CHARACTERISTICS AND GELLING PROPERTY OF GELATIN FROM SCALE OF SPOTTED GOLDEN GOATFISH (*PARUPENEUS HEPTACANTHUS*)

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SIRA CHUAYCHAN[1], SOOTTAWAT BENJAKUL[1*] AND HIDEKI KISHIMURA[2]

[1]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[2]Laboratory of Marine Products and Food Science, Research Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

*To whom correspondence should be addressed. Tel: 66-7428-6334

Fax: 66-7455-8866, E-mail: soottawat.b@psu.ac.th

ABSTRACT

Gelatins from the scales of spotted golden goatfish were extracted under different conditions and characterized. The gelatin yield, when extracted at 45, 60 and 75 C (for 6 or 12 h), was 2.3-2.6%, 8.6-9.3% and 9.9-10.1% on dry weight basis, respectively. All the gelatins had β- and α-chains as the dominant components, and had high imino acid contents (182-192 residues/1000 residues). Gel strength of the gelatin decreased and the gelatin solution became more turbid with increasing temperature and time of extraction. The gelling and melting temperatures of gelatin were 18.7-20.1 and 26.4-28.0 C, respectively, and these decreased with extraction temperature and time. The results suggest that the scales of spotted golden goatfish have potential to serve as the collagenous raw material for gelatin extraction.

Keywords: Scales, spotted golden goatfish, gelatin, yields, gel strength

PRACTICAL APPLICATIONS

Spotted golden goatfish (*Parupeneus heptacanthus*) is used in the manufacturing of frozen fish fillets in Thailand, and the scales with low market value are generated as byproducts. Large amounts of scales could potentially serve as raw material for gelatin production. However, the proper extraction conditions need to be determined experimentally, considering both yield and properties of the resulting gelatin.

INTRODUCTION

Gelatin is a fibrous protein obtained from collagenous materials subjected to thermal denaturation or partial degradation. It has been widely used in food and non-food (photographic, cosmetic, and pharmaceutical) industries [1]. Generally, gelatin is produced from skins and skeletons of land animals [2]. However, both bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) are human health concerns with gelatin from these sources. Furthermore, porcine and bovine gelatins are prohibited by some religions. Fish gelatin has therefore received increasing attention as an alternative to mammalian and avian gelatins. Fish skin, bones, and scales are by-products from fish processing, but they are collagenous materials with potential for gelatin production [3]. Gelatin has several functional properties, including gelling, foaming, emulsifying and wetting properties. Generally, the functional properties of gelatin and other food proteins are governed by many factors such as chain lengths or molecular weights, amino acid composition and hydrophobicity, etc. [4]. Additionally, the suitability of gelatin in a particular application is greatly influenced by the source of raw materials, varying by animal species, and also by the processing [4].

Spotted golden goatfish (*Parupeneus heptacanthus*) has been widely used in Thailand, in frozen fillet manufacturing. During descaling and dressing, large amounts of scales are generated as low-value by-products. Converting to or extracting value-added products, particularly gelatin, could both reduce waste and be an economic benefit. Gelatins have been extracted from the scales of several fish species, e.g., tilapia [5], bighead carp [6], and grass carp fish [7], and the characteristics of such gelatins have been assessed. However, there is no prior information available on gelatin from the scales of spotted golden goatfish, or on effects of extraction

conditions. Therefore, the aim of this study was to characterize gelatins with various extraction temperatures and times, using spotted golden goatfish scales as raw material.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulfate (SDS), Coomassie blue R-250 and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were purchased from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with 150-250 g bloom strength was obtained from Halagel (Thailand) Co., Ltd., (Bangkok, Thailand).

Collection and preparation of spotted golden goatfish scales

Scales of spotted golden goatfish with an average body weight of 100-120 g/fish were collected from Kingfisher Holding, LTD., Songkhla Province, Thailand. The scales were packaged in polyethylene bags and transported in ice to the Department of Food Technology, Prince of Songkla University, within approximately 1 h. Upon arrival, the scales were washed with tap water and drained before storing in polyethylene bags. The samples were kept at -20 C for storage times not exceeding 2 months. Before use, the frozen fish scales were tempered at 4 C overnight (8-10 h), and completely thawed using the running water for 2 min.

Pretreatment of spotted golden goatfish scales

The scales were suspended in 0.1 mol/L NaOH for 6 h, at the ratio 1:10 (w/v). The mixture was continuously stirred to remove non-collagenous proteins. The alkaline solution was

changed once at 3 h. Treated scales were then washed with tap water, until the wash water had neutral pH. Subsequently, the prepared scales were demineralized in 0.75 mol/L HCl, with the scale/solution ratio of 1:5 (w/v). The demineralization was performed at room temperature (28-30 C) with continuous stirring. Thereafter, the demineralized scales were washed until neutral pH of wash water was obtained.

Extraction of gelatin

The demineralized scales were suspended in distilled water at the ratio 1:10 (w/v). The extraction was conducted at different temperatures (45, 60 and 75 C) for various times (6 and 12 h), under continuous stirring. At the designated time to stop extraction, the mixture was filtered using a Buchner funnel with Whatman No.4 filter paper (Whatman International, Ltd., Maidstone, England). The filtrate was dried in a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark), and the gelatin sample obtained was subsequently subjected to analyses.

