition				Thermal unfolding			
	T_{gi}/K	T_{gp}/K	T_{ge}/K	ΔC_P/J/kg K	T_{ui}/K	T_{um}/K	T_{up}/K	ΔH_u/kJ/kg
LTLC	308.7bc (0.8)	312.1bc (2.4)	313.0bc (2.6)	1408ab	322.6def (0.6)	326.0cd (0.0)	329.7cd (0.2)	41.1b (8.6)
MTLC	302.5d (0.5)	305.9c (0.4)	308.0cd (1.0)	1098bc	319.2ef (0.2)	327.5cd (7.0)	328.3cd (0.4)	51.6a (3.7)
UTLC	279.5f (0.7)	285.8e (0.8)	393.3ef (1.7)	628cde	320.0def (3.2)	323.9cd (1.8)	329.0cd (0.7)	1.9c (0.8)
HTLC	279.0f (2.9)	285.9e (5.4)	295.7e (1.2)	602cde	323.4cde (1.8)	326.6cd (2.6)	330.8cd (1.8)	0.8c (0.4)
LTMC	269.7g (0.4)	273.3fg (0.1)	282.9gh (0.6)	669cde	320.3def (4.1)	325.7cd (2.8)	331.7cd (1.6)	0.8c (0.5)
MTMC	268.1g (2.2)	274.1fg (3.1)	283.1gh (6.1)	610cde	319.6def (3.2)	325.3cd (3.2)	330.2cd (0.5)	1.1c (0.6)
UTMC	262.6h (2.7)	275.8fg (4.8)	281.5h (2.9)	130e	320.4def (1.4)	323.6cd (1.6)	326.7d (1.4)	3.0c (0.5)
HTMC	260.3h (1.6)	272.5g (2.1)	278.8h (3.6)	411de	320.7def (1.0)	325.8cd (0.9)	331.5cd (0.1)	2.0c (0.4)
LTHC	267.9g (2.3)	278.7f (2.7)	288.2fg (1.7)	1705a	317.8f (4.0)	322.4d (6.3)	327.6cd (5.3)	0.7c (0.9)
MTHC	261.8h (1.8)	272.1g (1.6)	280.4h (2.2)	654cde	312.0g (5.9)	322.9d (3.3)	331.9c (5.5)	0.2c (0.1)
UTHC	261.7h (2.3)	269.9g (0.8)	278.6h (2.2)	657cde	308.6gh (2.7)	315.0e (4.0)	319.8e (4.6)	0.3c (0.2)
HTHC	261.5h (0.6)	273fg (1.9)	279.0h (2.7)	665cde	306.5h (0.8)	309.5e (0.7)	318.1e (5.0)	0.6c (0.1)
SDS	312.4b (5.0)	325.7a (2.9)	331.6a (2.0)	656cde	101.0a (1.9)	375.0a (0.4)	378.0a (0.9)	4.1c (3.8)
SDO	291.3e (3.7)	297.3d (6.5)	304.7d (2.3)	716cde	374.7cd (0.3)	329.7c (2.3)	332.4c (2.5)	0.8c (0.3)
BG	306.7cd (1.0)	312.4b (1.8)	316.2b (1.4)	810cd	328.6c (2.2)	327.8cd (0.7)	332.1cd (0.8)	1.0c (0.4)
PG	318.5a (2.4)	327.4a (5.6)	330.1a (6.6)	422de	358.3b (2.6)	361.8b (2.6)	365.1b (1.2)	3.6c (3.4)

Note: Values in a column followed by the same letter are not significantly different ($p < 0.05$). Values in the parentheses are standard deviations

mammalian gelatin [21, 25, 47]. It was argued in the literature that the lower thermal and mechanical characteristics were mainly due to the lower amount of proline, hydroxyproline, and glycine [7, 25, 48]. However, the results of this study showed that the difference in thermal and mechanical characteristics of gelatin extracted from fish skin as compared to mammalian gelatin could be reduced by manipulating the extraction conditions.

