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Comparative study on the gel properties and nanostructures of gelatins from chicken, porcine, and tilapia skin

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Abstract: To clarify the feasibility of replacing commercial gelatin with chicken skin gelatin, we investigated the gel properties and nanostructures of chicken skin gelatin (CG), commercial porcine skin gelatin (PG), and tilapia skin gelatin (FG). Compared with PG and FG, CG exhibited the better gel strength, hardness, chewiness, melting point, gelling temperature, and thermostability. The different physicochemical properties of CG might be caused by its higher imino acid content (25.43 residues/100 total residues), which make it more liable to form intramolecular H-bonds (lower amplitude of amide A wave number). In addition, atomic force microscopy (AFM) result was shown that CG contained larger spherical aggregates (483 nm) than PG and FG (334 and 224 nm, respectively), and the lack of chain and ring-like structure promoted the formation of a dense rigid gel. These results revealed that the intramolecular H-bond and the aggregation behavior are the fundamental explanations for the different gel properties of gelatins from three sources.

Practical Application: This research provides guidance for the application of chicken skin gelatin as a replacer for commercial gelatin. And the results provide a theoretical basis for the modification of chicken skin gelatin.

KEYWORDS
Gel properties, Atomic force microscopy (AFM), Nanostructure, gelatin, ingredient replacement

1 | INTRODUCTION

Gelatin is a biopolymer made from the partially hydrolyzed collagen from animal skin, bones, tendons, and other connective tissues (Sarbon et al., 2013; Sow, Tan et al., 2019). Gelatin has good film-forming, gelling, foaming, and emulsifying properties, thus it is often used in food, pharmaceutical, photographic, and cosmetic industries (Hernández-Nava et al., 2019; Sarbon et al., 2013; Yu et al., 2019). Worldwide, about 85% of gelatin is manufactured from porcine skin, cattle hides, and bovine bones (Sow & Yang, 2015). However, mammalian-based gelatin is often not suitable for Muslims who cannot consume porcine or bovine products. In recent years, as the demand for non-mammalian gelatin has increased, it has become increasingly important to develop alternative gelatin from other sources, including fish skin gelatin, such as from squid (Nagarajan et al., 2012), hoki (Mohtar et al., 2014; Mohtar et al., 2010), cod (Cai et al., 2018), horse mackerel (Le et al., 2015), and tilapia (Sow et al., 2019). In addition, by characterizing the physicochemical properties and microstructure of gelatin, the difference between fish skin gelatin and mammalian-based gelatin was analyzed. In general, fish skin gelatin has poorer gel strength and rheological properties compared with bovine or porcine gelatin, limiting its use as a substitute for mammalian-based gelatin (Feng et al., 2017; Huang, et al., 2020; Pang et al., 2017).

Therefore, new resources of gelatin, such as chicken gelatin, should be exploited. Chicken skin is a major source of by-product scraps from poultry processing, with general stronger gel strength and higher thermal stability than bovine gelatin, making it a good potential substitute for mammalian-based gelatin (Sarbon et al., 2013). Gelatin's gel strength, rheological properties, thermal stability, and its ultimate application are closely related to its amino acid composition, micromorphology, and nanostructure (Sow et al., 2019). Therefore, it is important to determine the microstructure characteristics and illustrate the effects of structure on the physicochemical properties of chicken skin gelatin for its future development and application.

The purpose of our study was to examine whether chicken skin gelatin could be a potential gelatin source, which meets the religious requirement for ethnic groups. The gel properties of chicken skin gelatin with those of commercially available porcine skin gelatin and fish skin gelatin were compared, including the determination of the gel strength, texture properties, viscosity, melting point, gelling temperature, and thermal properties. Meanwhile, the imino acid content, the secondary structure, and nanostructure of different gelatins were further analyzed. The results confirmed the differences between the gelatins from different sources and revealed the underlying causes.

Our work has theoretical significance for the modification and application of chicken skin gelatin.

2 | MATERIALS AND METHODS

2.1 | Sample preparation

2.1.1 | Tilapia skin gelatin and porcine skin gelatin

Commercial tilapia (*Oreochromis aureus*) skin gelatin (type B, 180 bloom), porcine skin gelatin (type B, 240 bloom) were purchased from Huachang Food Ingredients Company (Huachang, Co., Qingdao, Shandong, China).

2.1.2 | Chicken skin gelatin extraction

The preparation of chicken skins followed the extraction procedures of Yang et al. (2008) with slight modifications. Fresh chicken skins bought from the local market (Lianhua market, Zhengzhou, Henan, China) and delivered to the laboratory were immediately washed under running water. After removing the visible fat, the skins were cut into 2 to 3 cm squares, washed, and degreased with petroleum ether under running water, then wrapped in four layers of cheesecloth and squeezed to remove excess moisture.

The extraction method of chicken skin gelatin followed a previous report by Sarbon et al. (2013) with slight modifications. The defatted chicken skins were soaked in 1.0 mol/L NaOH solution (1:6 w/v) first, shaken evenly at 25 °C for 2.5 hr, then drained and squeezed using a cheesecloth. Drained skins were washed with running pH neutral water and soaked in 1.0 mol/L HCl solution (1:6 w/v). After that, the solution shaken evenly at 25 °C for 1 hr, then drained and rinsed thoroughly. Thereafter, the gelatin of chicken skin was extracted with deionized water (1:2 w/v, 55 °C, 3 hr). The extracted gelatin solution was filtered with a Büchner funnel and deionized by an Amberlite mixed bed resin (Life Technologies Corporation, Applied Biosystems AB, MB-6113). Finally, the filtrate was condensed to 1/10 of its original volume with a rotary evaporator, and then freeze-dried to obtain the chicken skin gelatin powder.

