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Influence of Drying Techniques on the Properties of Gelatin derived from Atlantic Salmon Skin

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Abstract

The properties of gelatin derived from fish processing by-products, such as Atlantic salmon (*Salmo salar*) skin, are strongly influenced by the drying method used during production. This study investigated the impacts of four drying methods on the extraction yield, physicochemical attributes, and gel characteristics of gelatin: freeze-dried gelatin (FDG), spray-dried gelatin (SDG), vacuum-dried gelatin (VDG), and hot air-dried gelatin (HDG) extracted from Atlantic salmon (*Salmo salar*) skin. The yields of FDG, VDG, and HDG were similar (15.75–16.45 g/100 g; $p > 0.05$), and significantly higher than that of SDG at 13.23 g/100 g ($p < 0.05$). Proximate analysis revealed that protein was the predominant component (94.60–95.03 g/100 g), with low fat (2.35–2.78 g/100 g) and ash (2.24–2.47 g/100 g) contents. SDG had the greatest lightness, followed by FDG, VDG, and HDG ($p < 0.05$), while HDG and VDG displayed more brownish, yellowish, and reddish colors. FDG exhibited the highest gel strength and viscosity, comparable to SDG ($p > 0.05$), while HDG had the lowest ($p < 0.05$). VDG showed the lowest turbidity, followed by FDG, HDG, and SDG ($p < 0.05$). All samples exhibited predominant α- and β-chains. FTIR spectra revealed similar patterns across all samples, with major peaks in the amide region. Overall, spray drying demonstrated strong potential as a practical and scalable alternative to freeze drying for gelatin production, offering high whiteness, desirable gel strength, and favorable viscosity while supporting sustainable applications in food and industrial sectors.

Keywords: Drying methods, Fish gelatin, Characteristics, Salmon skin

1. Introduction

Atlantic salmon (*Salmo salar*) plays a pivotal role in global aquaculture, contributing substantially to the fish processing industry. Worldwide production of salmon increased to nearly 3 million tons in 2023, and is expected to exceed this amount by 2024 [1]. This species is particularly prized for its distinct flavor, nutritional benefits, and widespread consumption [2,3]. However, high production volumes result in a substantial amount of by-products, with the skin, bones, head, and viscera constituting 40-50% of the processed salmon weight [4]. While these by-products are often repurposed into animal feed, this yields only minimal economic benefits. Thus, there is a growing need for sustainable strategies to utilize these by-products in value-added products, with gelatin extraction from fish skin emerging as a promising solution [5].

Gelatin, a biopolymer derived from the partial degradation or thermal denaturation of collagen in animal tissues, is used in many applications, including food, medicines, and cosmetics [6]. Traditionally, gelatin production has relied heavily on mammalian sources such as porcine and bovine materials, which together dominate more than 90% of the global supply [7]. Nonetheless, the utilization of mammalian-derived gelatin has limitations, including sociocultural and religious concerns, as well as safety issues associated with diseases like mad cow disease [8]. In response to these challenges, marine-derived gelatin, especially that extracted from fish skin, has emerged as a favorable alternative, offering compliance with halal and kosher standards [9]. Among marine sources, salmon skin is especially promising; it is a collagen-rich byproduct representing about 7% of the total mass generated during salmon processing [10] and has shown considerable potential for use in gelatin [6].

The gelatin production process involves several critical steps, including pre-treatment (using acid or alkali solutions), extraction, and drying [11]. Among these, drying is a particularly influential stage, as it significantly influences the quality and functional attributes

of the resulting gelatin. This stage can induce structural changes at the molecular level, including the disruption of covalent and non-covalent bonds, which may influence functional properties like gel strength, viscosity, solubility, and the ability to absorb water and fat [12]. Given that drying conditions significantly influence the final quality of gelatin, optimizing these conditions is essential to produce high-quality gelatin while maintaining cost-effectiveness. Although previous studies have provided valuable insights into the pre-treatment and extraction of gelatin from various fish sources, such as shark skin [13], featherback skin [14], salmon skin [15], perch skin [16], seabass skin [17], sturgeon skin [18], leatherjacket skin [19], and tilapia skin [20], research on the influence of various drying methods on the characteristics of fish-skin-derived gelatin remains limited. Freeze-drying, widely used for its ability to preserve the product quality, is commonly employed in gelatin manufacture. However, it is an expensive and time-consuming method, with challenges such as retention of undesirable odors [21]. In a prior study, we optimized the conditions for gelatin production from salmon skin using freeze-drying. While this approach proved effective, its high cost and lengthy processing time underscore the need for alternative drying techniques that can preserve gelatin quality while being more economically viable. Therefore, this study aims to compare the influences of four drying techniques, including freeze-drying, spray drying, vacuum drying, and hot air drying on the yield, physicochemical attributes, and gel characteristics of gelatin derived from Atlantic salmon skin.

2. Materials and methods

2.1. Chemicals

All chemicals utilized in this research were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Fish Skin Preparation

Atlantic salmon (*Salmo salar*) skins were provided by Thaveelarp Fisheries Ltd. (Trang, Thailand). Transportation to the laboratory was carried out in polystyrene containers using a 1:2 skin-to-ice ratio, with processing initiated within 2 h of delivery. Upon reaching the laboratory, residual muscle tissue and scales were manually removed, followed by rinsing the skins with chilled distilled water maintained at approximately 10°C. The cleaned skins were then sealed in polyethylene bags and kept at -20°C for a maximum duration of one month. For further processing, the frozen skins were uniformly sectioned into pieces of approximately 0.5 × 0.5 cm^2 by an electric saw, and these pieces were subsequently applied for gelatin extraction.

2.3. Gelatin Extraction

Gelatin extraction was performed using a modified method derived from Kuepethkaew et al. [22]. Before extraction, salmon skins underwent a series of pre-treatment steps. Initially, the skins were first immersed in 0.1 M NaOH solution at a skin-to-solution ratio of 1:15 (w/v), and the mixture was gently agitated at ambient temperature (150 rpm) for 2 h. The NaOH solution was replaced every 40 min to eliminate non-collagenous materials. After this, the skins were rinsed thoroughly with tap water until a neutral pH was achieved. Subsequently, they were treated with 0.0025 M HCl at a 1:20 (w/v) ratio for 10 min under gentle stirring to remove minerals. The skins were again rinsed with tap water until neutral pH was reached. Next, the skins were treated with 0.2 M acetic acid at a 1:10 (w/v) ratio for 20 min to allow collagen to swell. After this final pretreatment, the skins were again washed with tap water until neutral pH was restored.

For gelatin extraction, the swollen skins were submerged in distilled water at a 1:3 (w/v) ratio and subjected to heating at 50°C for 12 h in a water bath (WNB 22, Memmert, Schwabach, Germany), with constant stirring (200 rpm) using an overhead stirrer with a propeller

attachment. The resulting gelatin solution was initially filtered through a double layer of cheesecloth. Subsequently, activated carbon was introduced to the filtrate at a ratio of 1:100 (w/v), followed by 10 min of stirring. This mixture was centrifuged at 4,500×g for 20 min using a bench-top centrifuge (Orto Alresa, Magnus 22 R, Madrid, Spain). The resulting supernatant was filtered again using a Buchner funnel lined with Whatman No. 4 filter paper, and the resulting gelatin solution was collected for subsequent drying and analysis.

