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Properties of gelatin extracted from snakehead fish (*Chitala striata*) by-products at various temperatures and times

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Abstract

This study aimed to identify the properties of gelatin extracted from snakehead fish (*Channa striata*) by-products, including scale and a mixture of skin and scale. The gelatin extraction yield from fish scales was lower than that from fish skin and scales. Both gelatins showed similar gel strength (248 g in the mixture of fish skin and scale and 245 g in fish scale gelatin), while the viscosity and color of samples from fish skin and scale were better than those of fish scale samples extracted at 80 °C for 1 h. SDS-PAGE profile of fish scale protein degradation correlated with the increasing wavelength in amide I and III. Thus, a mixture of skin and scale can be used as a raw material in gelatin production.

Keywords: gelatin; snakehead fish; scale; the mixture of skin and scale.

Practical Application: Fish skin-scale gelatin had higher yields and lower protein degradation than fish-scale gelatin.

1 Introduction

Snakehead fish (Channa striata) have contributed significantly to freshwater fish production in southern Vietnam in recent years (Duong et al., 2019). Vietnam's production of cultured snakehead fish for domestic consumption increased to 40,000 tons per year (Quyen et al., 2016). Furthermore, other Asian countries have prevalently focused on its culture, the total global snakehead fish production in 2016 reached 92,523 tons (Food and Agriculture Organization of the United Nations, 2019), with an anticipated noticeable growth within the next few years. Consequently, huge amounts of by-products are discarded from the snakehead fish manufacturing industry. These by-products (fish head, skin, scales, and bone), which account for approximately 40% of the body weight (Ghaly et al., 2013), contain a high concentration of collagen, which is used to refine gelatin (Karim & Bhat, 2009). Indeed, the social demand for gelatin has increased over the last few decades because of its unique surface-active properties, allowing its wide application in the food, pharmaceutical, cosmetic, and other industries (Zhou & Regenstein, 2007). For these reasons, gelatin production from processing fish by-products has been a concern of broad interest to related-scientist groups, such as the skin of tra catfish (Thuy et al., 2022); wami tilapia skin (Alfaro et al., 2013); seabass skin (Tekle et al., 2022); and snakehead skin and bone (Rosmawati et al., 2021). However, there is no public consideration for gelatin extracted from snakehead fish by-products. Therefore, the purpose of this study was to extract and compare the gelatin properties of snakehead fish scale and a mixture of skin and scale to increase the value of fish by-products.

2 Materials and methods

2.1 Sample preparation

Snakehead fish by-products were collected from processing companies located in An Giang province, Vietnam. The samples were placed in a thermo insulated polystyrene box, covered with ice, and transported to the laboratory within 4 h. The samples were then washed in running water, cut into small pieces, placed in polyethylene bags, and kept at -20 °C until use.

2.2 Gelatin extraction

Gelatin from snakehead fish by-products was extracted according to the method described by Le et al. (2015), with minor adjustments. The fish scale and the mixture of skin and scale were soaked and gently stirred in EDTA-2Na 0.8 M for 24 h (the solution was changed every 12 h) at 4 °C with a material/solution ratio of 1/8 (w/v) for mineral removal. Gelatin extraction was conducted by soaking the samples in 7x distilled water (w/v) at various temperatures (60, 70, 80, and 90 °C) for different times (1, 2, and 3 h) with continuous stirring. The coarse solids from the samples at each extraction condition were removed by filtration with two layers of cheesecloth and continuously centrifuged at 16,000 × g for 30 min at a temperature of 20 °C to

Received 10 June, 2022

Accepted 08 Aug., 2022

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collect the supernatant and dried at 50 °C for 4 h (WTE Binder, Gallenkamp, Germany).

2.3 Gelatin yields

Based on the protein content in gelatin extracted by the Lowry method (Lowry et al., 1951) and the weight of the material, the yield of gelatin (YG) was calculated according to the method described by Le et al. (2015) as follows (Equation 1):

$$YG \ (\%) \frac{Protein \ concentration \ (mg \ / mL) \ x \ 7 \ times \ of \ distilled \ water \ (mL) \ x \ 100\%}{The \ weight \ of \ fish \ by - products \ samples \ (mg)}$$
(1)

2.4 Analysis of gel strength

Gelatin was dissolved completely in distilled water at 60 °C for 30 min to obtain a final concentration of 6.67% (w/v) for the gel strength determination, as described by Kittiphattanabawon et al. (2010), using a texture analyzer (Stable Micro Systems, Surrey, UK) with a load cell of 5 kg. The gelatin solution was poured into standard bloom bottles with a diameter of 3 cm and height of 2.5 cm. The gelatin gels were stored in the refrigerator at 4 °C for 16 h and placed centrally under a 1.27 cm diameter flat-faced cylindrical Teflon[®] plunger. Gel strength is expressed in grams (g).