2.2 | Physicochemical properties

2.2.1 | Proximate chemical compositions, pH, and turbidity

The proximate chemical compositions (moisture, ash, and protein content) of the gelatin samples were assayed

according the method reported by Muyonga et al. (2004), and a nitrogen conversion factor of 5.4 was applied (Eastoe & Eastoe, 1952). According to the method of Almeida and Lannes (2013), the pH of gelatin solutions (1% w/v) was determined using a pH meter (PHS-3C, Shanghai Leizi Company, Shanghai, China) at 25 °C. The modified method of Sow et al. (2017) was used to measure the turbidity of gelatin solutions (6.67%, w/v). The solutions were incubated at 10 °C for 1 hr, and their absorbance at 600 nm was determined using a UV-Spectrophotometer (SP-1920, Shanghai Guangpu, Shanghai, China). The calculation of turbidity was based on the following equation,

$$\tau = -\left(\frac{1}{L}\right) \mathrm{Ln}\left(\frac{I}{I_0}\right) \tag{1}$$

where, τ is the turbidity (cm^{-1}), L is the optical path length (cm), I is the transmitted radiation intensity, and I_0 is the incident radiation intensity (Sow, Chong et al., 2018).

2.2.2 | Gel strength and texture profile analysis

Different sources gelatin gels (6.67%, W/V) were prepared using the method reported by Sow, Toh et al. (2019) for testing gel strength and texture profile analysis (TPA). After swelling with distilled water, the gelatin power was heated and stirred in a water bath (60 °C, 30 min) until completely dissolved. Finally, all gelatin solutions were placed immediately in a flat bottom glass container (35 mm dia × 45 mm height) and stored at 10 ± 2 °C for 17 ± 1 hr. The gel strength and textural parameters of the gelatin were measured after the gels had matured evenly. Twenty gelatin gel from each source of gelatin were randomly selected for gel strength and TPA measurement.

A Model TA-XT plus Texture Analyzer (TA.XT.PLUS, Stable Micro System, Godalming, UK) was used to determine the gel strength and TPA of the gelatin gels (6.67%, w/v), which were placed in the flat bottom glass containers. The gelatin gels were measured at 10 °C according to the procedure described by Sow and Yang (2015). The gel strength was determined with the force used when a P/10 flat cylinder probe extrudes the sample downward at a height of 4 mm at a rate of 0.5 mm/s. For TPA, the gel samples were compressed using the P/10 flat cylinder probe until the deformation reached 40% of its original height. The detailed test parameters were: pretest speed: 1.0 mm/s; test speed: 0.5 mm/s; posttest speed: 0.5 mm/s; trigger force: 0.05 N; time: 5.0 s; trigger type: Auto. The hardness, springiness, cohesiveness, and chewiness of gelatin gels were obtained from the TPA curve.

2.2.3 | Viscosity

The viscosity (cP) of 30 mL of the gelatin solutions (6.67%, w/v) was determined using a viscometer (Brookfield DV II, AMETEK Brookfield, Middleboro, MA, USA) using the method described by Sow and Yang (2015). The test speed was 100 rpm, the temperature was 60 °C, and the time was 3 min. The calculation of viscosity was based on the following equation,

$$v = t \times c \times \rho \tag{2}$$

where, v is the viscosity (cP), t is the effluent time (s), c is the viscometer constant (cSt/s), and ρ is the density of the measured solution (g/mL).

2.2.4 | Melting temperature and gelling temperature

Melting temperature (T_{melting}) and gelling temperature (T_{gelling}) of the gel samples (6.67%, w/v, 10 ± 2 °C) were studied using a controlled stress rheometer (Model MARS 60, HAAKE, Vreden, Germany) following the method of Sow, Kong et al. (2018) with slight modifications. The gel samples were cut into disks (2.5 cm diameter, 1 mm thickness), then placed on a 3.5 cm diameter parallel plate. A temperature sweep process was performed from 10 to 40 °C (for T_{melting}) and back to 10 °C (for T_{gelling}) with a 1 °C/min change rate, 0.1592 Hz frequency, and 100 Pa stress. The T_{melting} and T_{gelling} were determined as the temperature when storage modulus (G') and loss modulus (G'') crossed over during heating and cooling temperature sweep, respectively. In order to confirm that the applied frequency and stress were within the linear viscoelastic range, the sweep tests were conducted from 0.1 to 10 Hz and 0.1 to 1,000 Pa at 10 °C prior to the test.

2.2.5 | Thermal properties

Accurately weighed gel samples (6.67%, w/v, 10 ± 2 °C) were sealed separately into an aluminum pan of a differential scanning calorimeter (DSC) (Model Q 20, TA Instruments, New Castle, DE, USA). The samples were scanned in inert atmosphere (100 mL/min of N$_2$) over a range of 10 to 50 °C with a 5 °C/min heating rate. The temperature at which an endothermic peak occurred was calculated as helix-coil transition temperature (T_d), and the transition enthalpy (ΔH_{m}) was depended on the area under the endothermic peak (Sarbon et al., 2013).

2.2.6 | Amino acid analysis

Amino acid composition was analyzed according to the method reported by Badii and Howell (2006) with slight modifications. Gelatins were hydrolyzed with HCl (6.0 mol/L) for 24 hr at 110 °C. The filtered hydrolysate (1 mL) was evaporated to dryness, and then dissolved in 1 mL of sodium citrate buffer. Then, the samples were filtered through a 0.45 µm membrane filter and analyzed on an amino acid analyzer (Model S-433D, Sykam, Eresing, Germany). The amino acids content was calculated and expressed as residues/100 total amino acid residues.