2.4. Drying Methods and Gelatin Yield

Following gelatin extraction, four drying methods were employed to evaluate their effects on gelatin yield and properties: freeze-drying, hot-air drying, vacuum drying, and spray-drying. In the freeze-drying method, the gelatin solution was initially frozen at -80°C for 6 h, then subjected to sublimation under a vacuum (100 Pa) in a CoolSafe Touch 100-9 freeze-dryer (Allerød, Denmark) set to -100°C for 72 h. In the spray-drying process, the gelatin solution was atomized using a YC-018 model spray dryer (Shanghai Pilotech Instrument & Equipment Co., Ltd., Shanghai, China) which operated at an inlet temperature of 160°C, an outlet temperature of 70°C, and a peristaltic pump speed of 10 rpm. For vacuum drying, the gelatin solution was processed in a vacuum shelf dryer (Nacres Co., Ltd, Bangkok, Thailand) for 10 h at 60°C and 70 cmHg. Hot-air drying was performed in a TD-10A tray dryer (Owner Foods Machinery Co., Ltd., Bangkok, Thailand) at 60°C for 24 h. The dried gelatins were labeled as freeze-dried gelatin (FDG), spray-dried gelatin (SDG), vacuum-dried gelatin (VDG), and hot-air-dried gelatin (HDG), respectively. The dried powders were stored in zip-lock bags within a vacuum-sealed plastic container at -20°C and used within one month. These samples were subsequently analyzed for various properties.

154 *2.5. Analyses*

155 *2.5.1. Gelatin Yield*

156 The extraction yield of gelatin was expressed on a dry weight basis and calculated using
157 the formula:

158 $$\text{Yield (g/ 100g)} = \frac{\text{Weight of dried gelatin (g)}}{\text{Weight of initial dry Atlantic salmon skin (g)}} \times 100 \quad \text{--------- (1)}$$

159

160 *2.5.2. Proximate Analysis*

161 The proximate composition of dried gelatin powders was measured following the
162 AOAC Official Methods [23], including determinations of moisture, ash, protein, and fat
163 content. The protein content was estimated by applying a nitrogen-to-protein conversion factor
164 of 5.55.

165

166 *2.5.3. Color Analysis*

167 Color characteristics of the dried gelatin samples, including lightness (L*), redness (a*),
168 and yellowness (b*), were assessed utilizing a colorimeter (CM-5, Konica Minolta, Tokyo,
169 Japan). The total color difference (ΔE*) was computed following the method outlined by
170 Kuepethkaew et al. [22], using the equation:

171 $$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \text{--------- (2)}$$

172 where ΔL^*, Δa^* and Δb^* denote the differences in each respective color parameter
173 compared to the white standard.

174

175 *2.5.4. Gel Strength*

176 Gel strength was evaluated following the method described by Kuepethkaew et al. [22].
177 A 6.67% (w/v) gelatin solution was prepared by dissolving the gelatin in distilled water at 60°C.
178 The solution was poured into cylindrical molds (3 cm in diameter, 2.5 cm in height) and

refrigerated at 4°C for 18 h to allow complete gelation. The strength of the formed gel was determined using a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a flat-faced cylindrical Teflon plunger (1.27 cm diameter) at 8-10°C. The probe was inserted to a depth of 4 mm at a rate of 1 mm/s. The peak force (in grams) recorded during penetration was taken as the gel strength.

2.5.5. Viscosity

Viscosity measurements were performed with a digital viscometer (Model DV E, Brookfield Engineering Laboratories, MA, USA) at 100 rpm using a No. 1 spindle. The measurement was conducted on a 6.67% (w/v) gelatin solution prepared as previously described. Results were expressed in centipoise (cP).

2.5.6. Turbidity

The turbidity of the gelatin solution (6.67% w/v) was assessed by determining absorbance at 600 nm utilizing a UV-visible spectrophotometer (Model UV-1900i, Shimadzu, Japan). Distilled water served as the reference blank. The procedure followed the method with slight modifications from Kuepethkaew et al. [22].

2.5.7. ATR-FTIR Spectroscopy

Infrared spectral analysis of the dried gelatin powders was performed using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. An FTIR spectrometer (Cary 630, Agilent Technologies, USA) was used to record the spectra. Sample preparation and data acquisition adhered to established procedures with reference to Kuepethkaew et al. [22]. Secondary structural compositions, including α-helix, β-sheets, β-turn

and random coil, were elucidated by deconvolution of amide I band (1700–1600 cm^{-1}) by peak fit (Seesolve, version 4.12) according to Ponnusamy et al. [24].

2.5.8. Protein Patterns

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was employed to investigate the protein distribution in the dried gelatin samples. The protocol was based on Laemmli [25], with preparation and electrophoresis conditions adapted from the method described by Sinthusamran et al. [26].

2.6. Statistical Analysis

All experiments were conducted in triplicate. A completely randomized design was applied, and the data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple-range test was employed to compare means, with significance set at $p < 0.05$. Analyses were performed with SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Yield

Gelatin yield is a crucial factor that must be considered, especially for its economic significance in large-scale industrial production [27]. Fig. 1 illustrates the influences of various drying techniques, including freeze drying, hot air drying, vacuum drying, and spray drying on gelatin yield (dry weight basis) extracted from Atlantic salmon skin. No significant differences ($p > 0.05$) were observed among the yields of FDG, HDG, and VDG, which ranged between 15.75 and 16.45 g/100 g. In contrast, SDG exhibited a significantly lower yield of 13.23 g/100 g ($p < 0.05$). Rasli et al. [28] noted no significant changes in the yield of chicken skin gelatin when comparing freeze-dried and vacuum-dried samples, which supports the findings of the

current investigation. Similarly, Rather et al. [29] found no substantial variation in gelatin yields from chicken feet using freeze-drying and hot air-drying techniques. However, Wan Omar and Sarbon [30] reported that the vacuum-dried method produced a higher yield than freeze-drying, attributing the difference to sample loss caused by gelatin sticking to the container during freeze drying. In the present study, the significantly lower yield of SDG is likely due to physical losses during spray drying. Fine gelatin droplets or powder particles may adhere to the chamber walls, cyclone separator, or collection vessels, resulting in reduced recovery efficiency. Kanwate et al. [8] reported a similar issue with gelatin extracted from *Labeo rohita* swim bladder, where lower yields from spray drying were attributed to powder sticking inside the dryer. Thus, different drying methods may not directly influence gelatin yield, except through physical losses such as product adhesion to equipment surfaces during drying.