Analyses

Yield

The yield of gelatin was calculated as percentage of the starting material (scales before pretreatment) by dry weight, as follows:

$$\text{Yield \%} = \frac{\text{Weight of freeze-dried gelatin (g)}}{\text{Weight of initial dry scales (g)}} \times 100$$

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein pattern in each gelatin sample was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), following the method of Laemmli [8] as modified by Sinthusamran et al. [9]. High-molecular-weight protein markers were used to estimate the molecular weights of the proteins.

Fourier transform infrared (FTIR) spectroscopic analysis

The FTIR spectra for all gelatin samples were obtained by total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, as described by Sinthusamran et al. [9]. Each gelatin sample was placed onto a crystal cell. The spectrum was acquired over the range 650–4000 cm^{-1} with 4 cm^{-1} resolution, by averaging 32 repeat scans, referenced against the background spectrum recorded for a clean empty cell at 25 C, using an FTIR spectrometer (Model Equinox 55, Bruker, Ettlingen, Germany). Deconvolution was performed on the average spectra for the amide I and II bands, using a resolution enhancement factor of 1.8 and full height bandwidth of 13 cm^{-1}. The analysis of spectral data was done with OPUS 3.0 data collection software (Bruker, Ettlingen, Germany).

Amino acid analysis

The amino acid composition of each gelatin sample was analyzed according to the method of Nagarajan et al. [10], with a slight modification. The content is expressed as residues/1000 residues.

Determination of gel strength

The gels were prepared by the method of Kittiphattanabawon et al. [11]. First, gelatin was dissolved in distilled water (60 C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until the gelatin was completely dissolved, and was poured into a cylindrical mold with 3 cm diameter and 2.5 cm height, where it was incubated at 4 C for 18 h prior to analysis. The gel strength was determined at 8–10 C using a texture analyzer (Stable Micro System, Surrey, UK) with a 5 kg load cell, cross-head speed set at 1 mm/s, and equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams) was recorded when the plunger had penetrated 4 mm into the gelatin gel.

Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of Boran et al. [12], using a controlled-stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany) with a stainless steel 60-mm-diameter parallel plate geometry. The gelatin solution (6.67%, w/v) was prepared as described previously. An amplitude sweep test revealed that 3 Pa stress at 1 Hz frequency was within the linear viscoelastic region (LVR) for the sample. The measurement was performed at a scan rate of 0.5 C/min, at 1 Hz frequency with 3 Pa stress amplitude, during cooling from 40 to 5 C and heating from 5 to 40 C. The gelling and melting temperatures were defined by the loss tangent value $\tan \delta = 1$, or equivalently $\delta = 45^{o}$.

Determination of turbidity

The turbidity of each gelatin solution was determined according to the method of Kittiphattanabawon et al. [11]. Gelatin was dissolved in distilled water (60 C) to obtain a final

concentration of 6.67% (w/v). The solution was stirred until the gelatin was completely dissolved. Turbidity was determined by measuring the absorbance at 360 nm with a spectrophotometer (UV-1800 Spectrophotometer, Shimadzu, Kyoto, Japan).

Microstructure analysis of gelatin gel

The microstructure of gelatin gels was imaged by a scanning electron microscopy (SEM). A gelatin gel sample (6.67%, w/v) with 2–3 mm thickness was fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The sample was then rinsed with distilled water for 1 h, and dehydrated stepwise in ethanol concentrations of 50, 70, 80, 90 and 100% (v/v). The dried sample was mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimen was then observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) set at 20 kV acceleration voltage.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test. For paired comparisons, a T-test was used. In statistical tests, p-values of less than 0.05 were considered significant. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Yield

The gelatin yield from the scales of spotted golden goatfish is shown in Table 1 for the various extraction conditions. The yield increased with extraction temperature ($P < 0.05$): at 45, 60 and 75 C, the gelatin yields were 2.27-2.57%, 8.63-9.27% and 9.90-10.07% on dry weight basis, respectively. The yield also increased with extraction time ($P < 0.05$), except at 75 C, the temperature that showed no significant difference between 6 h and 12 h extractions ($P > 0.05$). As temperature is increased, the bonds between α-chains in native collagen are effectively destroyed. As a consequence, the triple helix structure became amorphous and could be extracted with ease, giving high yields at elevated temperatures. Increasing the extraction time also favored the release of α and β-chains from the preprocessed fish scales [13]. The results qualitatively agree with Nagarajan et al. [10], who reported that the extraction yield of gelatin from splendid squid skin increased with extraction temperature and time. Arnesen and Gildberg [14] also found that increasing the extraction temperature increased the yield of gelatin from Atlantic salmon skin. Different yields of fish gelatin have been reported for sea bream scale (9.55%) [15], greater lizardfish skin (35.1%) [16] and nile perch skin (64.3%) [17]. It is noted that gelatin yield from the scales of spotted golden goatfish was much lower than the reported yields from fish skin. The scales have compact and dense structures, but the pretreatments, especially the demineralization, provided the scales with looser structures [18]. As a result, hot water could penetrate the scales to extract gelatin. The gelatin yield depends on the type of raw material and on the gelatin extraction process, including the pretreatments [11].

Protein patterns

The protein patterns of gelatin are illustrated in Fig. 1, for the gelatins from scales of spotted golden goatfish, with various temperatures and durations of extraction. All the gelatin samples contained β- and α-chains with MWs of 192 and 123-112 kDa, respectively, as the major constituents. The protein patterns were similar for samples extracted at 45 and 60 C, except that α_2-chains in the latter showed lower band intensity. Extraction at 75 C gave noticeably lower band intensities for most proteins, except for the α_1-chains that remained unchanged. However, no marked differences in protein patterns were observed between 6 and 12 h extractions of gelatin. The results suggest that elevated temperatures induced degradation of protein chains. This is in accordance with Kaewruang et al. [2], who found the pronounced degradation of unicorn leatherjacket skin gelatin with elevated extraction temperatures. The content of α-chains in a gelatin determines its main functional properties, such as gelling, emulsifying and foaming properties [4]. The extraction conditions, both temperature and duration, clearly affected the constituents of gelatin from spotted golden goatfish scales.