2.5 Determination of viscosity

Gelatin viscosity was measured at 100 rpm using a Brookfield DV (RVDV-11+CP, USA) based on the method described by Jamilah et al. (2011) with slight modifications. A gelatin solution of 6.67% concentration (w/v) was prepared as described in section 2.4. Viscosity values were recorded in triplicates and expressed as cP.

2.6 Color measurement

The color of the gelatin powder was evaluated using a colorimeter (PCE–CSM 2, China) following the method of Saeleaw & Benjakul (2015). The values of L^* (lightness/brightness), a^* (redness/greenness) and b^* (yellowness/blueness) were recorded. Simultaneously, the total difference in the color of gelatin (ΔE) compared to the white standard ($L^* = 93,52$; $a^* = -0,3$; and $b^* = 1,57$) was calculated using the following Equation 2:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \tag{2}$$

2.7 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein patterns of gelatin extracted from snakehead fish by-products were determined as described by Thuy et al. (2022), with minor modifications. Gelatin in the supernatant extracted at 80 °C for 1 h was mixed with buffer (Tris-HCl 0.5 M at pH 6.8, with 20% (v/v) glycerol and 10% (w/v) SDS in the presence of 10% (v/v) mercaptoethanol) at a gelatin/buffer ratio of 1:2 (v/v). The polyacrylamide gel (7.5%) was used by loading approximately 10 μ L samples with protein markers (Sigma Chemical Co., St. Louis, MO, USA) for electrophoresis at a constant current of 20 mA, followed by staining and distaining. The molecular weight of gelatin was estimated by comparison with that of standard protein markers.

2.8 Analysis of amino acid

The amino acid composition of gelatin extracted from snakehead fish byproducts was analyzed using an amino acid analyzer system (Biochrom 32+, USA). The amino acid content was recorded as the number of amino acid residues per thousand residues.

2.9 Fourier-Transform Infrared (FTIR) spectra

FTIR analysis of gelatin extracted from the by-products of snakehead fish was performed using a PerkinElmer MIR/NIR Frontier spectrometer in the MIR mode. The spectrum was recorded using an MIR/NIR Frontier spectrometer, with spectral wavenumbers ranging from 4000 to 400 cm⁻¹.

2.10 Statistical analysis

All data are the means with standard deviation errors of triplicate experiments. The SPSS software package (SPSS 16.0; SPSS Inc., Chicago, IL, USA) was used for data analysis. The level of significant variance of measurements was determined and evaluated using one-way analysis of variance (ANOVA) and Duncan's multiple range test at 95% probability.

3 Results and discussion

3.1 Extraction of gelatin yield

Gelatin yields, extracted from the snakehead fish scale and the mixture of skin and scale, clearly increased in extraction condition of both rising temperature and time, as shown in Table 1. The gelatin yield extracted from the scale (2.12% to 14.3%) was lower than that from the mixture of skin and scale gelatin (12.1% to 24.3%), depending on the extraction conditions. The increase in the gelatin yield with increasing extraction temperature and time can be explained by the stabilization of hydrogen bonds in the collagen structure by extraction at a longer extraction time and higher temperature, resulting in a helix-to-coil transition

Table 1. The extraction yield (%) of gelatin from snakehead fish scale and the mixture of skin and scale at various extraction temperatures and times.