3.2. Proximate composition

The proximate composition of gelatins extracted from Atlantic salmon skin using different drying methods was measured, and the results are displayed in Table 1. Significant variation in moisture content was observed among the samples ($p < 0.05$), with values of 11.50, 8.04, 8.77, and 9.54 g/100 g for FDG, HDG, VDG, and SDG, respectively. FDG exhibited the highest moisture content, while HDG had the lowest. Despite these variations, all samples contained moisture levels much below the maximum limit of 15% recommended for consumable gelatin [31]. According to Shyni et al. [32], gelatin is highly hygroscopic, and determining its physicochemical attributes becomes challenging when moisture content falls within the 6–8% range. Therefore, the differences observed here may be attributed to the specific drying temperatures and durations used in each method [8]. The notably lower moisture content in HDG and VDG likely reflects more effective moisture removal typically

253 associated with these drying techniques. On a dry weight basis, all gelatin samples exhibited
254 high protein content ranging from 94.60 to 95.03 g/100 g, with no significant differences
255 observed ($p > 0.05$). This indicates that the drying methods did not substantially affect the
256 protein concentration in the gelatin, which is consistent with gelatin's primary function as a
257 protein-based product. For fat content (dry basis), the results were as follows: FDG (2.35 g/100
258 g), HDG (2.72 g/100 g), VDG (2.70 g/100 g), and SDG (2.78 g/100 g). Marked disparities were
259 noted between FDG and the other samples, with FDG exhibiting the lowest fat level ($p > 0.05$).
260 The slightly higher fat levels in HDG, VDG, and SDG may be attributed to residual lipid
261 retention during the drying process. Freeze-drying entails rapid freezing and sublimation in
262 vacuum, which can induce phase separation between water, proteins, and lipids [33], possibly
263 leading to partial migration or co-evaporation of lipids. Additionally, the porous and expanded
264 structure of freeze-dried matrices, resulting from minimal structural collapse, may reduce the
265 entrapment of residual lipids compared to the denser matrices formed in hot air, vacuum, or
266 spray drying [34]. These structural and physical differences likely contribute to the more
267 efficient removal of fat during freeze-drying. A similar trend was observed in freeze-dried
268 swim bladder gelatin, which exhibited lower fat content compared to vacuum- and spray-dried
269 counterparts [8]. Nevertheless, all gelatin samples exhibited low fat content, consistent with
270 previous reports that lipid leaching during extraction can result in minor fat contamination in
271 the final product [35]. Ash content (dry basis) ranged from 2.24 to 2.47 g/100 g, with no
272 statistically significant variations among treatments ($p > 0.05$). All values fell below the
273 maximum allowable ash content of 2.6 g/100 g for edible gelatin [36]. This suggests that the
274 mineral content of the gelatin remained relatively consistent across drying methods. The
275 protein, fat, and ash contents of gelatin can differ depending on raw material and processing
276 methods, as noted by Rafieian et al. [37].
277

3.3. Gel strength and viscosity

Gel strength and viscosity are critical parameters for evaluating the functional characteristics of gelatin, especially in applications requiring specific textural or rheological properties. As illustrated in Fig. 2, the gel strength values of gelatin samples dried using various drying techniques varied significantly depending on the drying method ($p < 0.05$). FDG demonstrated the highest gel strength, although this value was not significantly different from that of SDG ($p > 0.05$). However, FDG exhibited significantly higher gel strength than VDG and HDG ($p < 0.05$). SDG also did not differ significantly from VDG but was significantly higher than HDG. These findings align with earlier reports by Mad-Ali et al. [38], who found comparable gel strength between freeze-dried and spray-dried goat skin gelatin, and Kanwate et al. [8], who observed higher gel strength in freeze-dried and spray-dried gelatin than in vacuum-dried samples. The observed differences may be attributed to variations in molecular integrity due to thermal exposure during drying. Prolonged or intense heat, particularly in hot-air drying, can cause the degradation of gelatin molecules and reduced functional performance. Supporting this, the molecular weight distribution profile (Fig. 4) indicated a reduction in β-chain fractions for VDG and HDG, suggesting partial breakdown of protein chains [39]. Gel strength is known to correlate with molecular weight and the degree of hydrolysis—factors influenced by the drying process [21,40]. The lower gel strength in VDG and HDG may thus result from increased protein degradation during extended drying periods, while FDG and SDG preserved more intact protein structures. In addition, differences in intrinsic factors such as molecular weight distribution, protein chain length, and amino acid composition may potentially influence variances in gel strength [38].

In terms of viscosity, all gelatin solutions ranged from 17.58 to 23.49 cP. The lowest viscosity was recorded for HDG ($p < 0.05$), while FDG, SDG, and VDG exhibited statistically similar viscosity values ($p > 0.05$). A decline in viscosity is typically associated with a

303 reduction in high molecular weight chains due to thermal degradation [39,41]. Furthermore,
304 changes in hydrogen bonding and protein cross-linking in solution may also impact viscosity
305 [42]. Therefore, drying methods significantly influence the gel strength and viscosity of gelatin
306 derived from Atlantic salmon skin, with freeze-drying and spray-drying better preserving
307 functional characteristics compared to hot-air drying.
308
309 *3.4. Color characteristics of gelatin powder*
310 Color is an important quality attribute in gelatin, particularly for food applications
311 where a light, neutral appearance is preferred to avoid altering the color of the final product
312 [43]. While color does not directly influence the functional characteristics of gelatin, it can
313 determine the suitability of gelatin in specific formulations. The color characteristics of gelatin
314 powders varied significantly depending on the drying technique, as presented in Table 2. Visual
315 appearance indicated that both SDG and FDG exhibited a whitish color, while VDG and HDG
316 appeared light brown. This visual distinction aligns with the Instrumental color values.
317 Regarding color parameters, SDG exhibited the highest lightness (L*) value (92.23), followed
318 by FDG (89.35), VDG (69.80), and HDG (66.72) ($p < 0.05$). The higher L* value in SDG
319 reflects its lighter color, which can be attributed to the short exposure time and rapid drying
320 process that minimizes thermal degradation [44]. Interestingly, despite the absence of thermal
321 stress in freeze-drying, FDG had a slightly lower L* value than SDG. Similar results have been
322 reported for seabass skin gelatin [44], though contrary findings were observed in splendid squid
323 gelatin [21]. The values for redness (a*) and yellowness (b*) were notably higher in HDG (a =
324 0.20; b = 14.85), followed by VDG (a = –0.09; b = 9.95), FDG (a = –1.56; b = 3.45), and SDG
325 (a = –1.60; b = 2.45) ($p < 0.05$). These results indicate that HDG and VDG produced gelatin
326 with more yellowish to brownish hues, while SDG and FDG retained a near-white appearance.
327 The total color difference (ΔE*) supported these observations, with HDG showing the highest

ΔE* (36.09) and SDG the lowest (7.88) ($p < 0.05$). The elevated ΔE* value in HDG corresponds with its low L* and high a* and b* values, highlighting its deviation from a neutral white color. The increase in redness and yellowness is likely associated with prolonged thermal exposure during drying, which can induce non-enzymatic browning reactions involving amino acids [45]. Extended drying at elevated temperatures promotes protein breakdown and the liberation of free amino acids, enhancing Maillard reactions and contributing to a darker color [21]. In contrast, the rapid drying of spray-drying and the low-temperature environment of freeze-drying minimized such degradation, resulting in gelatin with a lighter, whitish appearance. In conclusion, both visual appearance and instrumental color values clearly demonstrated the influence of drying technique on gelatin color. Spray-drying and freeze-drying effectively preserved a bright, neutral color, whereas vacuum and hot-air drying produced more yellowish powders due to increased thermal effects. These findings emphasize necessity of choosing suitable drying techniques according to the intended visual quality, especially for applications requiring a light-colored gelatin product.