Fourier transform infrared (FTIR) spectra

The FTIR spectra are shown in Fig. 2 for gelatin from scales of spotted golden goatfish with various temperatures and durations of extraction. FTIR spectroscopy has been used to determine the functional groups and the secondary structures in gelatin [17]: the major peaks of gelatins were situated in Amide I, II, III, A and B regions. The amide I primary vibration mode is a C=O stretching vibrations coupled to contributions from CN stretching, CCN deformation, and in-plane NH bending modes [19]. Similar amide I band was found in all the gelatin samples, appearing over wavenumbers 1640-1643 cm^{-1}. Kittiphattanabawon et al. [11] reported that with

higher temperatures and longer times of extraction, the amide I band of gelatin from shark skin shifted towards higher wavenumbers [11]. The difference between Amide I of gelatin from spotted golden goatfish and other gelatins might be due to different thermal stabilities of gelatins from various sources. It was found that the α_1-chains in gelatin from scales of spotted golden goatfish showed high thermal stability, as evidenced by this component persisting at elevated extraction temperatures (Fig. 1). Sinthusamran et al. [13] and Kaewruang et al. [2] suggested that the loss of triple helix structure was due to disruption of interchain interactions under harsh conditions. The amide II band of gelatins extracted at 45, 60 and 75 C (for both extraction durations) were observed at wavenumbers 1540-1543 cm^{-1}. The amide II vibration mode is attributed to an out-of-phase combination of CN stretching and in-plane NH deformation modes of the peptide group [19]. All gelatin samples exhibited generally similar spectra, both in terms of wavenumbers and amplitudes. Additionally, the absorption bands of all gelatins in the amide-III region appeared at similar wavenumbers ranging within 1539-1541cm^{-1}. The amide III band represents the combination of CN stretching vibrations and NH deformations at the amide linkages, as well as absorption by wagging vibrations of CH_2 groups in the glycine backbone and in the proline side-chains. It has been used to indicate the disorder from α-helical to random-coil structures [19].

Furthermore, the amide A band was at 3290, 3292 and 3294 cm^{-1} for gelatins extracted at 45, 60 and 75 C for 6 h, at 3291, 3294 and 3296 cm^{-1} for gelatins extracted for 12 h in the same order of temperatures. The amide A band is associated with the N–H stretching vibrations and indicates hydrogen bonds. Generally, free N–H stretching vibrations occur in the range of 3400–3440 cm^{-1}. When the N–H group of a peptide is involved in a hydrogen bond, the resonance shifts to lower frequencies. The amide A band shifted to higher wavenumbers with increased

253 extraction temperature and with longer extraction times. At elevated temperatures of extraction,
254 the interchain bonds between α- or β-chains were increasingly disrupted, matching the higher
255 yields observed (Table 1). The amide B band was observed at wavenumbers 3086, 3077 and
256 3075 cm^{-1} for gelatin extracted at 45, 60 and 75 C for 6 h; and at 3081, 3076 and 3072 cm^{-1} for
257 gelatin extracted at 45, 60 and 75 C for 12 h, respectively. Gelatin extracted at a higher
258 temperature for a longer time exhibited the lower wavenumbers. The amide B band vibration
259 mode is asymmetric stretching vibrations of =C–H as well as of NH_3^+. The observations suggest
260 that the interaction of $^-NH_3$ groups between peptide chains was more pronounced in gelatin
261 extracted at higher temperature for a longer time [10]. Therefore, the secondary structures and
262 functional groups of gelatins obtained from scales of spotted golden goatfish were affected by
263 extraction temperature and time.
264
265 **Amino acid composition**
266 The amino acid composition of gelatin extracted under the various extraction conditions
267 is shown in Table 2. All the gelatin samples showed similar amino acid compositions, in which
268 glycine was the major amino acid (334–335 residues/1000 residues). Glycine is located at every
269 third position of an α-chain and represents nearly one third of its total residues [1]. All the gelatin
270 samples contained imino acids, including proline (108-113 residues/1000 residues) and
271 hydroxyproline (74-79 residues/1000 residues). Different imino acid contents have been reported
272 for gelatin from various fish species, such as from sea bream scales (185 residues/1000 residues)
273 [15], from grass carp scales (157 residues/1000 residues) [7] and from cod skin (154
274 residues/1000 residues) [14]. With high content of hydroxyproline, gelatin is believed to have
275 highly viscoelastic properties and to develop strong gel structures [4]. Hydroxyproline also plays

an essential role in the stabilization of triple helix strands in the mother collagen via hydrogen bonding at its OH group [1]. In gelatin, the OH groups of hydroxyproline might be involved in hydrogen bonds of α- or β-chains, thereby strengthening the gel network. In the present study, it was found that both hydroxyproline and proline contents of gelatin decreased with the extraction temperature. The lowest imino acid content was obtained for gelatin extracted at 75 C. Alanine was also found at high contents (133-136 residues/1000 residues). However, very low cysteine levels were obtained for gelatin from spotted golden goatfish scales. Collagen and gelatin have been known to be free of cysteine [15]. The results suggest that extraction temperature and time affected the amino acid composition of gelatin from the scales of spotted golden goatfish.