Temperature (°C)	Time (h)	Fish scale	Fish skin and scale mixture
60	1	$2.12\pm0.240^{\rm a}$	12.1 ± 0.243^{a}
60	2	$2.62\pm0.213^{\text{a}}$	$12.6\pm0.111^{\text{a}}$
60	3	$3.51\pm0.032^{\rm b}$	$13.5\pm0.031^{\rm b}$
70	1	$7.02 \pm 0.035^{\circ}$	$16.2\pm0.314^{\circ}$
70	2	$7.15 \pm 0.032^{\circ}$	$17.1\pm0.042^{\rm d}$
70	3	$8.09\pm0.350^{\rm e}$	$18.1\pm0.122^{\text{e}}$
80	1	$7.84\pm0.584^{\rm d}$	$17.8\pm0.281^{\rm de}$
80	2	$9.34\pm0.462^{\rm f}$	$19.3\pm0.412^{\rm f}$
80	3	11.1 ± 0.352^{g}	$21.1\pm0.247^{\rm g}$
90	1	$8.22\pm0.362^{\rm e}$	$22.2\pm0.160^{\rm h}$
90	2	$12.6\pm0.402^{\rm h}$	$22.9\pm0.231^{\rm h}$
90	3	$14.3\pm0.310^{\rm i}$	$24.3\pm0.340^{\text{j}}$

Data are expressed as mean \pm standard deviation (n = 3). Different superscripts in the same column indicate statistical differences (p < 0.05).

and soluble gelatin formation from collagen (Sinthusamran et al., 2014). The overheating condition during gelatin extraction caused partial destruction of the triple helix configuration of collagen, followed by the formation of shorter peptides that are released into water, but it may lead to the degradation of gelatin properties (Nagarajan et al., 2012; Sinthusamran et al., 2014). These results are consistent with those of previous research by Nagarajan et al. (2012) and Le et al. (2015), who observed an increase of gelatin yield from splendid squid skin and horse mackerel scale with an increase in extraction temperature and time, respectively.

3.2 Gel strength of gelatin

The gel strengths of gelatin extracted from scale and mixture of snakehead skin and scale at different extraction conditions are shown in Table 2. The increase in gel strength of gelatin extracted from snakehead fish by-products was observed at the extraction temperature range of 70 to 80 °C and a decrease in the gel strength of gelatin was observed at temperature higher than 90 °C and time longer than 2 h. The time extension combined with temperature rise would cause protein degradation, which could be largely detrimental to the gel network formation of gelatin (Kittiphattanabawon et al., 2010; Rosmawati et al., 2021). The gel strength of gelatin reached its highest value at 248 g and 245 g when the same extraction at 80 °C for 1 h was observed in the snakehead scale and skin and scale mixture, respectively. These data were consistent with that of the studies by Zuraida & Pamungkas (2020) for gelatin extracted from snakehead scales (229 g) and Thuy et al. (2022) for tra catfish skin gelatin (208 g).

3.3 Viscosity of gelatin

The changes in viscosity of gelatin extracted from the snakehead fish scale and mixture of skin and scale are shown in Table 3. The viscosity of gelatin extracted from the mixture of skin and scale (11.8 cP) was higher than that from scale (9.78 cP) at 80 °C for 1 h. A slight reduction in viscosity with increase in time was observed, which was explained by the low molecular

Table 2. The gel strength (%) of gelatin from snakehead fish scale and the mixture of skin and scale at various extraction temperatures and times.

Temperature (°C)	Time (h)	Fish scale	Fish skin and scale mixture
60	1	$137 \pm 3.76^{\circ}$	$147 \pm 2.76^{\rm bc}$
60	2	$129\pm6.47^{\rm bc}$	$159 \pm 6.47^{\circ}$
60	3	$125\pm4.10^{\rm b}$	$146 \pm 2.20^{\mathrm{b}}$
70	1	$234\pm3.40^{\rm hi}$	$239\pm3.40^{\text{gh}}$
70	2	$230\pm4.88^{\rm h}$	$233 \pm 4.37^{\rm g}$
70	3	$179\pm3.74^{\rm f}$	178 ± 3.92^{d}
80	1	$245\pm1.33^{\rm i}$	$248\pm6.08^{\rm h}$
80	2	$218 \pm 1.70^{\rm g}$	$220\pm3.22^{\rm f}$
80	3	$174 \pm 2.51^{\circ}$	176 ± 3.55^{d}
90	1	$180\pm5.72^{\rm f}$	196 ± 4.12^{e}
90	2	166 ± 2.14^{d}	165 ± 2.14^{e}
90	3	108 ± 9.21^{a}	123 ± 2.40^{a}

Data are expressed as mean \pm standard deviation (n = 3). Different superscripts in the same column indicate statistical differences (p < 0.05).