3.5. Turbidity of gelatin solution

Turbidity is an important visual attribute of gelatin solutions and may influence its suitability for specific applications, particularly those requiring transparency, such as in beverages or pharmaceuticals [46]. The turbidity is generally caused by the presence of residual inorganic matter, protein fragments, and mucosubstance impurities that remain after the extraction process [47]. As displayed in Fig. 3, the turbidity of gelatin solutions significantly differed based on the drying method used ($p < 0.05$). Among the samples, SDG had the greatest turbidity, followed by HDG, FDG and VDG. These results suggest that high-temperature drying, particularly under rapid conditions such as spray-drying, may promote structural changes that enhance molecular aggregation, thereby increasing turbidity. This observation

aligns with Mad-Ali et al. [48], who reported greater turbidity in spray-dried gelatin compared to freeze-dried counterparts. The higher turbidity in SDG may result from partial denaturation and exposure of hydrophobic regions, which encourage aggregation during spray-drying at higher temperature [49]. Interestingly, although VDG and HDG were both dried at 60°C, the turbidity in VDG was significantly lower. This could be attributed to the shorter drying duration in vacuum drying, which may have minimized thermal damage and aggregation. Surprisingly, FDG, which underwent drying without thermal exposure, did not show the lowest turbidity, as might be expected. This suggests that non-thermal factors such as protein aggregation during freezing or the effect of ice crystal formation may also influence turbidity. Consequently, drying techniques significantly affect the turbidity of gelatin solutions from Atlantic salmon skin.

3.6. SDS-PAGE Patterns

The SDS-PAGE profiles of gelatin derived from Atlantic salmon skin and processed through various drying techniques are presented in Fig. 4. All gelatin samples exhibited typical gelatin protein patterns, consisting of γ-chains, β-chains, and α-chains (α1 and α2), which are known to be the principal constituents of gelatin [22]. While the overall banding profiles appeared similar across treatments, differences in band intensities were evident. In particular, the β-chain bands of VDG and HDG showed slightly reduced intensities compared to those of FDG and SDG, indicating partial degradation of high molecular weight proteins during drying. This degradation is likely a consequence of prolonged thermal exposure associated with vacuum and hot-air drying. Conversely, despite the higher inlet temperature used in spray-drying, the short residence time may have limited protein damage, resulting in a more preserved β-chain intensity in SDG. The findings are consistent with the research of Kanwate et al. [8], which indicated that freeze-dried and spray-dried gelatin maintained more intact γ-, β-, and α-

chain structures than gelatin processed using vacuum drying. Collectively, the SDS-PAGE results confirm that the drying process significantly influences the molecular integrity of gelatin, with both temperature and exposure duration playing critical roles in determining protein stability.

3.7. Fourier transform infrared (FTIR) spectra

FTIR analysis was conducted to investigate the functional groups and assess the secondary structure of gelatin extracted from Atlantic salmon skin using different drying methods (Fig. 5A). All gelatin samples exhibited similar FTIR spectral patterns, featuring characteristic amide bands typical of gelatin. The amide A band, attributed to N–H stretching vibrations, was found in the range of 3256–3281 cm^{-1}. This shift from the free N–H stretch region (3400–3440 cm^{-1}) indicates the involvement of hydrogen bonding, particularly among shorter peptide chains in HDG, VDG, and SDG, which exhibited slightly lower wavenumbers than FDG [17]. The amide B bands, which are indicative of asymmetric stretching of =C–H and $–NH_3^+$ groups, were observed near 2923–2938 cm^{-1}. The amide I band, arising primarily from C=O stretching vibrations (1600–1700 cm^{-1}), is a key indicator of protein secondary structure. All samples displayed amide I bands around 1627–1636 cm^{-1}, consistent with a random coil structure, typical for gelatin [50]. Notably, HDG showed a slightly lower amide I wavenumber (1627 cm^{-1}), suggesting greater conformational disruption in its polypeptide chains due to thermal degradation. The amide II band (1523–1541 cm^{-1}), reflecting N–H bending and C–N stretching, and the amide III band (1235–1239 cm^{-1}), representing C–N stretching and N–H deformation, were present in all samples. However, the band intensities of HDG and VDG were noticeably lower at the amide I, II, and III regions compared to FDG and SDG. This reduction in absorbance indicates a higher degree of structural disorder and possible breakdown of the triple helix or partial denaturation of gelatin molecules. These results suggest

that freeze-drying and spray-drying better preserved the molecular structure of gelatin, while vacuum-drying and especially hot-air drying led to greater disruption of secondary structures due to extended thermal exposure. This aligns with previous studies reporting that drying conditions significantly influence the structural integrity of gelatin [28,51].

The secondary structure of gelatin, particularly within the Amide I region (1700–1600 cm^{-1}), is a critical determinant of its functional properties, including gel strength, thermal stability, and solubility [39]. The results demonstrate that the drying method significantly influences the secondary structural composition of gelatin extracted from Atlantic salmon skin, with notable variations in β-sheets, β-turns, and random coils, while no α-helical content was detected in any sample (Fig. 5B). The complete lack of α-helical structures in all samples aligns with gelatin's nature as a denatured form of collagen [24]. The triple-helical structure of native collagen is disrupted during extraction, resulting in gelatin that primarily consists of β-sheets, turns, and random coils rather than α-helices [24]. This structural profile is typical of gelatin and explains its functional differences from intact collagen.

β-sheets were the dominant secondary structure across all gelatin samples, with percentages ranging from 54.53% (VDG) to 59.35% (SDG). The highest β-sheet content was observed in SDG, likely due to the rapid dehydration process, which promotes the formation of tightly packed, hydrogen-bonded structures. FDG also exhibited a high β-sheet content (57.43%), as the sublimation process under low temperatures minimizes thermal degradation, preserving the protein's ordered structure. In contrast, HDG and VDG showed slightly lower β-sheet percentages, possibly due to prolonged exposure to heat or slower dehydration, which may lead to partial protein unfolding and reduced structural order.

The β-turn content varied between 18.70% and 21.97%, while random coil structures ranged from 21.03% to 24.83%. The highest β-turn content in VDG suggests that vacuum drying, which operates under reduced pressure and moderate temperatures, may facilitate the

formation of bends and loops in the protein backbone. Conversely, HDG exhibited the highest random coil percentage (24.83%), likely due to thermal denaturation disrupting secondary structures and increasing disordered regions. This higher proportion of random coils could enhance solubility but may compromise gelation properties. Meanwhile, SDG had the lowest β-turn content (18.70%), reinforcing the idea that rapid drying favors rigid β-sheet.