Gel strength of gelatin

The gel strength is shown in Table 1, for gelatin extracted from spotted golden goatfish scales under the various extraction conditions. At a fixed extraction time, the gel strength of gelatin decreased with the extraction temperatures ($P < 0.05$). For gelatins extracted at a fixed temperature, the lower gel strength was observed with the longer extraction time ($P < 0.05$). Among the cases tested, gelatin extracted at 45 C for 6 h showed the highest gel strength (286.6 g). The gel strength correlated with the band intensities of β- and γ-chains (Fig. 1). The amounts of β- and γ-components have been reported as the major factor governing the gelation of gelatin [16]. Additionally, the imino acids played a role in gel formation. Hydroxyl groups of hydroxyproline are involved in inter-chain hydrogen bonding, both via a bridging water molecule as well as by direct hydrogen bonding to the carbonyl group [1]. Different gel strengths have been reported for gelatins from different fish species, including from sea bream scales (126 g) [15], and from lizardfish scales (268 g) [20]. It is noted that gelatin extracted at the highest

299 temperature tested, especially for 12 h, showed comparatively low gel strength ($P < 0.05$). This
300 coincides with the lowest imino acid content of this case (182 residues/1000 residues). The
301 differences in gel strength between the cases could stem from such intrinsic characteristics as
302 molecular weight distribution and amino acid composition.
303
304 **Gelling and melting temperatures**
305 Thermal transitions were monitored by changes in the phase angle (δ) as shown in Fig 3,
306 for dissolved gelatin from the scales of spotted golden goatfish, both during cooling (40-5 C) and
307 during subsequent heating (5-40 C). The gelling temperatures of gelatin were in the range of
308 18.7- 20.1C. The formation of junction zones and a three-dimensional gel network during
309 cooling of gelatin can be monitored via the phase angle [13]. Nagarajan et al. [10] reported that
310 the amounts of γ-, β-, and α-chain components influenced the gelling point of gelatin. Gelling
311 temperatures have been reported earlier for gelatins from different sources, such as scales of sea
312 bream (20.8 C) [15] and scales of grass carp (20.8 C) [7]. In the current study, gelatin extracted
313 at higher temperatures, especially for the longer time, showed lower gelling temperatures,
314 coinciding with low imino contents (Table 2). Generally, fish gelatin has lower gelling and
315 melting temperatures than its mammalian counterparts. This could be due to the low imino acid
316 content of fish gelatin [21].
317 The melting temperature of gelatin from spotted golden goatfish scales ranged from 26.4
318 to 28.0 C. The gelatin samples extracted at low temperature and short time had the higher
319 melting temperatures. This suggests that these gels could be maintained in semi-solid state for a
320 longer time when heat is applied, e.g. during chewing in the mouth. Conversely, gelatins
321 extracted at higher temperatures showed lower melting temperatures. The melting temperature of

gelatin samples correlated positively with the gel strength (Table 1). Varying melting temperatures have been reported for gelatin from various sources, such as from grass carp scales (26.9 C) [7] and from sea bream scales (26.0 C) [15]. Generally, the thermal stability of gelatin gel has been shown to directly correlate with the number and stability of Pro-rich regions in the collagen or gelatin molecules, which are considerably lower in cold water fish than in warm blooded animals. Temperature of the environment that the animals inhabit affects the gelling and melting temperatures of the resultant gelatin [21].

Turbidity

Turbidity of the gelatin solutions is shown in Table 1, expressed as $A_{360,}$ for the various extraction conditions from spotted golden goatfish scales. The turbidity increased with the extraction temperature ($P < 0.05$). At a fixed extraction temperature, the turbidity increased with extraction time ($P < 0.05$), except for 45 C, the temperature that gave no difference in turbidity between 6 and 12 h extractions. It is noted that the turbidity of gelatin solutions from spotted golden goatfish was higher than that of the bovine gelatin solution ($p < 0.05$). Random aggregation of the gelatin molecules from spotted golden goatfish scales might cause the increased turbidity. When proteins are subjected to elevated temperatures, aggregation can be induced, resulting in increased turbidity. This is in agreement with the shift of amide B spectral band to lower wavenumbers (Fig. 2). [11] also reported that gelatin solutions from the skin of blacktip shark had increased turbidity with increasing extraction temperature and time. In the present study, the solutions were not clarified, whereas commercial gelatin solutions are commonly clarified using activated charcoal [22]. Therefore, the extraction conditions affected the appearance of gelatin solution as well as the resulting gels.

Microstructures of gelatin gels

Gel microstructures for the gelatins from the spotted golden goatfish scales, extracted at different temperatures for various times, are shown in Fig. 4. For a fixed extraction time, gelatin extracted at higher temperature yielded the coarser gel network with larger voids. Furthermore, the gel from gelatin extracted over a longer time was less uniform with coarser structure. Among all the cases, gelatin extracted at 45 C for 6 h exhibited the finest gel network with a high connectivity. Conversely, the largest strands and voids were found in the case of gelatin extracted at 75 C for 12 h. In general, fine network structure of gelatin gel positively correlated with gel strength (Table 1). Gelatin extracted at low temperature over a short time most likely had chains with comparatively high molecular weight (Fig. 1). Sinthusamran et al. [13] reported that denser strands in gel structure were governed by the content of high molecular weight constituents (γ- and β-chains) in gelatin, while loose strands in the gel matrix were found for gelatins containing comparatively small and short chains. The long chains could form junction zones with ordered alignment in the gel, leading to strong aggregation with connectivity. The results reveal that the extraction condition impacted the molecular arrangement of gelatin chains in the gel network, thereby determining the properties of gels.