3.4 Color of gelatin powder

The total difference in color (ΔE^*) of gelatin extracted from snakehead fish byproducts under different extraction conditions is shown in Table 4. The results showed that there was a significant increase in ΔE^* of gelatin with an increase in temperature and time during extraction. The temperature rise facilitated extremely high extent of non-enzymatic browning reaction, contributing to the increased yellowness (b^*) and concomitantly the darker lightness by retaining the minimal fragments via damage of gelatin molecules (Thuy et al., 2022). The gelatin samples of the fish scale and the skin and scale mixture extracted at 80 °C for 1 h in showed a low total difference in color value (ΔE^*) with high lightness (L^* - values) when the extraction temperature was lower than 90 °C and time was not longer than 2 h.

3.5 SDS-PAGE profile of gelatin extracted from snakehead fish by-products

Figure 1 shows the protein patterns of fish-scale gelatin and a mixture of skin and scale extracted at 80 °C for 1 h. The molecular weight of the protein (consisting of α and β components) was observed in both gelatin from scale and the skin and scale mixture. However, degradation of protein patterns was observed in fish-scale gelatin when compared to gelatin from skin and scale mixtures, indicating an association between the transition of α -helix by uncoupling of intermolecular cross-links and interruption of intramolecular bonding (Kittiphattanabawon et al., 2012). The relationship between protein degradation and the increase in amide I and amide III bands in FTIR spectra has been described in studies by Kittiphattanabawon et al. (2012) and Thuy et al. (2022), who reported that gelatin from shark skin and tra catfish skin with a lower wavenumber in amide I and amide III showed a high degree of protein denaturation.

 Table 3. The viscosity (cP) of gelatin from snakehead fish scale and skin-scale mixture.

Temperature (°C)	Time (h)	Fish scale	Fish skin and scale mixture
60	1	7.03 ± 0.032^{bc}	$7.05 \pm 0.042^{\circ}$
60	2	$6.80\pm0.057^{\rm b}$	$6.83\pm0.076^{\text{b}}$
60	3	$6.45\pm0.350^{\text{a}}$	$6.35\pm0.050^{\text{a}}$
70	1	$9.01\pm0.359^{\rm d}$	$10.3\pm0.095^{\rm i}$
70	2	$8.72\pm0.465^{\rm cd}$	$9.01\pm0.359^{\text{gh}}$
70	3	$7.01\pm0.186^{\rm bc}$	$8.73\pm0.176^{\rm fg}$
80	1	$9.78\pm0.189^{\rm g}$	$11.8\pm0.304^{\rm k}$
80	2	$9.32\pm0.076^{\rm f}$	$9.32\pm0.076^{\rm h}$
80	3	$8.73\pm0.176^{\rm cd}$	$8.30\pm0.333^{\rm ef}$
90	1	$9.17\pm0.202^{\text{e}}$	$9.07\pm0.126^{\text{gh}}$
90	2	$8.38\pm0.225^{\circ}$	$8.18\pm0.202^{\text{e}}$
90	3	$7.75\pm0.726^{\text{e}}$	$7.85\pm0.278^{\rm d}$

Data are expressed as mean \pm standard deviation (n = 3). Different superscripts in the same column indicate statistical differences (p < 0.05).