Figure 2c shows that the onset glass transition temperature decreased with the increase of extraction temperature up to 323 K and then remained nearly constant. This indicated that structural breakdown reached maximum at

Table 5 Thermal characteristics of gelatin from different sources containing water at 16.0 g/100 sample

	Melting of solids				
	T_{mi}/K	T_{mm}/K	T_{mp}/K	T_{me}/K	ΔH_m/kJ/kg
LTLC	436.3ab (3.4)	457.0abc (4.7)	479.0ab (1.7)	531.0a (0.4)	254.0ab (14.8)
MTLC	455.3a (15.3)	470.0a (19.1)	479.0a (14.9)	517.0ab (26.2)	217.0abcde (68.5)
UTLC	392.6abc (31.1)	405.0abcd (39.8)	413.0bcd (34.7)	479.0bcde (28.9)	239.0abc (81.9)
HTLC	390.2abc (50.0)	405.0abcd (47.2)	424.0abcd (45.0)	501.0abcd (29.6)	213.0abcde (46.9)
LTMC	399.9abc (41.3)	414.0abcd (41.8)	437.0abcd (34.8)	501.0abcd (16.6)	236.0abcd (9.7)
MTMC	422.4abc (46.8)	437.0abcd (46.0)	453.0abcd (48.4)	497.0abcde (17.7)	211.0abcde (61.0)
UTMC	422.0abc (37.1)	452.0abcd (59.2)	467.0abcd (48.1)	492.0abcde (32.8)	122.0de (57.7)
HTMC	428.9ab (41.5)	455.0ab (29.5)	462.0abcd (28.4)	491.0abcde (26.8)	280.0a (30.9)
LTHC	388.0abc (6.8)	391.0bcd (3.97)	407.0bcd (2.8)	461.0cde (3.63)	283.0a (141.0)
MTHC	389.7abc (22.3)	394.0bcd (22.7)	410.0bcd (10.8)	458.0de (9.8)	121.0e (18.7)
UTHC	444.0a (56.1)	447.0abcd (63.8)	459.0abcd (56.1)	528.0a (19.2)	146.0cde (23.3)
HTHC	441.5a (63.3)	457.0abc (62.5)	470.0abc (56.2)	505.0abc (34.2)	136.0bcde (49.8)
SDS	351.6c (1.8)	382.0bcd (2.2)	400.0cd (1.1)	466.0cde (3.2)	243.0abc (19.2)
SDO	350.6c (8.2)	372.5d (9.7)	397.0d (1.04)	467.0cde (6.1)	215.0abcde (13.7)
BG	364.4bc (8.1)	376.0cd (9.0)	395.0d (6.3)	452.0e (30.2)	227abcde (72.1)
PG	405.4abc (43.3)	415.0abcd (43.6)	424.0abcd (44.5)	479.0bcde (32.4)	162.0bcde (38.0)

Note: Values in a column followed by the same letter are not significantly different ($p < 0.05$). Values in the parentheses are standard deviations

323 K. Moreover the decrease in glass transition was more pronounced at 0.01 N concentration compared to 0.1 and 1 N samples. The increase of acid concentration during extraction shifted the curve toward lower temperature indicating increasing concentration decreased the glass transition temperature (Fig. 2c). More plasticized samples were formed with the increase of acid concentration. Similarly, pH of whey protein films affected its glass transition temperature [49]. Gelatin is a very complex molecule and its characteristics depend on many factors, such as the collagen type, tissue, animal species, and age. The type and concentration of acid strongly influence swelling properties and solubilization of collagen, leading to variations in molecular weight distribution in the resultant gelatins, depending on the persistence of some of the cross-links between collagen-chains [50]. According to Asghar and Henrickson [51], the lyotropic effect of carboxylic acids on collagen seems to dominate the swelling capacity, rather than a specific ion effect, since it is the non-ionized acid that acts as the swelling agent by competing with the peptide group involved in intermolecular linking of protein chain, mainly because of the hydrogen bonding power of the acid.

Table 5 shows unfolding temperature decreased exponentially with the increase of extraction temperature in the case of higher (1.0 N) concentrated samples. The curves of unfolding temperature also shifted to lower temperature (Fig. 2d). In contrast 0.1 and 0.01 N samples did not have an effect on the unfolding temperature. The extraction concentration and temperature did not show significant effect on the onset solids-melting temperature (Table 5). The value of T_{mi} was 422 K compared to the 427 K in the case of gelatin extracted from tuna skin when extraction concentration and temperature were at 0.1 N and 323 K (UTMC), respectively [21]. A generic trend could not be observed in the case of solids-melting temperature as a function of extraction temperature and concentration. In addition more variability in the data was observed as compared to that of glass transition and unfolding temperature as evidenced from the standard deviations. Similarly high variability was also observed in the case of gelatin from different sources [21]. However, solids-melting temperature of skin was significantly lower compared to the extracted gelatin from shaari skin ($p < 0.05$) (Table 5).