2.3 | Structure characterization

2.3.1 | Fourier transform infrared spectroscopy

The gelatin samples from different sources were examined using a Fourier transform infrared spectroscopy (FTIR) spectrometer (Model WQF-510, Beijing Beifen-Ruili Analytical Instrument [Group] Company Limited, Beijing China) according to the method of Zhou and Yang (2019) with some modifications. The gelatin samples were lyophilized and grinded with KBr at least five repetitions performed in scanning. The scan range was set between 4,000 and 450 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.2 | Atomic force microscopy (AFM)

Different sources gelatin gels (1.0%, w/v) were heated in a 60 °C water bath for 1 hr to dissolve completely and homogenized with a Vortex mixer (Fisher Scientific, Pittsburgh, PA, USA). After that, the gelatin solutions were diluted to 10 to 40 µg/mL and then rapidly pipetted onto a freshly cleaved mica sheet surface using a rubber pipette bulb (Sow et al., 2017; Yang et al., 2007). The mica sheet was dried in a desiccator before imaging. The micromorphology of gelatin was observed using AFM (MicroNano AFM-III, Shanghai Zhuolunwei Nanometer Equipment Company Limited, Shanghai, China). The tapping mode and the line profile extraction function were used for imaging and quantitative analysis, respectively.

2.4 | Statistical analysis

Three independent experiments were conducted and at least triplicate samples within each run were collected. In order to obtain reliable, representative and statistically valid results, dozens of parallel AFM images were con-

TABLE 1 Proximate chemical compositions, pH, turbidity, gel strength, viscosity, and texture profile analysis (TPA) parameters of gelatins from different sources

Samples	PG	FG	CG
Moisture (%)	12.10 ± 0.00^a	11.90 ± 0.10^a	8.68 ± 0.01^b
Ash (%)	0.14 ± 0.02^c	0.76 ± 0.02^b	1.74 ± 0.02^a
Protein (%)	87.30 ± 0.10^a	86.56 ± 0.53^a	87.62 ± 1.12^a
pH	5.3 ± 0.0^b	5.7 ± 0.0^a	5.0 ± 0.0^c
Turbidity (cm^{-1})	0.29 ± 0.01^c	2.73 ± 0.01^b	14.05 ± 0.08^a
Gel strength (g)	289 ± 2^b	184 ± 1^c	366 ± 1^a
Viscosity (cP)	25.67 ± 0.33^b	24.67 ± 0.33^b	30.00 ± 0.58^a
Hardness (g)	743 ± 49^b	660 ± 22^c	2233 ± 7^a
Springiness	0.979 ± 0.010^a	1.005 ± 0.008^a	1.002 ± 0.014^a
Cohesiveness	0.63 ± 0.06^a	0.38 ± 0.01^b	0.62 ± 0.02^a
Chewiness (g)	459 ± 14b	250 ± 11^c	631 ± 4^a

Different lowercase superscript letters in the same row indicate significant differences within the different groups ($P < 0.05$).

PG, porcine skin gelatin; FG, tilapia skin gelatin; CG, chicken skin gelatin.

ducted. The results are reported as the mean ± standards deviation. Statistical analysis was performed using the SPSS Statistics 20 software (IBM Corporation, Armonk, NY, USA). The differences in the results among the gelatin from different sources were determined using one-way analysis of variance (ANOVA) ($P < 0.05$) and Duncan's multiple range tests.

3 | RESULTS AND DISCUSSION

3.1 | Proximate chemical compositions, pH, and turbidity

The proximate chemical compositions, pH, turbidity of PG, FG, and CG are presented in Table 1. The moisture content of CG was significantly lower compared with those of PG and FG ($P < 0.05$). And the ash content of CG was significantly higher than those of PG and FG ($P < 0.05$). Even so, the natural ash in chicken skin gelatin in this study did not exceed the recommended maximum ash content (2.6%) for edible gelatin (Muyonga et al., 2004). It was noted that the protein content of CG was similar to those of PG and FG. The results of proximate chemical compositions indicated that chicken skin has similar protein content with porcine and tilapia skin, which makes chicken skin have the potential to be a source of commercial gelatin. The pH values of gelatin solutions from different sources were distributed between 5.0 and 6.0. These differences in pH values were the result of the chemical treatments employed during the extraction procedure (Almeida & Lannes, 2013). In addition, the chicken skin gelatin was more turbid than PG and FG. This might have resulted from the influence

TABLE 2 Melting temperature ($T_{melting}$), gelling temperature ($T_{gelling}$), denaturation temperature (T_d), and enthalpy value (ΔH_m) of gelatins from different sources

Samples	$T_{melting}$ (°C)	$T_{gelling}$ (°C)	$\underline{Td}$ (°C)	ΔH_m (J/g)
PG	34.90 ± 0.07[b]	18.82 ± 0.06[b]	28.14 ± 0.28[b]	0.31 ± 0.09[a,b]
FG	31.21 ± 0.04[c]	14.49 ± 0.02[c]	25.98 ± 0.82[c]	0.12 ± 0.05[b]
CG	37.58 ± 0.04[a]	21.83 ± 0.04[a]	32.75 ± 0.18[a]	0.43 ± 0.09[a]

Different lowercase superscript letters in the same column indicate significant differences within the different groups ($P < 0.05$).

PG, porcine skin gelatin; FG, tilapia skin gelatin; CG, chicken skin gelatin.

of inorganic proteinaceous and mucosubstance contaminants that are difficult to remove during gelatin extraction (Elavarasan et al., 2016).

3.2 | Gel strength, viscosity, and TPA

The gel strength and texture parameters are important quality characteristics of gel products (Cai et al., 2016). As shown in Table 1, compared with PG and FG, the gel strength of CG gels had markedly higher values ($P < 0.05$). This phenomenon was probably controlled by the intrinsic characteristics of different sources of skin, such as the size of the protein chains, the protein molecular weight distribution, and the imino acid content (Ali et al., 2018; Sarbon et al., 2013; Tu et al., 2015). Furthermore, the chemical treatment during the extraction process, the gel concentration, the gelation time, and the temperature also affect gel strength (Babin & Dickinson, 2001).