CONCLUSIONS

Gelatin from the scales of spotted golden goatfish was extracted using water at different temperatures for various durations, and this affected the gelatin characteristics and properties. Extraction at higher temperatures for longer times resulted in higher yields; however, the gel became weaker. The scales of spotted golden goatfish could serve as raw material for gelatin

extraction, and the extraction conditions should be appropriately selected for the intended use of the gelatin.

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FIGURE CAPTIONS

FIGURE 1 SDS-PAGE PATTERNS OF GELATIN FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT VARIOUS TEMPERATURES FOR VARIOUS TIMES. M DENOTES HIGH MOLECULAR WEIGHT MARKERS.

FIGURE 2 FTIR SPECTRA OF GELATIN FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT VARIOUS TEMPERATURE FOR VARIOUS TIMES.

FIGURE 3 CHANGES IN THE PHASE ANGLE (Δ, ^{O}) OF GELATIN SOLUTION (6.67%, W/V) FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT VARIOUS TEMPERATURES FOR VARIOUS TIMES, DURING COOLING (A) AND SUBSEQUENT HEATING (B).

FIGURE 4 MICROSTRUCTURES OF GELATIN GELS FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT VARIOUS TEMPERATURES FOR VARIOUS TIMES. MAGNIFICATION: 3000×

TABLE 1

EXTRACTION YIELD (% DRY WEIGHT BASIS), GEL STRENGTH AND TURBIDITY OF GELATIN FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT DIFFERENT TEMPERATURES FOR VARIOUS TIMES.

Temperature (^{o}C)	**Time (h)**	**Yields (%)**	**Gel strength (g)**	**Turbidity (OD_{360})**
45	6	2.27 ± 0.12^{AaX}	286.61 ± 1.02^{BfZ}	0.791 ± 0.81^{AaX}
	12	2.57 ± 0.29^{BbX}	249.62 ± 0.53^{AeZ}	0.806 ± 0.83^{AaX}
60	6	8.63 ± 0.22^{AcY}	227.77 ± 1.52^{BdY}	0.939 ± 0.97^{AbY}
	12	9.27 ± 0.43^{BdY}	206.63 ± 0.91^{AcY}	1.194 ± 0.62^{BcY}
75	6	9.90 ± 1.76^{AeZ}	154.07 ± 1.20^{BbX}	1.493 ± 0.96^{AdZ}
	12	10.07 ± 0.93^{AeZ}	124.86 ± 1.38^{AaX}	1.700 ± 0.65^{BeZ}

Values are presented as mean ± SD (n = 3).

Different uppercase letters (A and B) in the same column under the same temperature indicate significant differences ($P < 0.05$). Different lowercase letters in the same column indicate significant differences ($P < 0.05$). Different uppercase letters (X, Y and Z) in the same column for the same time indicate significant differences ($P < 0.05$).

TABLE 2

AMINO ACID COMPOSITION OF GELATIN FROM THE SCALES OF SPOTTED GOLDEN GOATFISH, EXTRACTED AT DIFFERENT TEMPERATURES FOR VARIOUS TIMES.

Amino acids	**Extraction temperature / time**					
	45 °C		**60 °C**		**75 °C**	
	6 h	**12 h**	**6 h**	**12 h**	**6 h**	**12 h**
Alanine	135	135	134	136	133	135
Arginine	53	54	54	53	54	53
Aspartic acid/asparagine	44	43	43	43	43	43
Cysteine	2	1	1	1	1	1
Glutamic acid /glutamine	71	72	72	72	72	75
Glycine	335	335	335	335	335	334
Histidine	6	6	7	6	7	8
Isoleucine	7	7	8	7	7	7
Leucine	17	19	19	19	19	19
Lysine	26	26	29	27	27	27
Hydroxylysine	6	6	6	7	6	6
Methionine	13	14	13	14	14	14
Phenylalanine	14	13	14	12	16	14
Hydroxyproline	79	78	76	77	75	74
Proline	113	112	109	110	109	108
Serine	36	36	37	37	36	38
Threonine	23	23	23	23	25	23
Tyrosine	2	2	2	3	3	3
Valine	18	18	18	18	18	18
Total	1000	1000	1000	1000	1000	1000
Imino acid	192	190	185	187	184	182