Gelatin from snakehead fish by-products

Temperature (°C)	Time (h)	L*	a*	b*	ΔE
		Gelatin f	rom scale		
60	1	$83.1\pm0.935^{\mathrm{i}}$	2.85 ± 0.286^{ab}	22.8 ± 0.560^{a}	23.9 ± 0.874^{a}
60	2	80.5 ± 0.501^{gh}	3.15 ± 0.275^{bc}	$23.9\pm0.739^{\rm bcd}$	$26.1\pm0.436^{\rm bc}$
60	3	$78.4\pm0.621^{\rm ef}$	2.85 ± 0.162^{ab}	23.5 ± 0.696^{abc}	26.8 ± 0.503^{cd}
70	1	$84.1\pm0.523^{\rm i}$	$2.91\pm0.010^{\rm ab}$	23.4 ± 0.538^{ab}	$24.0\pm0.520^{\rm a}$
70	2	$79.5 \pm 1.329^{\text{fg}}$	2.57 ± 0.199^{a}	$23.5\pm0.685^{\text{abc}}$	$26.2\pm1.01^{\rm bc}$
70	3	$76.1\pm0.489^{\rm d}$	2.76 ± 0.215^{ab}	$24.4\pm0.561^{\rm bcd}$	$28.9\pm0.755^{\rm fg}$
80	1	$83.8\pm0.308^{\rm i}$	$3.44\pm0.191^{\rm cd}$	$24.7\pm0.220^{\rm d}$	$25.4\pm0.231^{\rm b}$
80	2	$81.2\pm0.762^{\rm h}$	2.77 ± 0.126^{ab}	$26.0\pm0.390^{\circ}$	$27.6\pm0.416^{\rm de}$
80	3	$77.9 \pm 0.806^{\circ}$	$3.53\pm0.127^{\rm d}$	$24.5\pm0.499^{\rm cd}$	$28.0\pm0.896^{\rm ef}$
90	1	$78.6 \pm 0.761^{\rm ef}$	$3.63\pm0.060^{\rm d}$	$27.0 \pm 0.666^{\circ}$	29.7 ± 0.252^{g}
90	2	74.1 ± 0.393°	$4.76\pm0.131^{\rm f}$	$30.0\pm0.711^{\rm f}$	$34.8\pm0.404^{\rm i}$
90	3	$72.7\pm0.619^{\mathrm{b}}$	$4.50\pm0.137^{\rm f}$	$30.1 \pm 0.775^{\rm f}$	$36.0\pm0.808^{\rm j}$
Temperature (°C)	Time (h)	L*	a*	b*	ΔΕ
		Gelatin from the mix	ture of skin and scale		
60	1	$84.8\pm0.620^{\rm g}$	2.86 ± 0.265^{de}	$21.8\pm0.586^{\text{d}}$	22.3 ± 0.306^{c}
60	2	$80.7 \pm 0.787^{\rm f}$	$3.08\pm0.181^{\rm ef}$	$23.2\pm0.375^{\rm ef}$	$25.4\pm0.696^{\rm de}$
60	3	$78.3 \pm 0.506^{\circ}$	$2.85\pm0.148^{\rm de}$	$22.5\pm0.542^{\rm de}$	$26.1 \pm 0.711^{\text{ef}}$
70	1	$83.7\pm0.492^{\rm g}$	$1.90\pm0.015^{\rm b}$	$16.8\pm0.336^{\rm a}$	$18.3\pm0.079^{\rm a}$
70	2	$81.9\pm0.078^{\rm f}$	$1.33\pm0.203^{\rm a}$	$16.4\pm0.381^{\rm a}$	$20.8\pm0.223^{\rm b}$
70	3	$76.2\pm0.682^{\rm d}$	1.10 ± 0.059^{a}	$16.4\pm0.441^{\rm a}$	$22.9\pm0.498^{\circ}$
80	1	$84.0\pm0.310^{\rm g}$	$3.30\pm0.107^{\rm fg}$	$24.3\pm0.734^{\rm g}$	$24.9\pm0.549^{\rm d}$
80	2	$81.6\pm0.248^{\rm f}$	2.77 ± 0.076^{cd}	$25.7\pm0.436^{\rm h}$	$27.1\pm0.393^{\rm fg}$
80	3	$78.2\pm1.448^{\rm e}$	$3.43\pm0.068^{\text{g}}$	$24.1\pm0.549^{\rm g}$	$27.5\pm1.069^{\rm g}$
90	1	75.1 ± 0.329°	$4.65 \pm 0.050^{\circ}$	$29.4\pm0.682^{\rm j}$	$33.7\pm0.641^{\rm k}$
90	2	$73.7\pm0.195^{\rm b}$	$4.29\pm0.308^{\rm i}$	$27.3\pm0.965^{\rm i}$	32.8 ± 0.613^{jk}
90	3	72.1 ± 0.654^{a}	4.50 ± 0.200^{ij}	24.6 ± 0.454^{g}	31.8 ± 0.539^{i}

Data are expressed as mean \pm standard deviation (n = 3). Different superscripts in the same column indicate statistical differences (p < 0.05).

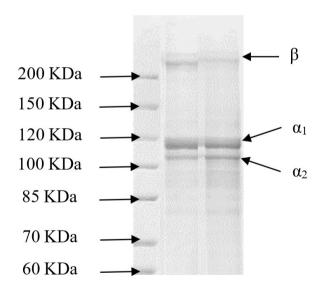


Figure 1. Protein patterns of gelatin from snakehead fish by-products. Lane 1, high molecular weight marker; lane 2, skin and scale mixture; lane 3, fish scale.