To further study the TPA of the gelatin gels from different sources, hardness, springiness, cohesiveness, and chewiness were evaluated. As shown in Table 1, CG had higher hardness and chewiness than the other samples ($P < 0.05$). The hardness and chewiness value represents the maximum force required to compress the gel and the work required to masticate the gel into a ready-to-swallow state, respectively (Sow & Yang, 2015). Thus, the higher hardness and chewiness of CG suggests a firmer texture of gel. In addition, there was no difference between the cohesiveness of CG and that of PG, which was significantly higher than of FG. This phenomenon indicated that compared with FG, CG and PG could maintain the original structure when the gels were compressed, and have a better ability to reform the structure after compression.

The viscosity values of gelatins from all sources are also given in Table 1. Among the different gelatin samples, the viscosity of CG was the highest, followed by PG and FG. The molecular weight and molecular size distribution of proteins in skin are related to viscosity (Zhou et al., 2006). Overall, CG had high gel strength, hardness, chewiness, and viscosity, suggesting a strong gel.

3.3 | Melting temperature ($T_{melting}$), gelling temperature ($T_{gelling}$), denaturation temperature (T_d), and enthalpy value (ΔH_m)

The results of $T_{melting}$ and $T_{gelling}$ analyses of the gelatins from different sources are summarized in Table 2. Among all the samples, the $T_{melting}$ and $T_{gelling}$ measured in CG was highest, respectively, while that of FG was the lowest ($P < 0.05$). From Table 2, it can also be seen that the highest T_d measured was for CG, which was significantly higher than that for PG and FG ($P < 0.05$). This result might be caused by an acid-base interaction during the gelatin extraction procedure, which would inhibit the degradation of sub components, an important factor for the stability of gelatin (Yang et al., 2008). Similarly, compared with the enthalpy values (ΔH_m) of FG, CG, and PG had significantly higher values ($P < 0.05$). The ΔH_m of CG was 0.43 J/g, which was consistent with the result of Sarbon et al. (2013).

The $T_{gelling}$ is related to the number of cross-linked hydrogen bonds in the gel structure (Sarbon et al., 2013). And the higher $T_{melting}$ indicates that more energy required to break the cross-linked junction zones during heating, indicating high thermal stability (Sow et al., 2018). Moreover, the higher T_d and ΔH_m suggested fewer helix-coil transitions during temperature variation, which indicated a more stable collagen structure. Gelatin from different sources have different thermodynamic properties, which are mainly affected by their imino acid composition (Gómez-Guillén et al., 2002). The amino groups of proline and the hydroxyl group of hydroxyproline in gelatin can form hydrogen bonds with the side chains of other amino acids and water molecules during gel formation. These hydrogen bond interaction have a positive effect on stabilizing the triple helix structure, which has a significant effect on the thermal stability of gelatin (Gómez-Guillén et al., 2011).

The results detailed in Tables 1 and 2 showed that CG had the highest gel strength, hardness, chewiness, melting point, gelling temperature, and denaturation temperature. Therefore, it is necessary to further elucidate the essential reasons for the gelatin properties of CG.

TABLE 3 Amino acid analysis of gelatins from different sources (residues/100 total amino acid residues)

| Amino/%	PG	FG	CG
Asp	5.31 ± 0.02^a	5.34 ± 0.04^a	5.41 ± 0.07^a
Glu	10.14 ± 0.04^a	10.02 ± 0.07^{ab}	9.96 ± 0.02^b
Ser	2.60 ± 0.00^b	2.70 ± 0.01^a	2.13 ± 0.03^c
Arg	7.91 ± 0.05^a	7.70 ± 0.06^b	7.82 ± 0.10^{ab}
Gly	22.76 ± 0.08^a	22.72 ± 0.12^a	21.67 ± 0.18^b
Thr	1.68 ± 0.00^c	1.83 ± 0.01^b	1.88 ± 0.03^a
Pro	13.36 ± 0.08^a	11.57 ± 0.12^c	12.84 ± 0.18^b
Ala	8.65 ± 0.04^a	8.73 ± 0.05^a	8.71 ± 0.11^a
Val	2.64 ± 0.04^a	2.39 ± 0.02^c	2.44 ± 0.00^{bc}
Met	0.55 ± 0.01^b	0.54 ± 0.01^b	0.70 ± 0.01^a
Cys	0.15 ± 0.00^b	0.17 ± 0.01^a	0.12 ± 0.00^c
Ile	1.14 ± 0.00^c	1.43 ± 0.02^a	1.37 ± 0.02^b
Leu	3.03 ± 0.01^b	2.97 ± 0.03^b	3.17 ± 0.04^a
Phe	1.99 ± 0.02^b	1.91 ± 0.01^c	2.06 ± 0.00^a
His	1.01 ± 0.01^b	0.96 ± 0.01^a	1.05 ± 0.00^a
Lys	3.66 ± 0.01^b	3.56 ± 0.02^c	3.79 ± 0.05^a
Tyr	0.58 ± 0.00^a	0.45 ± 0.00^b	0.61 ± 0.01^b
Hyp	11.34 ± 0.03^b	10.70 ± 0.10^c	12.59 ± 0.17^a
Total imino acid	24.70 ± 0.10^b	22.27 ± 0.21^c	25.43 ± 0.35^a

Different lowercase superscript letters in the same row indicate significant differences within the different groups ($P < 0.05$).

P, porcine skin gelatin; FG, tilapia skin gelatin; CG, chicken skin gelatin.

3.4 | Amino acid analysis

The results of amino acid compositions of gelatins from different sources are shown in Table 3. Glycine (21.67% to 22.76%) was the major amino acid of gelatin, followed by proline (11.57% to 13.36%), and hydroxyproline (10.70% to 12.59%), respectively. The main amino acids of gelatin also included glutamic acid, alanine, and aspartic acid, with less of the essential amino acids, which is a characteristic of Nile perch skin, bone gelatin, and gelatins from other sources (Alfaro et al., 2014; Muyonga et al., 2004). In addition, among all gelatin samples, CG had the highest imino acid (proline and hydroxyproline) content (25.43%, PG: 24.70%, FG: 22.27%).