Figure1

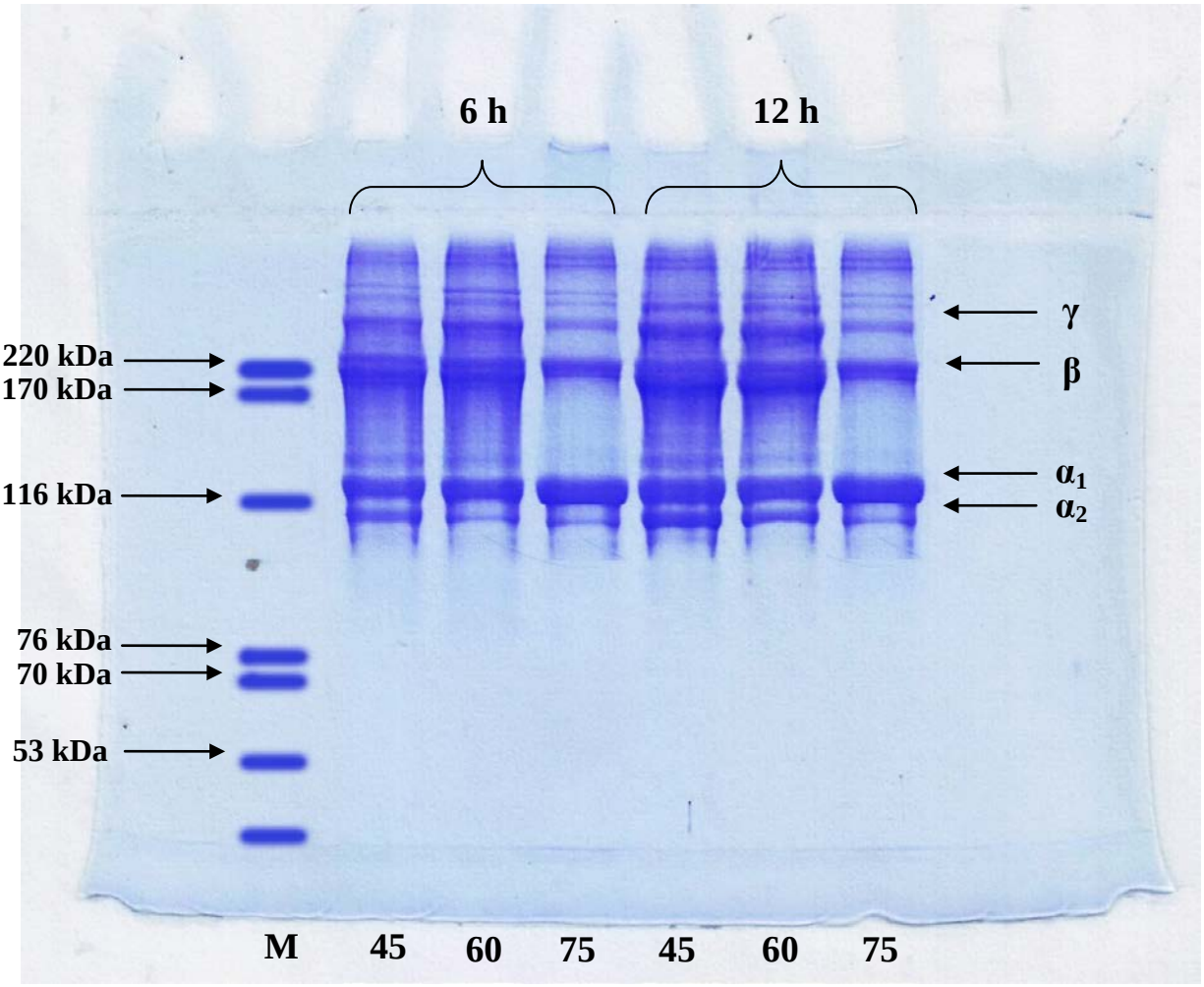

6 h
12 h
220 kDa
170 kDa
116 kDa
76 kDa
70 kDa
53 kDa
γ
β
α1
α2
M
45
60
75
45
60
75
Extraction temperature (C)