4. Conclusions

Drying techniques significantly impacted the yield, physicochemical attributes, and gel characteristics of gelatin extracted from Atlantic salmon skin. Among the techniques, hot-air drying led to the greatest quality deterioration, particularly in color, gel strength, and viscosity, likely due to prolonged heat exposure. In contrast, spray-drying produced gelatin with functional properties comparable to freeze-drying, including favorable gel strength, turbidity, and molecular integrity. This study highlights the untapped potential of Atlantic salmon skin as a sustainable and valuable alternative source for gelatin production. By demonstrating the effectiveness of spray drying as a viable method, these findings offer new perspectives for optimizing gelatin extraction from underutilized fish by-products. Furthermore, the approach contributes to reducing food processing waste and supports environmentally responsible practices in line with global sustainability goals.

CRediT authorship contribution statement

Panumas Somjid: Investigation, Writing- original draft. **Sappasith Klomklao:** Conceptualization, Methodology, Validation, Funding acquisition, Writing- review & editing. **Soottawat Benjakul:** Supervision. **Hideki Kishimura:** Methodology.

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Declaration of competing interest

The authors affirm that there are no competing interests or conflicts of interest related to this publication.

References

[1] Food and Agriculture Organization of the United Nations, FAO globefish. https://www.fao.org/in-action/globefish/news-events/news/news-detail/Salmon---Main-producers-see-record-breaking-exports/en, 2023 (accessed 13 January 2025).

[2] J.P. Monteiro, T. Sousa, T. Melo, C. Pires, A. Marques, M.L. Nunes, R. Calado, M.R. Domingues, Unveiling the lipid features and valorization potential of Atlantic salmon (*Salmo Salar*) heads, Mar. Drugs. 22 (2024) 518. https://doi.org/10.3390/md22110518

[3] K. Nilsuwan, K. Fusang, P. Pripatnanont, S. Benjakul, Properties and characteristics of acid-soluble collagen from salmon skin defatted with the aid of ultrasonication, Fishes, 7 (2022) 51. https://doi.org/10.3390/fishes7010051

[4] Y. Liu, V.V. Ramakrishnan, D. Dave, Enzymatic hydrolysis of farmed Atlantic salmon by-products: Investigation of operational parameters on extracted oil yield and quality, Process Biochem. 100 (2021) 10-19. https://doi.org/10.1016/j.procbio.2020.09.019

[5] C. V. T. Rigueto, R. de Oliveira, K.S. Gomes, I. Alessandretti, M. T. Nazari, M. Rosseto, D. D. C. Krein, , R. A. Loss, A. Dettmer, From waste to value-added products: A review of opportunities for fish waste valorization, Environ. Qual. Manag. 33 (2023) 203-221. https://doi.org/10.1002/tqem.22040

[6] R. de Oliveira, C. V. T. Rigueto, M. Rosseto, D. D. C. Krein, K. S. Gomes, R. A. Loss, A. Dettmer, Influence of enzymatic crosslinking on Tambaqui (*Colossoma macropomum*) skin gelatin film properties, Polym. Bull. 81(2024) 4539-4556. https://doi.org/10.1007/s00289-023-04924-0

[7] T. Zhang, R. Sun, M. Ding, L. Tao, L. Liu, N. Tao, X. Wang, J. Zhong, Effect of extraction methods on the structural characteristics, functional properties, and emulsion

stabilization ability of Tilapia skin gelatins, Food Chem. 328 (2020) 127114. https://doi.org/10.1016/j.foodchem.2020.127114

[8] B.W. Kanwate, R.V. Ballari, T.G. Kudre, Influence of spray-drying, freeze-drying and vacuum-drying on physicochemical and functional properties of gelatin from Labeo rohita swim bladder, Int. J. Biol. Macromol. 121 (2019) 135-141. https://doi.org/10.1016/j.ijbiomac.2018.10.015

[9] C. V. T. Rigueto, M. Rosseto, I. Alessandretti, R. de Oliveira, D. A. R. Wohlmuth, J. F. Menezes, R.A. Loss, A. Dettmer, I.R. Pizzutti, Gelatin films from wastes: A review of production, characterization, and application trends in food preservation and agriculture, Food Res. Int. 162 (2022) 112114. https://.org/10.1016/j.foodres.2022.112114

[10] A.N. Aryee, B.K. Simpson, L.E. Phillip, R.I. Cue, Effect of temperature and time on the stability of salmon skin oil during storage, J. Am. Oil Chem. Soc. 89 (2012) 287-292. https://doi.org/10.1007/s11746-011-1902-0

[11] Ü. Cansu, Utilization of infrared drying as alternative to spray-and freeze-drying for low energy consumption in the production of powdered gelatin, Gels, 10 (2024) 522. https://doi.org/10.3390/gels10080522

[12] D. Dehnad, S.M. Jafari, M. Afrasiabi, Influence of drying on functional properties of food biopolymers: From traditional to novel dehydration techniques, Trends Food Sci. Technol. 57 (2016) 116-131. https://doi.org/10.1016/j.tifs.2016.09.002

[13] A. Salem, O. Abdelhedi, H. Sebii, F.B. Taheur, N. Fakhfakh, M. Jridi, N. Zouari, F. Debeaufort, Techno-functional characterization of gelatin extracted from the smooth-hound shark skins: Impact of pretreatments and drying methods, Heliyon, 9 (2023). https://doi.org/10.1016/j.heliyon.2023.e19620

514 [14] P. Kittiphattanabawon, S. Benjakul, S. Sinthusamran, H. Kishimura, Gelatin from clown
515 featherback skin: Extraction conditions, LWT - Food Sci. Technol. 66 (2016) 186-192.
516 [15] Y. Liu, L. Xia, H. Jia, Q. Li, W. Jin, X. Dong, J. Pan, Physiochemical and functional
517 properties of chum salmon (*Oncorhynchus keta*) skin gelatin extracted at different
518 temperatures, J. Sci. Food Agric. 97 (2017) 5406-5413. https://doi.org/10.1002/jsfa.
519 8431
520 [16] J. Muyonga, C. Cole, K. Duodu, Extraction and physico-chemical characterisation of Nile
521 perch (*Lates niloticus*) skin and bone gelatin, Food Hydrocoll. 18 (2004) 581-592.
522 https://doi.org/10.1016/j.foodhyd.2003.08.009
523 [17] S. Sinthusamran, S. Benjakul, H. Kishimura, Characteristics and gel properties of gelatin
524 from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions, Food
525 chem. 152 (2014) 276-284. https://doi.org/10.1016/j.foodchem.2013.11.109
526 [18] M. Nikoo, S. Benjakul, A. Ehsani, J. Li, F. Wu, N. Yang, B. Xu, Z. Jin, X. Xu, Antioxidant
527 and cryoprotective effects of a tetrapeptide isolated from Amur sturgeon skin gelatin,
528 J. Funct. Foods. 7 (2014) 609-620. https://doi.org/10.1016/j.jff.2013.12.024
529 [19] P. Kaewruang, S. Benjakul, T. Prodpran, S. Nalinanon, Physicochemical and functional
530 properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as
531 affected by extraction conditions, Food Biosci. 2 (2013) 1-9. https://doi.org/10.1016/
532 j.fbio. 2013.03.002
533 [20] C.C. Tan, A.A. Karim, U. Uthumporn, F.C. Ghazali, Effect extraction temperature on the
534 emulsifying properties of gelatin from black tilapia (*Oreochromis mossambicus*) skin,
535 Food Hydrocoll. 108 (2020) 106024. https://doi.org/10.1016/j.foodhyd.2020.106024
536 [21] A. Hamzeh, S. Benjakul, T. Sae-Leaw, S. Sinthusamran, Effect of drying methods on
537 gelatin from splendid squid (*Loligo formosana*) skins, Food Biosci. 26 (2018) 96-103.
538 https://doi.org/10.1016/j.fbio.2018.10.001