It has been reported that the content of proline and hydroxyproline significantly affects the stability of the gel helix structure when forming gel networks (Farris et al., 2011). In addition, the pyrrolidine rings of the imino acids and the hydrogen bonds formed between amino acid residues are also crucial for the stability of the gelatin gel (Chen et al., 2014). Therefore, its higher imino acid content allows the CG to form a better gel helix structure, which is essential for a stable gel, and promotes an increase in the melting temperature and denaturation temperature,
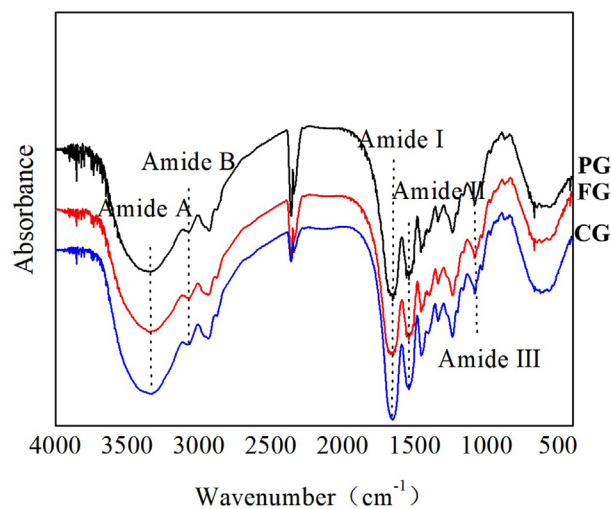
FIGURE 1 Fourier transform infrared spectroscopy (FTIR) spectra of porcine skin gelatin (PG), tilapia skin gelatin (FG), and chicken skin gelatin (CG)

thereby enhancing the gel strength and texture properties of gelatin (Tables 1 and 2) (Chakka et al., 2016).

3.5 | FTIR spectroscopy analysis

The major amide bands (amide A, B, I, II, III) detected in the FTIR spectra of gelatin from different sources are shown in Figure 1. Though there was no significant shifting of amide B, I, II, and III among the samples, the amide A band of CG was found at wave numbers of 3,326 cm^{-1}, which was lower than that of FG (3,347 cm^{-1}) and PG (3,334 cm^{-1}). According to Sow and Yang (2015), the amide A band (in the range of 3,400 to 3,440 cm^{-1}) is related to a free N-H stretching vibration coupled with intramolecular H-bonds. The shifting of the amide A bands to a lower wave number indicated more hydrogen bonds and the higher density of H-bonds in the sample (Cai et al., 2016). The H-bonds could be formed between the -NH residues and -C=O groups on the gelatin's peptide chains (Sow & Yang 2015; Sow et al., 2017). In this study, the chicken skin gelatin contained more imino acids (proline and hydroxyproline) (Table 3), making it more prone to form hydrogen bonds. Therefore, the location of amide A was found to be at lower wave number than in the other gelatin.

3.6 | Nanostructural analysis

The macroscale properties of the gel molecules depend not only on the amino acid composition and secondary structure of the gelatin, but also on the microaggregation state of the gelatin. AFM is an effective method for
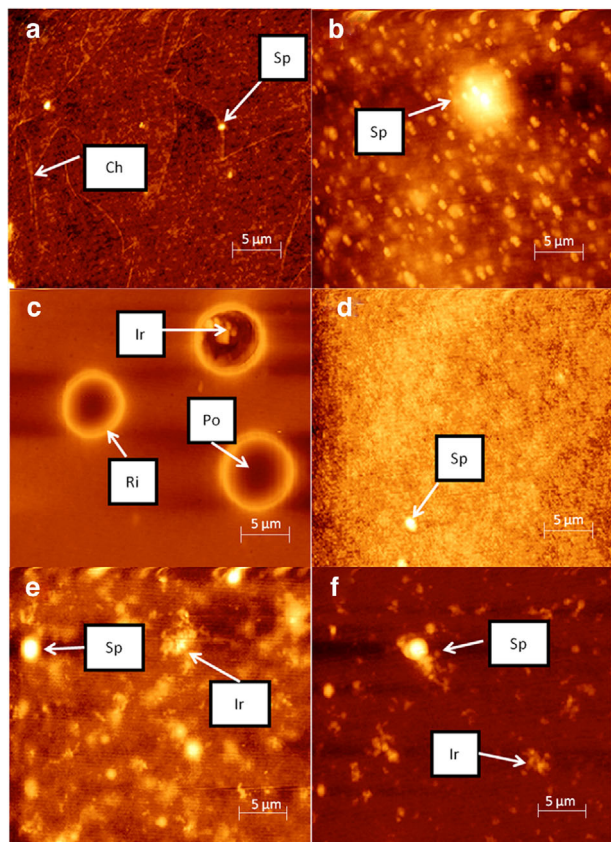
FIGURE 2 Nanostructure morphology of (a, b) porcine skin gelatin, (c, d) tilapia skin gelatin, (e, f) chicken skin gelatin imaged by atomic force microscopy. Sp, spherical aggregate; Ch, chain structure; Ir, irregular aggregate; Po, pore structure; Ri, ring-like structure

TABLE 4 Summary of nanostructures present in different sources of gelatins

Nanostructures	PG	FG	CG
Spherical aggregate	+	+	+
Diameter (nm)	334 ± 12^{b}	224 ± 15^{c}	483 ± 85^{a}
Height (nm)	3.5 ± 0.4^{b}	0.6 ± 0.0^{b}	18.8 ± 7.4^{a}
Irregular aggregate	+	+	+
Chain	+	−	−
Pore	−	+	−
Ring	−	+	−

PG, porcine skin gelatin; FG, tilapia skin gelatin; CG, chicken skin gelatin. Different lowercase superscript letters in the same row indicate significant differences within the different groups ($P < 0.05$).
"+" indicates the presence of a structure. "−" indicates the absence of a structure.