Figure 2

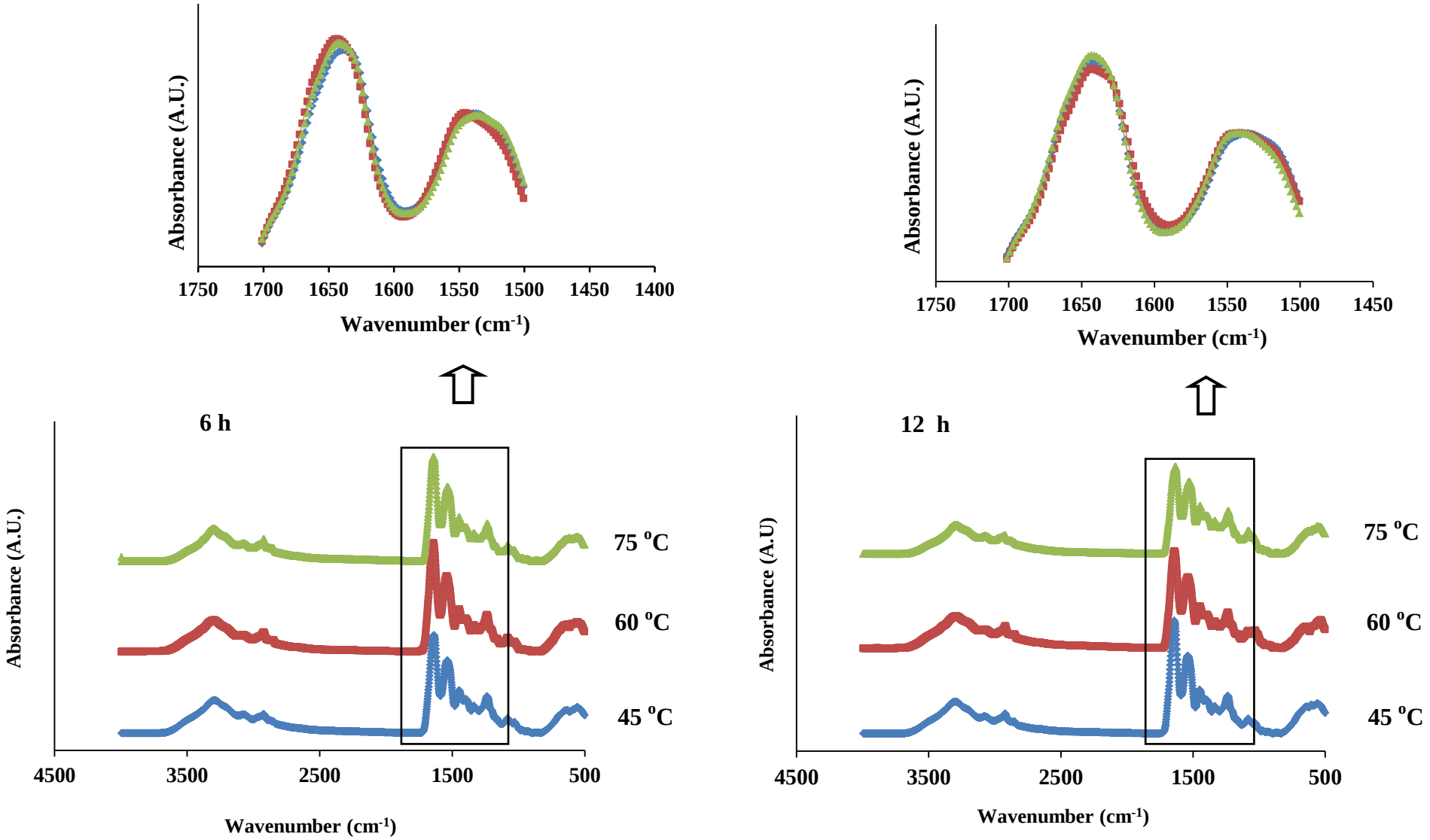
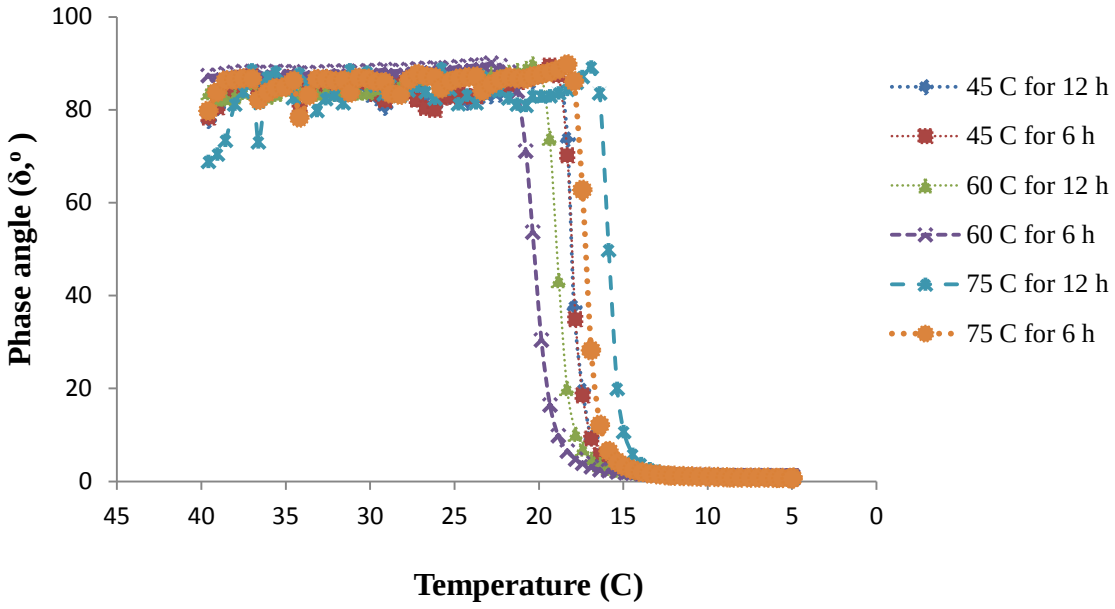

Absorbance (A.U.)
1750 1700 1650 1600 1550 1500 1450 1400
Wavenumber (cm-1)
Absorbance (A.U.)
1750 1700 1650 1600 1550 1500 1450
Wavenumber (cm-1)
6 h
12 h
Absorbance (A.U.)
Absorbance (A.U)
75 °C
60 °C
45 °C
4500 3500 2500 1500 500
Wavenumber (cm-1)

Figure 3

A


Phase angle (δ,°)
100
80
60
40
20
0
45
40
35
30
25
20
15
10
5
0
Temperature (C)
45 C for 12 h
45 C for 6 h
60 C for 12 h
60 C for 6 h
75 C for 12 h
75 C for 6 h