539 [22] S. Kuepethkaew, S. Klomklao, Y. Zhang, A.N. Aryee, S. Benjakul, M. Betti, B.K.
540 Simpson, Characteristics of gelatin from lizardfish (*Saurida micropectoralis*) and
541 threadfin bream (*Nemipterus hexodon*) skins as influenced by extraction conditions, J.
542 Food Sci. Technol. 60 (2023) 2813-2824. https://doi.org/10.1007/s13197-023-05799-9
543 [23] AOAC. Offcial Methods of Analysis of AOAC International (20th.), International
544 Association of Offcial Analytical Chemists International, (2016).
545 [24] A. Ponnusamy, K. Niluswan, T. Prodpran, J. T. Kim, J. W. Rhim, S. Benjakul, Storage
546 stability of Asian seabass oil-in-water Pickering emulsion packed in pouches made
547 from electrospun and solvent casted bilayer films from poly lactic acid/chitosan-gelatin
548 blend containing epigallocatechin gallate, Int. J. Biol. Macromol. 265 (2024) 130760.
549 https://doi.org/10.1016/j.ijbiomac.2024.130760
550 [25] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of
551 bacteriophage T4, nature, 227 (1970) 680-685. https://doi.org/10.1038/227680a0
552 [26] S. Sinthusamran, S. Benjakul, Y. Hemar, H. Kishimura, Characteristics and properties of
553 gelatin from seabass (*Lates calcarifer*) swim bladder: impact of extraction temperatures,
554 Waste Biomass Valorization. 9 (2018) 315-325. https://doi.org/10.1007/
555 s12649-016-9817-5
556 [27] T.K. Kim, Y.K. Ham, D.M. Shin, H.W. Kim, H.W. Jang, Y.B. Kim, Y.S. Choi, Extraction
557 of crude gelatin from duck skin: effects of heating methods on gelatin yield, Poult. Sci.
558 99 (2020) 590-596. https://doi.org/10.3382/ps/pez519
559 [28] H. Rasli, N. Sarbon, Effects of different drying methods on the rheological, functional and
560 structural properties of chicken skin gelatin compared to bovine gelatin, Int. Food Res.
561 J. 22 (2015) 584. http://www.ifrj.upm.edu.my/
562 [29] J.A. Rather, S.D. Majid, A.H. Dar, T. Amin, H. Makroo, S.A. Mir, F.J. Barba, B.N. Dar,
563 Extraction of gelatin from poultry byproduct: influence of drying method on structural,

564 thermal, functional, and rheological characteristics of the dried gelatin powder, Front.
565 Nutr. 9 (2022) 895197. https://doi.org/10.3389/fnut.2022.895197
566 [30] W.H. Wan Omar, N. Sarbon, Effect of drying method on functional properties and
567 antioxidant activities of chicken skin gelatin hydrolysate, J. Food Sci. Technol. 53
568 (2016) 3928-3938. https://doi.org/10.1007/s13197-016-2379-5
569 [31] Monograph, G.M.E., Standard method for the testing of edible gelatin, Gelatin Monogr.
570 Gelatin Manufacturers of Europe, 2005.
571 [32] K. Shyni, G. Hema, G. Ninan, S. Mathew, C.G. Joshy, P. Lakshmanan, Isolation and
572 characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog
573 shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*), Food hydrocoll, 39 (2014) 68-
574 76. https://doi.org/10.1016/j.foodhyd.2013.12.008
575 [33] D. Nowak, E. Jakubczyk, The freeze-drying of foods—The characteristic of the process
576 course and the effect of its parameters on the physical properties of food materials,
577 Foods, 9 (2020) 1488. https://doi.org/10.3390/foods9101488
578 [34] V.P. Oikonomopoulou, M.K. Krokida, V.T. Karathanos, The influence of freeze drying
579 conditions on microstructural changes of food products, Procedia Food Sci. 1 (2011)
580 647-654. https://doi.org/10.1016/j.profoo.2011.09.097
581 [35] S. Mad-Ali, S. Benjakul, T. Prodpran, S. Maqsood, Interfacial properties of gelatin from
582 goat skin as influenced by drying methods, LWT, 73 (2016) 102-107. https://doi.org/10.
583 1016/j.lwt.2016.05.048
584 [36] N.R. Jones, Uses of gelatin in edible products, in: The Science and Technology of Gelatin,
585 Academic Press, London, 1977 pp. 366–392.
586 [37] F. Rafieian, J. Keramat, M. Shahedi, Physicochemical properties of gelatin extracted from
587 chicken deboner residue, LWT - Food Sci. Technol. 64 (2015) 1370-1375.
588 https://doi.org/10.1016/j.lwt.2015.04.050