the analysis of the heterogeneity of aggregation structures in the biomacromolecules (Zhong & Yan, 2015). As shown in Figure 2, different micromorphologies were observed in gelatins from different source. The morphology of aggregates is determined by hydrogen bonding, the hydrophobic interaction of gelatin peptides in aqueous solution, and the electrostatic interactions between oppositely charged peptide fragments (Pang et al., 2014). Various kinds of gelatins contain more spherical aggregation structures, which are formed based on the triple helical structure of the gelatin (Mackie et al., 1998). Interestingly, CG contained spherical aggregates (Figures 2e, 2f) (average diameter = 483 nm) that were larger than those of PG and FG (334 and 224 nm, respectively) (Table 4). In addition, for PG, a low amount of chain structure was observed, which was considered to represent unaggregated free peptides (Figure 2a). These free peptide chains may be related to the low molecular weight molecules formed after the degradation of the subunits of gelatin, which is not conducive to the formation of a high-quality gel. Meanwhile, a ring-like structure with an annular pore at the center

was discovered in FG (Figure 2c). This phenomenon was similar to previous report by Sow and Yang (2015), which indicated conjoined multimeric aggregates formed during gelation.

3.7 | Schematic model

From the different gel properties, imino acid content, intramolecular hydrogen bonds, and nanostructure images of gelatins, we deduced that gelatins from different sources exhibited different molecular aggregation behaviors, which are the fundamental reasons for the different gel properties of the gelatins (Figure 3). We speculated that minimal aggregated water and ions penetrate into the CG molecules during hydrolysis (Yang et al, 2007), and then the CG molecules are inclined to form irregular aggregates and spherical aggregates after water evaporation (Figure 3a). Based on our experiments, CG had the highest content of imino acids in all samples (Table 3), which would promote the formation of intramolecular hydrogen bonds (lower amplitude of amide A wave number, Figure 1). Therefore, compared with PG and FG, the strong hydrogen bonding force of the CG peptide chains are more likely to allow the CG chains to gather and form larger size spherical aggregation structures (Duconseille et al., 2015). Finally, a dense rigid gel network could form, with the highest gel strength, hardness, chewiness, melting point, $T_{gelling}$, and denaturation temperature (Tables 1 and 2).

PG was not only crosslinked as irregular and spherical aggregates, but also formed small amounts of chain structures, representing the unaggregated free peptides of PG. These aggregate structures allow PG to form a moderately strong gel network that is weaker than that of the CG gel (Figure 3b). However, for FG, because of its low content
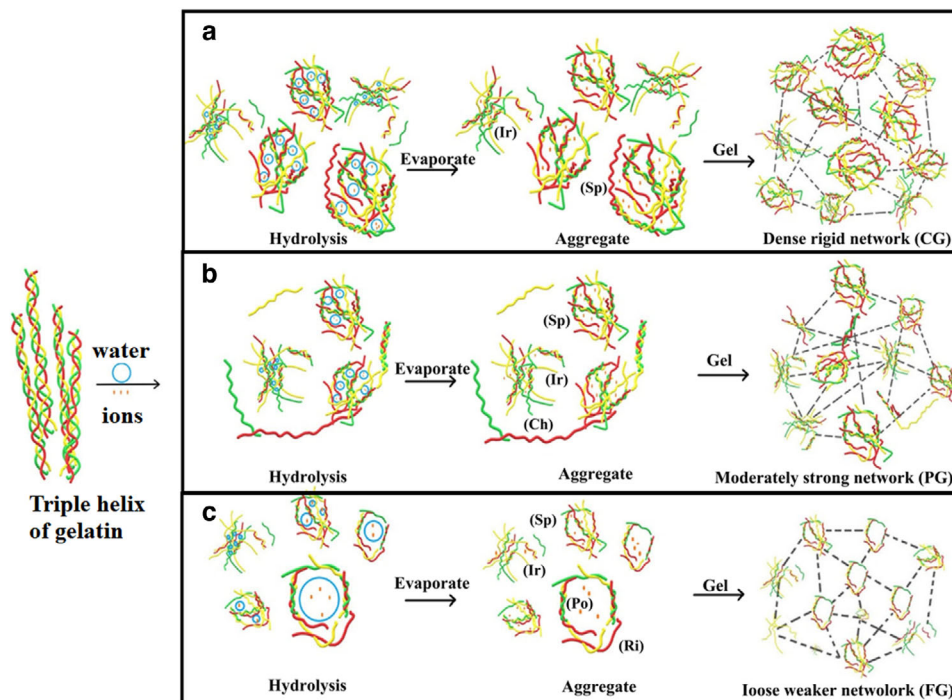
FIGURE 3 Hypothetical schematic images for the behavior of (a) chicken skin gelatin (CG), (b) porcine skin gelatin (PG), (c) tilapia skin gelatin (FG) to form nanostructures. Sp, spherical aggregate; Ch, chain structure; Ir, irregular aggregate; Po, pore structure; Ri, ring-like structure

of imino acids and weak intramolecular hydrogen bonding, plenty of water with salt ions would enter into the gelatin molecules during hydration, forming large water pools. After evaporation, the FG molecules that accumulate around the water pool and the annular pores in the center form a ring-like structure, which leads to the formation of a loose and weak gel (Figure 3c). The loose network structure results in poor gel strength, hardness, chewiness, melting point, $T_{gelling}$, and thermostability. These results revealed that the intramolecular H-bond and the molecular crosslinking behavior are the fundamental explanations for the varying gel properties of the gelatin from different sources.

Of course, further research is needed to verify and modify this proposed model. It is necessary to systematically determine the subunit composition, sol-gel transformation behavior, network structure and water holding ability of gelatins from different sources. In addition, the coil-helix transformation closely related to gel formation and stabilization also needs to be clarified.