3.6 The amino acid composition of gelatin

A similar trend in the amino acid composition of gelatin extracted from snakehead fish by-products at 80 °C for 1 h is presented in Table 5. Glycine, the principal amino acid in fish

Table 5. Amino acid composition of gelatin from snakehead fish scale
and the scale-skin mixture.

	Amino acid content (residues/1000 residues)		
Amino acid	Fish scale gelatin	The mixture of skin and scale	
Aspartic acid	54	50	
Threonine	20	21	
Serine	38	36	
Glutamic acid	74	75	
Glycine	305	311	
Alanine	89	91	
Valine	25	23	
Cystein	2	2	
Methionine	13	12	
Tryptophan	0	0	
Isoleucine	10	9	
Leucine	28	25	
Tyrosine	5	4	
Phenylalanine	19	17	
Hydrolysine	6	6	
Lysine	32	31	
Histidine	6	6	
Arginine	55	56	
Hydroxyproline	93	95	
Proline	126	130	
Imino acid (Proline and Hydroxyproline)	219	225	

scale and the mixture of skin and scale gelatin, accounted for 30% of the total amino acid content. In general, the total amount of proline and hydroxyproline (imino acid) contributes to the stabilization of gelatin by maintaining the triple-helix structure and influencing the gel strength of gelatin (Nikoo et al., 2011). The amount of imino acid in gelatin from scale (219 residues) and the mixture of skin and scale (225 residues) was higher than that in gelatin from horse mackerel scale (178/1000 residues) (Le et al., 2015). In particular, the imino acid content in the gelatin of snakehead fish by-products was equal to that of mammalian gelatin (216-225 residues per 1000 residues) (Avena-Bustillos et al., 2006). This may improve the gel strength of snakehead fish by-product gelatin (245-248 g), similar to that of pork skin gelatin (240 g) (Wasswa et al., 2007).

3.7 FTIR spectroscopy of gelatin from by-products of snakehead fish

The FTIR spectra of gelatin extracted at 80 °C for 1 h from fish-scale and the mixture of skin and scale are presented in Figure 2. Similar patterns in the FTIR spectrum were observed between gelatin from the scale and skin and scale mixture. Amide I of gelatin from scale and skin and scale mixture was observed at wavenumbers of 1645 and 1641 cm⁻¹, respectively. The amide I band (1600-1700 cm⁻¹), with stretching vibrations of the C=O groups in peptides, has a great influence on the secondary structure of proteins (Nikoo et al., 2014). Amide III bands were observed at 1239 and 1236 cm⁻¹ with fish-scale gelatin, fish skin, and scale gelatin, respectively. Amide III plays an important role in the transfer of collagen to gelatin by disrupting the collagen triple-helix structure (Muyonga et al., 2004). The bands of amide I and amide III from snakehead fish-scale gelatin were stronger than those of gelatin from the mixture of skin and scale. This result indicated that under similar extraction conditions, the denaturation of α -helix by uncoupling of intermolecular cross-links and interruption of intramolecular bonding in fishscale gelatin was higher than that in the gelatin mixture. This result was in agreement with that of Kittiphattanabawon et al. (2012) and Le et al. (2015), who reported that the increase in the wavelength number of amide I and III of gelatin from shark skin and scale of horse mackerel, respectively, is related to the denaturation of the α -helix in the gelatin structure.

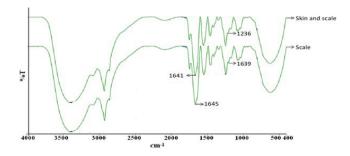


Figure 2. FTIR of gelatin from scale and skin-scale mixture of snakehead fish.

4 Conclusion

The properties of gelatin extracted from the snakehead scale and a mixture of skin and scale were specified. The extraction yield and viscosity of gelatin from fish skin and scale were higher than those from fish scale. Furthermore, the protein denaturation of fish scale gelatin was higher than that of the skin and scale mixture, as indicated by the SDS-PAGE profile and FTIR spectra. The results indicated gelatin from the mixture with high extraction yield and showed the stabilization in the structure under higher temperature and time during extracted. Therefore, a mixture of skin and scales from snakehead fish could be used for gelatin production.

Ethical approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

Conflict of interest

There are no potential conflicts of interest to disclose.

Author contributions

Before publishing the final version, the manuscript was conceptualized, written, and revised thoroughly by all authors.

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