589 [38] S. Mad-Ali, S. Benjakul, T. Prodpran, S. Maqsood, Characteristics and gel properties of
590 gelatin from goat skin as affected by spray drying, Dry. Technol. 35 (2017) 218-226.
591 https://doi.org/10.1007/s13197-017-2597-5
592 [39] C. V. T. Rigueto, M. Rosseto, K. S. Gomes, R. A. Loss, B. Biduski, C. Manera, M.
593 Godinho, V. B. Brião A. Dettmer, I. R. Pizzutti, Steam explosion pretreatment for
594 bovine limed hide waste gelatin extraction, Food Hydrocoll. 142 (2023) 108854.
595 https://doi.org/10.1016/j.foodhyd.2023.108854
596 [40] Ü. Cansu, Comparative evaluation of different separation and concentration procedures
597 on some quality and functional properties of fish gelatin, Innov. Food Sci. Emerg.
598 Technol. 83 (2023) 103237. https://doi.org/10.1016/j.ifset.2022.103237
599 [41] T. Ahmad, A. Ismail, S.A. Ahmad, K.A. Khalil, Y. Kumar, K.D. Adeyemi, A.Q. Sazili,
600 Recent advances on the role of process variables affecting gelatin yield and
601 characteristics with special reference to enzymatic extraction: A review, Food
602 hydrocoll. 63 (2017) 85-96. https://doi.org/10.1016/j.foodhyd.2016.08.007
603 [42] X. Chen, L. Ma, T. Guo, Y. Yu, X. Li, W. Xia, Y. Zhang, Effects of freezing-thawing
604 pretreatment combined with liquid nitrogen and dilute acid on the gelatinization of
605 collagen, Int. J. Biol. Macromol. 118 (2018) 435-441. https://doi.org/10.1016/
606 j.ijbiomac.2018.06.106
607 [43] C. da Silva Araújo, E. Pino-Hernández, J.T. Souza Batista, M.R. Sarkis Peixoto Joele, J.
608 de Arimateia Rodrigues do Rego, L.d.F. Henriques Lourenço, Optimization of fish
609 gelatin drying processes and characterization of its properties, Sci. Rep. 11 (2021)
610 20655. https://doi.org/10.1038/s41598-021-99085-3
611 [44] T. Sae-Leaw, S. Benjakul, N.M. O'Brien, Effect of pretreatments and drying methods on
612 the properties and fishy odor/flavor of gelatin from seabass (*Lates calcarifer*) skin, Dry.
613 Technol. 34 (2016) 53-65. https://doi.org/10.1080/07373937.2014.1003071

614 [45] A. Salem, N. Fakhfakh, M. Jridi, O. Abdelhedi, M. Nasri, F. Debeaufort, N. Zouari,
615 Microstructure and characteristic properties of dogfish skin gelatin gels prepared by
616 freeze/spray-drying methods Int. J. Biol. Macromol. 162 (2020) 1-10. https://doi.org/
617 10.1016/j.ijbiomac.2020.06.033
618 [46] A.d.T. Alfaro, E. Balbinot, C.I. Weber, I.B. Tonial, A. Machado-Lunkes, Fish gelatin:
619 characteristics, functional properties, applications and future potentials, Food Eng. Rev.
620 7 (2015) 33-44. https://doi.org/10.1007/s12393-014-9096-5
621 [47] Z. Zarai, R. Balti, H. Mejdoub, Y. Gargouri, A. Sayari, Process for extracting gelatin from
622 marine snail (*Hexaplex trunculus*): Chemical composition and functional properties,
623 Process Biochem. 47 (2012) 1779-1784. https://doi.org/10.1016/ j.procbio.2012.06.007
624 [48] S. Mad-Ali, S. Benjakul, T. Prodpran, S. Maqsood, Characteristics and gelling properties
625 of gelatin from goat skin as affected by drying methods, J. Food Sci. Technol. 54 (2017)
626 1646-1654. https://doi.org/10.1007/s13197-017-2597-5
627 [49] T.M. Johnson, M.E. Zabik, Egg albumen proteins interactions in an angel food cake
628 system, J. Food Sci. 46 (1981) 1231-1236. https://doi.org/10.1111/j.13652621.1981.
629 tb03029.x
630 [50] I. Yakimets, N. Wellner, A.C. Smith, R.H. Wilson, I. Farhat, J. Mitchell, Mechanical
631 properties with respect to water content of gelatin films in glassy state, Polymer, 46
632 (2005) 12577-12585. https://doi.org/10.1016/j.polymer.2005.10.090
633 [51] C.G. da Silva, A.S. Rodrigues, A.C. Lima, R. de Oliveira Mello, F.D.P. Morisso, R.C.P.
634 Dornelles, E.H. Kubota, Gelatin extracted from jundiá skin (*Rhamdia quelen*): An
635 alternative to the discarded by-product, Food Res. Int. 161 (2022) 111829.
636 https://doi.org/10.1016/j.foodres.2022.111829
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Figure Legends:

Fig. 1. Yield of gelatin extracted from Atlantic salmon skin as affected by different drying techniques. Bars are given as means ± standard deviation (n = 3). Different letters on the bars indicate significant differences ($p < 0.05$).

Fig. 2. Gel strength and viscosity of gelatin extracted from Atlantic salmon skin as affected by different drying techniques. Bars are given as means ± standard deviation (n = 3). Different letters on the bars within the same parameter indicate significant differences ($p < 0.05$).

Fig. 3. Turbidity of gelatin solution extracted from the skin of Atlantic salmon as affected by different drying techniques. Bars are given as means ± standard deviation (n = 3). Different letters on the bars indicate significant differences ($p < 0.05$).

Fig. 4. SDS-PAGE of gelatin extracted from Atlantic salmon skin as affected by different drying techniques. M: Marker; FDG: Freeze-dried gelatin; HDG: hot air-dried gelatin; VDG: Vacuum-dried gelatin; SDG: Spray-dried gelatin.

Fig. 5. FTIR spectra (A) and secondary structure analysis in amide I region (B) of gelatin extracted from Atlantic salmon skin as affected by different drying techniques. FDG: Freeze-dried gelatin; HDG: hot air-dried gelatin; VDG: Vacuum-dried gelatin; SDG: Spray-dried gelatin. *Regions: α-helix, 1650-1660 cm^{-1}; β-sheet, 1610-1640 $cm^{-1}$and 1670-1690 cm^{-1}; β-turn, 1660-1670 cm^{-1} and 1690-1700 cm^{-1}; random coil, 1640-1650 cm^{-1}.

Table 1. Proximate composition of gelatin extracted from Atlantic salmon skin as affected by different drying methods.

Composition (g/100 g)	FDG	HDG	VDG	SDG
Moisture	11.56 ± 0.04^{a}	8.04 ± 0.14^{d}	8.77 ± 0.38^{c}	9.54 ± 0.03^{b}
Protein*	94.84 ± 0.65^{a}	95.03 ± 0.39^{a}	94.84 ± 0.16^{a}	94.60 ± 0.30^{a}
Fat*	2.35 ± 0.11^{b}	2.72 ± 0.12^{ab}	2.70 ± 0.17^{ab}	2.78 ± 0.06^{a}
Ash*	2.41 ± 0.12^{a}	2.24 ± 0.00^{a}	2.30 ± 0.03^{a}	2.47 ± 0.38^{a}

Values are given as means ± SD (n=3). The different lowercase letters in the same row indicate significant differences ($p < 0.05$). FDG: Freeze-dried gelatin; HDG: hot air-dried gelatin; VDG: Vacuum-dried gelatin; SDG: Spray-dried gelatin. *Dry weight basis.

663 **Table 2.** Appearance and color attributes of gelatin from Atlantic salmon skin as affected by

664 different drying techniques.