4 | CONCLUSIONS

Compared with commercial porcine skin gelatin and tilapia skin gelatin, chicken skin gelatin exhibited higher gel strength, hardness, chewiness, melting point, $T_{gelling}$

, and denaturation temperature ($P < 0.05$). The different physicochemical properties of CG might reflect its higher imino acid content (25.43 residues/100 total residues), which would promote the formation of intramolecular hydrogen bonds (lower amplitude of amide A wave number). From the nanostructure images of the gelatin, we deduced that minimal aggregated water and ions penetrate into CG molecules during hydrolysis, allowing the CG molecules to form irregular aggregates and spherical aggregates (average diameter was 483 nm) that were larger than those of PG and FG (334 and 224 nm, respectively) after water evaporation. However, the PG and FG also form small amounts of chain structure and ring-like structure, respectively, because of the unaggregated free peptides of PG and the large water pools penetrates into FG. These results revealed that the intramolecular H-bonds and the aggregation behavior could explain the varying gel properties of the gelatin from different sources. The results indicated that chicken skin gelatin has the potential to become a commercial gelatin which meets the religious requirement for ethnic groups.

AUTHOR CONTRIBUTIONS

Hongshun Yang designed the study and interpreted the results. Ying Xin collected test data and drafted the manuscript. Mengyang Chai carried out the experiment in detail. Yucheng Hou assisted in completing the experiment. Fusheng Chen, Shaojuan Lai and Hongshun Yang revised the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Alfaro, A. D. T., Balbinot, E., Weber, C. I., Tonial, I. B., & Machado-Lunkes, A. (2014). Fish Gelatin: characteristics, functional properties, applications and future potentials. *Food Engineering Reviews*, *7*, 33–44. https://doi.org/10.1007/s12393-014-9096-5

Ali, A. M. M., Kishimura, H., & Benjakul, S. (2018). Physicochemical and molecular properties of gelatin from skin of golden carp (*Probarbus Jullieni*) as influenced by acid pretreatment and prior-ultrasonication. *Food Hydrocolloids*, *82*, 164–172. https://doi.org/10.1016/j.foodhyd.2018.03.052

Almeida, P. F., & Lannes, S. C. D. S. (2013). Extraction and physicochemical characterization of gelatin from chicken by-product. *Journal of Food Process Engineering*, *36*, 824–833. https://doi.org/10.1111/jfpe.12051

Babin, H., & Dickinson, E. (2001). Influence of transglutaminase treatment on the thermoreversible gelation of gelatin. *Food Hydrocolloids*, *15*, 271–276. https://doi.org/10.1016/s0268-005x(01)00025-x

Badii, F., & Howell, N. (2006). Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. *Food Hydrocolloids*, *20*, 630–640. https://doi.org/10.1016/j.foodhyd.2005.06.006

Cai, L., Feng, J., Peng, X., Regenstein, J. M., Li, X., Li, J., & Wei, Z. (2016). Effect of egg albumen protein addition on physicochemical properties and nanostructure of gelatin from fish skin. *Journal of Food Science & Technology*, *53*, 4224–4233. https://doi.org/10.1007/s13197-016-2416-4

Cai, L., Nian, L., Cao, A., Wu, W., Wang, J., Wang, Y., & Li, J. (2018). Effects of xylitol and stevioside on the physical and rheological properties of gelatin from cod skin. *Food Science & Technology International*, *24*, 639–650. https://doi.org/10.1177/1082013218784389

Chakka, A. K., Muhammed, A., Sakhare, P. Z., & Bhaskar, N. (2016). Poultry processing waste as an alternative source for mammalian gelatin: extraction and characterization of gelatin from chicken feet using food grade acids. *Waste and Biomass Valorization*, *8*, 1–11. https://doi.org/10.1007/s12649-016-9756-1

Chen, L., Liang, M., Zhou, M., Liu, Y., & Zhang, Y. (2014). Effects of pressure on gelatinization of collagen and properties of extracted gelatin. *Food Hydrocolloids*, *36*, 316–322. https://doi.org/10.1016/j.foodhyd.2013.10.012

Duconseille, A., Astruc, T., Quintana, N., Meersman, F., & Sante-Lhoutellier, V. (2015). Gelatin structure and composition linked to hard capsule dissolution: A review. *Food Hydrocolloids*, *43*, 360–376. https://doi.org/10.1016/j.foodhyd.2014.06.006

Eastoe, J. E., & Eastoe, B. (1952). A method for the determination of total nitrogen in proteins. *The British Gelatine and Glue Research Association Research Report, Series B*, *5*, 1–17.

Elavarasan, K., Kumar, A., Uchoi, D., Tejpal, C. S., Ninan, G., & Zynudheen, A. A. (2016). Extraction and characterization of gelatin from the head waste of tiger tooth croaker (*Otolithesruber*). *Waste and Biomass Valorization*, *8*, 851–858. https://doi.org/10.1007/s12649-016-9639-5

Farris, S., Schaich, K. M., Liu, L. S., Cooke, P. H., Piergiovanni, L., & Yamb, K. L. (2011). Gelatin-pectin composite films from polyion-complex hydrogels. *Food Hydrocolloid*, *25*, 61–70. https://doi.org/10.1016/j.foodhyd.2010.05.006

Feng, X., Ng, V. K., Miks-Krajnik, M., & Yang, H. (2017). Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. *Food and Bioprocess Technology*, *10*, 89–102. https://doi.org/10.1007/s11947-016-1798-7

Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A., & Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*, *16*, 25–34. https://doi.org/10.1016/S0268-005X(01)00035-2

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*, *25*, 1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

Huang, T., Tu, Z., Zou, Z., Shangguan, X., Wang, H., & Bansal, N. (2020). Glycosylated fish gelatin emulsion: Rheological, tribological properties and its application as model coffee creamers. *Food Hydrocolloids*, *102*, 105552. https://doi.org/10.1016/j.foodhyd.2019.105552

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2019). Complex coacervation between gelatin and chia mucilage as an alternative of encapsulating agents. *Journal of Food Science*, *84*, 1–7. https://doi.org/10.1111/1750-3841.14605