B

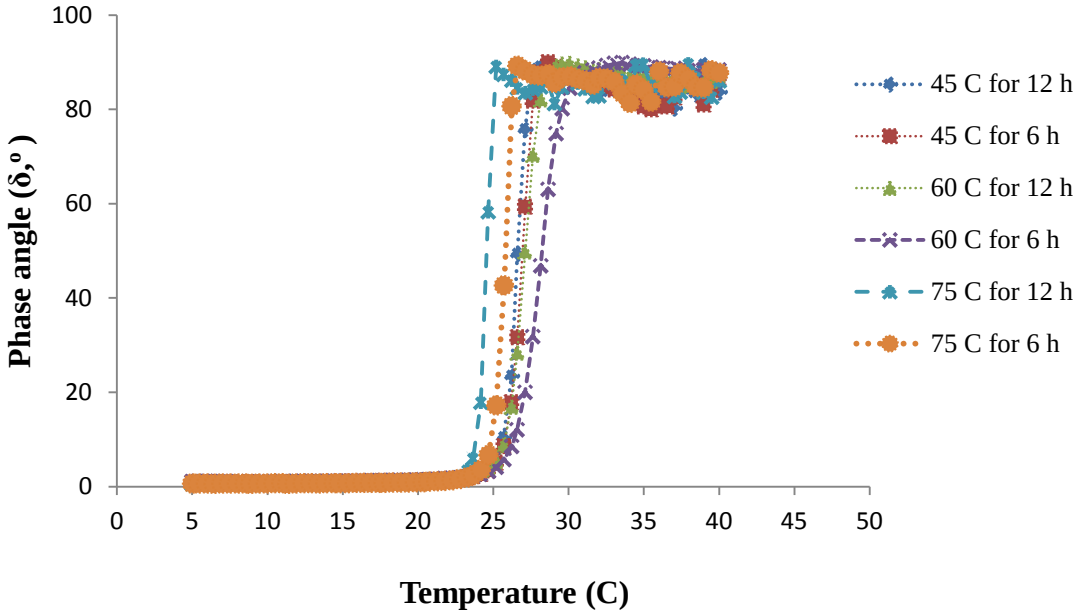
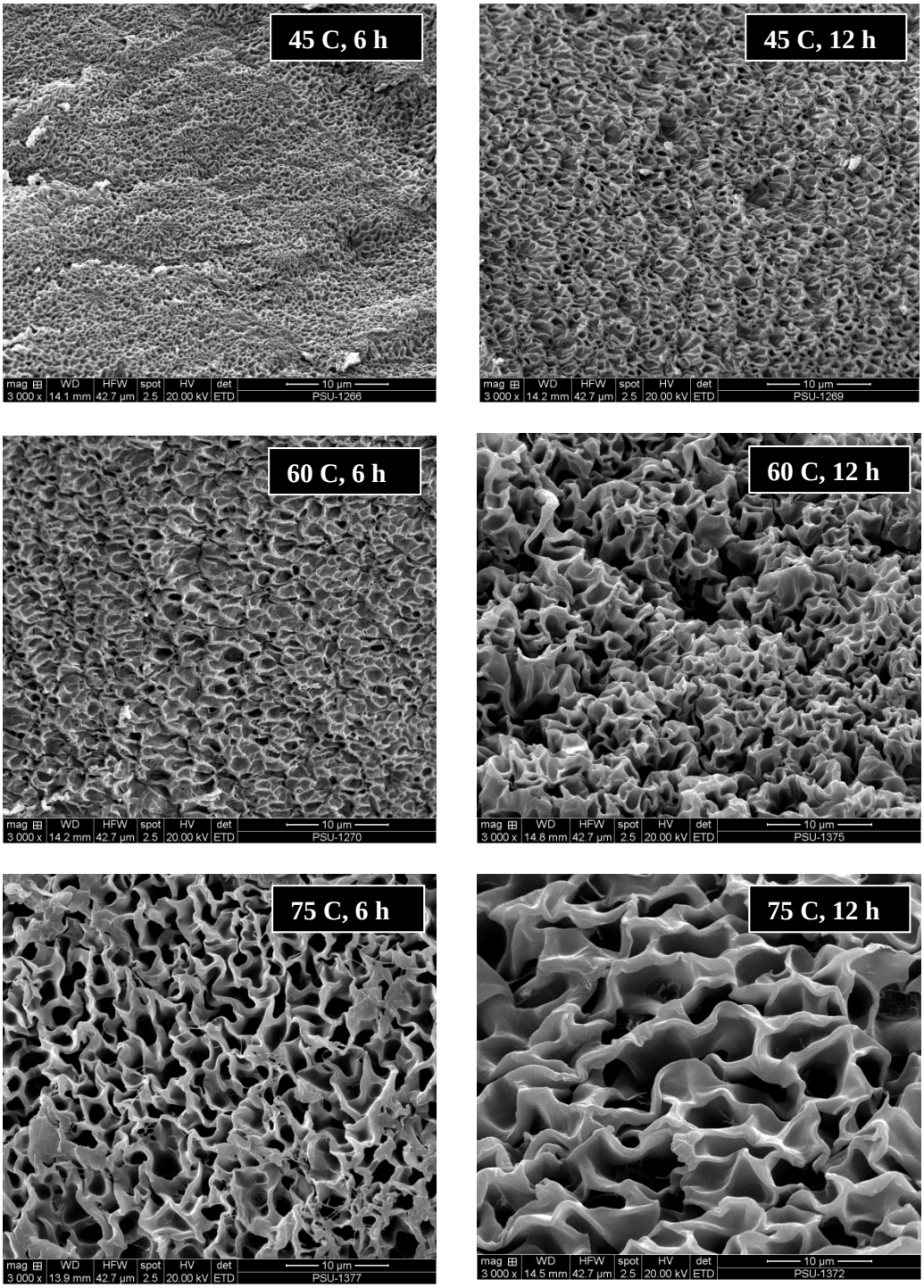

Phase angle (δ,°)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
Temperature (C)
45 C for 12 h
45 C for 6 h
60 C for 12 h
60 C for 6 h
75 C for 12 h
75 C for 6 h

Figure 4


45 C, 6 h
mag WD HFW spot HV det
3 000 x 14.1 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1266
45 C, 12 h
mag WD HFW spot HV det
3 000 x 14.2 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1269
60 C, 6 h
mag WD HFW spot HV det
3 000 x 14.2 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1270
60 C, 12 h
mag WD HFW spot HV det
3 000 x 14.8 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1375
75 C, 6 h
mag WD HFW spot HV det
3 000 x 13.9 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1377
75 C, 12 h
mag WD HFW spot HV det
3 000 x 14.5 mm 42.7 µm 2.5 20.00 kV ETD
10 µm
PSU-1372