Conclusions

The effects of acid concentration and temperature of extraction of fish gelatin on the physico-chemical characteristics (chemical composition and color) and thermal characteristics (glass transition, unfolding, and solids-melting) were studied and compared to mammalian gelatins. Results showed that in general extraction conditions had an effect on the yield, protein content, and color. This study showed also a difference in thermal and mechanical characteristics of gelatin extracted from fish skin as compared to mammalian gelatin. This difference could be reduced by manipulating the extraction conditions. It was

possible to develop gelatin from shaari skin with comparable onset glass transition temperature of mammalian gelatin (bovine and porcine) by selecting appropriate concentration of acetic acid (i.e., 0.01 N at 277 or 293 K).

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References

1. Karim AA, Bhat R. Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends Food Sci Technol. 2008;19(12):644–56.
2. Montero P, Gómez-Guillén MC. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. J Food Sci. 2000;65:434–8.
3. Wainewright FW. Physical tests for gelatin and gelatin products. In: Ward AG, Courts A, editors. The science and technology of gelatin. New York: Academic Press; 1977. p. 507–34.
4. Hinterwaldner R. Raw material. In: Ward AG, Courts A, editors. The science and technology of gelatin. New York: Academic Press; 1977. p. 295–314.
5. Gennadios A, McHugh TH, Weller CL, Krochta JM. Edible coatings and films based on proteins. In: Krochta JM, Baldwin EA, Nisperos-Carriedo M, editors. Edible coatings and films to improve food quality. Lancaster: Technomic Publishing Company; 1994. p. 201–78.
6. Slade L, Levine H. Polymer-chemical properties of gelatin in foods. In: Pearson AM, Dutson TR, Bailey AJ, editors. Collagen as food: vol 4. Advances in meat research, vol. 4. New York: Van Nostrand Reinhold; 1987. p. 251–66.
7. Haug IJ, Draget KI, Smidsrod O. Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocoll. 2004;18:203–13.
8. Leuenberger BH. Investigation of viscosity and gelation properties of different mammalian and fish gelatins. Food Hydrocoll. 1991;54:353–61.
9. Zhang S, Wang Y, Herring JL, Oh JH. Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. J Food Sci. 2007; 72(9):C498–503.
10. Yang H, Wang Y, Zhou P, Regenstein JM. Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. Food Hydrocoll. 2008;22:1541–50.
11. Zhou P, Regenstein JM. Optimization of extraction conditions for Pollock skin gelatin. J Food Sci. 2004;69(5):C393–8.
12. Rahman MS. State diagram of date flesh using differential scanning calorimetry (DSC). Int J Food Prop. 2004;7(3):407–28.
13. Rahman MS. State diagram of foods: its potential use in food processing and product stability. Trends Food Sci Technol. 2006;17:129–41.
14. Rahman MS. Food stability beyond water activity and glass transition: macro-micro region concept in the state diagram. Int J Food Prop. 2009;12(4):726–40.
15. D'Cruzand NM, Bell LN. Thermal unfolding of gelatin in solids as affected by the glass transition. J Food Sci. 2005;70(2):E64–8.
16. Bell L, Touma DE. Glass transition temperatures determined using a temperature-cycling differential scanning calorimetry. J Food Sci. 1996;61(4):807–28.
17. Marshall AS, Petrie SEB. Thermal transitions in gelatin and aqueous gelatin solutions. J Photogr Sci. 1980;28:128–34.
18. Sablani SS, Kasapis S, Al-Rahbi Y, Al-Mugheiry M. Water sorption isotherms and glass transition properties of gelatin. Dry Technol. 2002;20(10):2081–92.
19. Sobral PJA, Habitante AMQB. Phase transitions of pigskin gelatin. Food Hydrocoll. 2001;15:377–82.
20. Vanin FM, Sobral PJA, Menegalli FC, Carvalho RA, Habitante AMQB. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocoll. 2005;19:899–907.
21. Rahman MS, Al-Saidi GS, Guizani N. Thermal characterization of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin. Food Chem. 2008;108:472–81.
22. Gomez-Guillen M, Montero P. Method for the production of gelatin of marine origin and product thus obtained. International Patent PCT/S01/00275. 2001.
23. AOAC. Official methods of analysis. 15th ed. Washington, DC: Association of Official Analytical Chemists; 1990.
24. Pico-Tag. Amino acid analysis system. Midford: Waters Corporation; 1984.
25. Rahman MS, Al-Mahrouqi AI. Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. Int J Food Sci Nutr. 2009;60(S7):229–42.
26. Gudmundsson M, Hafsteinsson H. Gelatin from cod skins as affected by chemical treatments. J Food Sci. 1997;62:37–47.
27. Jamilah B, Harvinder KG. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chem. 2002;77:81–4.
28. Gomez-Guillen MC, Turnay J, Fernandez-Diaz MD, Ulmo N, Lizarbe MA, Montero P. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll. 2002;16:25–34.
29. Mizuta S, Yoshinaka R, Sato M, Sakaguchi M. Hot-water solubility of collagen from the muscle of kuruma prawn. Fisheries Sci. 1995;61:536–7.
30. Peterson BR, Yates JR. Gelatin extraction. United-States-Patent. 4 064 008. Novo Industri AS. 1977.
31. Eysturskaro J, Haug IJ, Elharfaoui N, Djabourov M, Draget KI. Structural and mechanical properties of fish gelatin as a function of extraction conditions. Food Hydrocoll. 2009;23:1702–11.
32. Djabourov M, Lechaire J, Gaill F. Structure and rheology of gelatin and collagen gels. Biorheology. 1993;30:191–205.
33. De Batista TM, Martins VCA, De Guzzi Plepis AM. Thermal behavior of in vitro mineralized anionic collagen matrices. J Therm Anal Calorim. 2009;95(3):945–9.
34. Joly-Duhamel C, Hellio D, Djabourov M. All gelatin networks: 1. Biodiversity and physical chemistry. Langmuir. 2002;18:7208–17.
35. Arnesen JA, Gildberg A. Extraction and characterisation of gelatine from Atlantic salmon (*Salmo salar*) skin. Bioresour Technol. 2007;98:53–7.
36. Zhou P, Regenstein JM. Effects of Alkaline and acid pretreatments on Alask Pollock skin gelatin extraction. J Food Sci. 2005;70(6):C392–6.
37. Balian G, Bowes JH. The structure and properties of collagen. In: Ward AG, Courts A, editors. The science and technology of gelatin. New York: Academic Press; 1977. p. 1–31.
38. Rother J. Edible gelatin types, properties, use and application in the food industry. Food Tech Europe. 1995;1(5):32–42.
39. Sarabia AI, Gomez-Guillen MC, Montero P. The effect of added salts on the viscoelastic properties of fish skin gelatin. Food Chem. 2000;70:71–6.
40. Arnesen JA, Gildberg A. Preparation and characterisation of gelatine from the skin of harp seal (*Phoca groenlandica*). Bioresour Technol. 2002;82:191–4.