Le, T., Maki, H., Takahashi, K., Okazaki, E., & Osako, K. (2015). Properties of gelatin film from Horse mackerel (*Trachurus Japonicus*) Scale. *Journal of Food Science*, *80*, E734–E741. https://doi.org/10.1111/1750-3841.12806

Mackie, A. R., Gunning, A. P., Ridout, M. J., & Morris, V. J. (1998). Gelation of gelatin observation in the bulk and at the air-water interface. *Biopolymers*, *46*, 245–252. https://doi.org/10.1002/(SICI)1097-0282(19981005)46:43.0.CO;2-P

Mohtar, N. F., Perera, C. O., & Hemar, Y. (2014). Chemical modification of New Zealand hoki (*Macruronus novaezelandiae*) skin gelatin and its properties. *Food Chemistry*, *155*, 64–73. https://doi.org/10.1016/j.foodchem.2014.01.043

Mohtar, N. F., Perera, C. O., & Quek, S. Y. (2010). Optimisation of gelatin extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS–PAGE. *Food Chemistry*, *122*, 307–313. https://doi.org/10.1016/j.foodchem.2010.02.027

Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Extraction and physico-chemical characterisation of Nile perch (*Latesniloticus*) skin and bone gelatin. *Food Hydrocolloids*, *18*, 581–592. https://doi.org/10.1016/j.foodhyd.2003.08.009

Nagarajan, M., Benjakul, S., & Prodpran, T. (2012). Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures. *Food Hydrocolloid*, *29*, 389–397. https://doi.org/10.1016/j.foodhyd.2012.04.001

Pang, Z., Deeth, H., Sopade, P., Sharma, R., & Bansal, N. (2014). Rheology, texture and microstructure of gelatin gels with and without milk proteins. *Food Hydrocolloids*, *35*, 484–493. https://doi.org/10.1016/j.foodhyd.2013.07.007

Pang, Z., Deeth, H., Yang, H., Prakash, S., & Bansal, N. (2017). Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt. *Journal of Dairy Science*, *100*, 3436–3447. https://doi.org/10.3168/jds.2016-11881

Sarbon, N. M., Badii, F., & Howell, N. K. (2013). Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin. *Food Hydrocolloids*, *30*, 143–151. https://doi.org/10.1016/j.foodhyd.2012.05.009

Sow, L. C., Chong, J. M. N., Liao, Q. X., & Yang, H. (2018). Effects of κ-carrageenan on the structure and rheological properties of fish gelatin. *Journal of Food Engineering*, *239*, 92–103. https://doi.org/10.1016/j.jfoodeng.2018.05.035

Sow, L. C., Peh, Y. R., Pekerti, B. N., Fu, C., Bansal, N., & Yang, H. (2017). Nanostructural analysis and textural modification of tilapia fish gelatin affected by gellan and calcium chloride addition. *LWT-Food Science and Technology*, *85*, 137–145. https://doi.org/10.1016/j.lwt.2017.07.014

Sow, L. C., Kong, K., & Yang, H. (2018). Structural modification of fish gelatin by the addition of gellan, κ-carrageenan, and salts mimics the critical physicochemical properties of pork gelatin. *Journal of Food Science*, *83*, 1280–1291. https://doi.org/10.1111/1750-3841.14123

Sow, L. C., Toh, N. Z. Y., Wong, C. W., & Yang, H. (2019). Combination of sodium alginate with tilapia fish gelatin for improved texture properties and nanostructure modification. *Food Hydrocolloids*, *94*, 459–467. https://doi.org/10.1016/j.foodhyd.2019.03.041

Sow, L. C., Tan, S. J., & Yang, H. (2019). Rheological properties and structure modification in liquid and gel of tilapia skin gelatin by the addition of low acyl gellan. *Food Hydrocolloids*, *90*, 9–18. https://doi.org/10.1016/j.foodhyd.2018.12.006

Sow, L. C., & Yang, H. (2015). Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin. *Food Hydrocolloids*, *45*, 72–82. https://doi.org/10.1016/j.foodhyd.2014.10.021

Tu, Z. C., Huang, T., Wang, H., Sha, X. M., Shi, Y., Huang, X. Q., Man, Z. Z., & Li, D. J. (2015). Physico-chemical properties of gelatin from bighead carp (*Hypophthalmichthys nobilis*) scales by ultrasound-assisted extraction. *Journal of Food Science and Technology*, *52*, 2166–2174. https://doi.org/10.1016/j.foodhyd.2014.10.021

Yang, H., Wang, Y., Zhou, P., & Regenstein, J. M. (2008). Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. *Food Hydrocolloids*, *22*, 1541–1550. https://doi.org/10.1016/j.foodhyd.2007.10.007

Yang, H., Wang, Y., Regenstein, J. M., & Rouse, D. B. (2007). Nanostructural characterization of catfish skin gelatin using atomic force microscopy. *Journal of Food Science*, *72*, 430–440. https://doi.org/10.1111/j.1750-3841.2007.00480.x

Yu, D., Regenstein, J. M., & Xia, W. (2019). Bio-based edible coatings for the preservation of fishery products: A review. *Critical Reviews in Food Science and Nutrition*, *59*, 2481–2493. https://doi.org/10.1080/10408398.2018.1457623

Zhou, P., Mulvaney, S. J., & Regenstein, J. M. (2006). Properties of Alaska pollock skin gelatin: a comparison with tilapia and pork skin gelatin. *Journal of Food Science*, *71*, C313–C321. https://doi.org/10.1111/j.1750-3841.2006.00065.x

Zhong, J., & Yan, J. (2015). Seeing is believing: atomic force microscopy imaging for nanomaterial research. *RSC Advances*, *6*, 1–52. https://doi.org/10.1039/C5RA22186B

Zhou, Y., & Yang, H. (2019). Effects of calcium ion on gel properties and gelation of tilapia (*Oreochromis niloticus*) protein isolates processed with pH shift method. *Food Chemistry*, *277*, 327–335. https://doi.org/10.1016/j.foodchem.2018.10.110