41. Badii F, Howell NK. Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. Food Hydrocoll. 2006;20:630–40.
42. Johnston-Banks FA. Gelatin. In: Harris P, editor. Food gels. London: Elsevier Applied Science Publ; 1990. p. 233–89.
43. Ledward DA. Gelation of gelatin. In: Mitchell JR, Ledward DA, editors. Functional properties of food macromolecules. London: Elsevier Applied Science Publ; 1986. p. 171–201.
44. Burjandze TV. Hydroxy-proline content and location in relation to collagen thermal stability. Biopolymers. 1979;18:931–6.
45. Mizuno K, Hayashi T, Bachinger HP. Hydroxylation-induced stabilization of the collagen triple helix. Further characterization of peptides with 4(R)-hydroxyproline in the Xaa position. J Biol Chem. 2003;278:32373–9.
46. Badea E, Miu L, Budrugeac P, Giurginca M, Masic A, Badea N, Gatta GD. Study of deterioration of historical parchments by various thermal analysis techniques complemented by SEM, FTIR, UV–VIS–NIR and unilateral NMR Investigations. J Therm Anal Calorim. 2008;91:17–27.
47. Norland RE. Fish gelatin technical aspects and applications. In: Bland SJ, editor. Photographic gelatin. London: Royal Photographic Society; 1987. p. 266–81.
48. Gilsenan PM, Ross-Murphy SB. Rheological characterization of gelatins from mammalian and marine sources. Food Hydrocoll. 2000;14:191–5.
49. Anker M, Stading M, Hermansson A. Effects of pH and gel state on the mechanical properties, moisture contents, and glass transition temperatures of whey protein films. J Agric Food Chem. 1999;47:1878–86.
50. Gimenez B, Turnay J, Lizarbe MA, Montero P, Gómez-Guillén MC. Use of lactic acid for extraction of fish skin gelatin. Food Hydrocoll. 2005;19:941–50.
51. Asghar A, Henrickson RL. Chemical, biochemical, functional, and nutritional characteristics of collagen in food system. Adv Food Res. 1982;28:231–372.