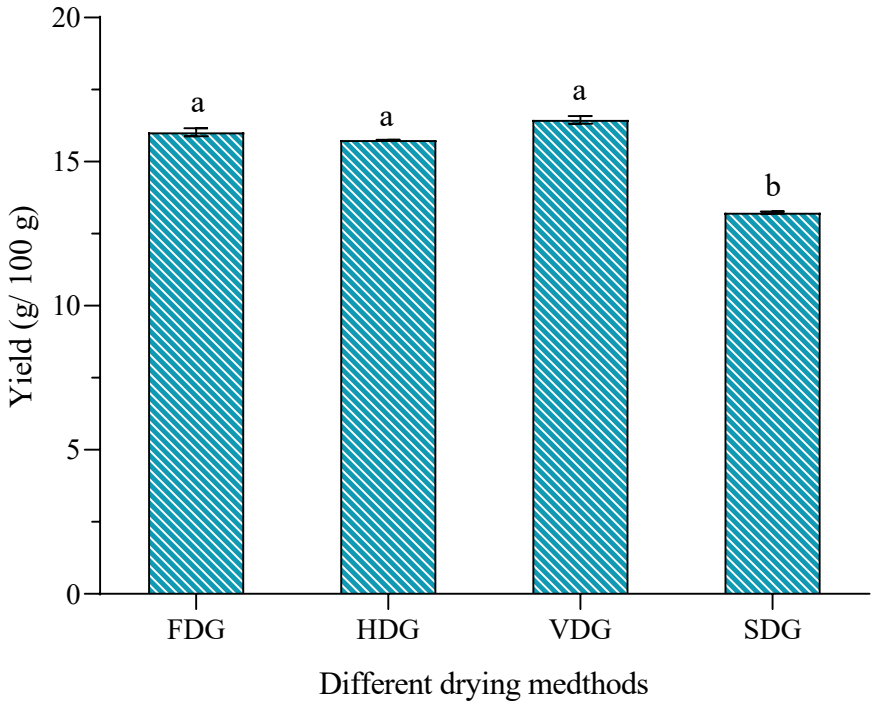
Sample	FDG	HDG	VDG	SDG
Appearance				
Color				
*L**	89.35 ± 0.80^{b}	66.72 ± 0.56^{d}	69.80 ± 0.31^{c}	92.23 ± 0.11^{a}
*a**	-1.56 ± 0.05^{c}	0.20 ±0.04^{a}	-0.09 ± 0.02^{b}	-1.60 ± 0.01^{c}
*b**	3.45 ± 0.15^{c}	14.85 ± 0.10^{a}	9.95 ±0.10^{b}	2.45 ± 0.03^{d}
ΔE*	10.88 ± 0.76^{c}	36.09 ± 0.52^{a}	31.38 ± 0.27^{b}	7.88 ± 0.10^{d}

665 Values are given as means ± SD (n=3). The different lowercase letters in the same row indicate

666 significant differences ($p < 0.05$). FDG: Freeze-dried gelatin; HDG: hot air-dried gelatin;

667 VDG: Vacuum-dried gelatin; SDG: Spray-dried gelatin.

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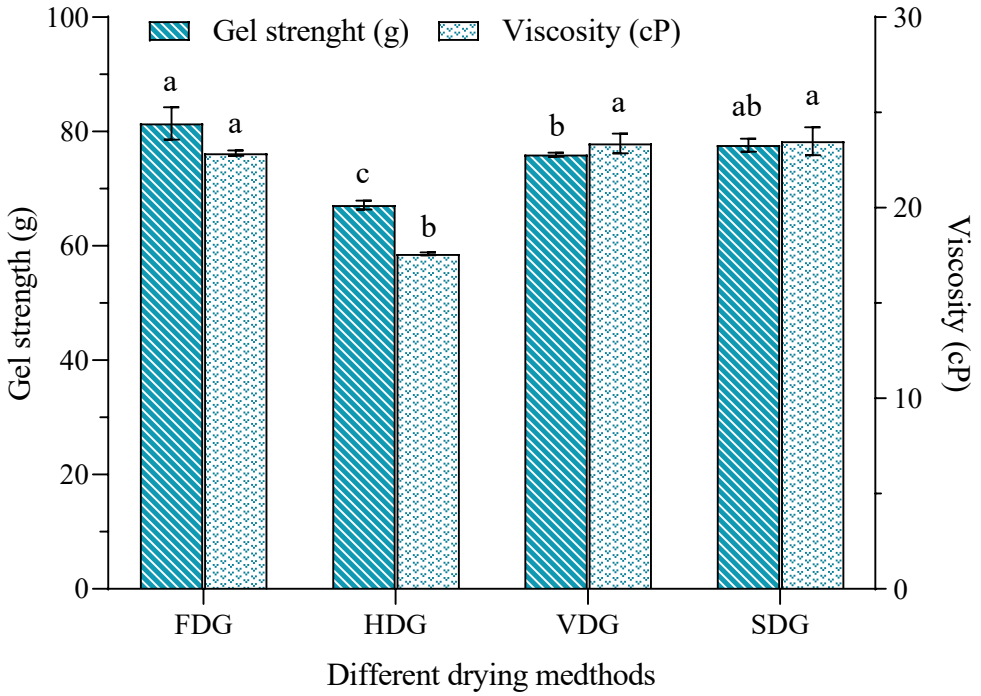


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670 **Fig. 1.**

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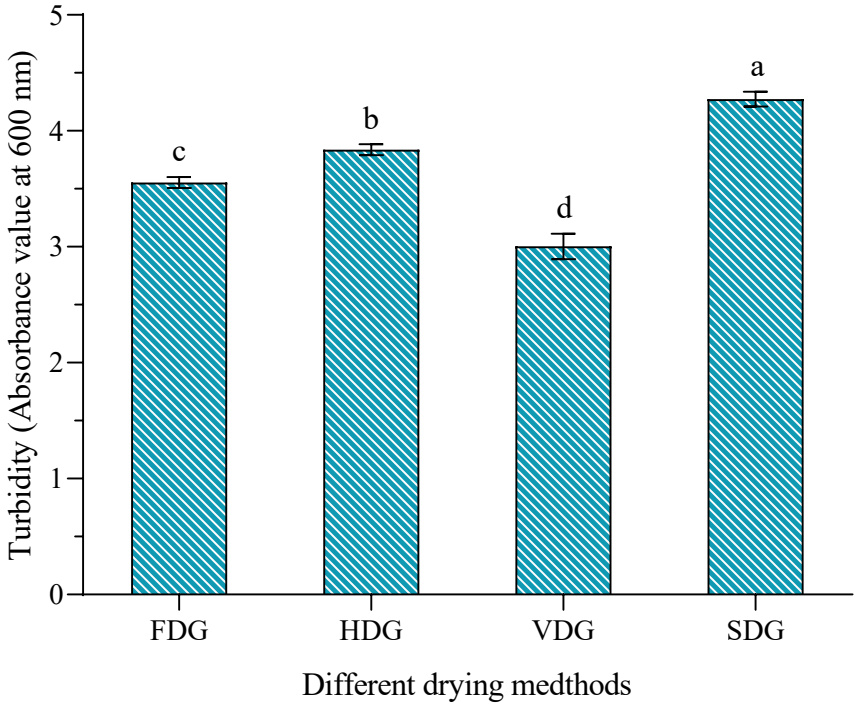


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674 **Fig. 2.**

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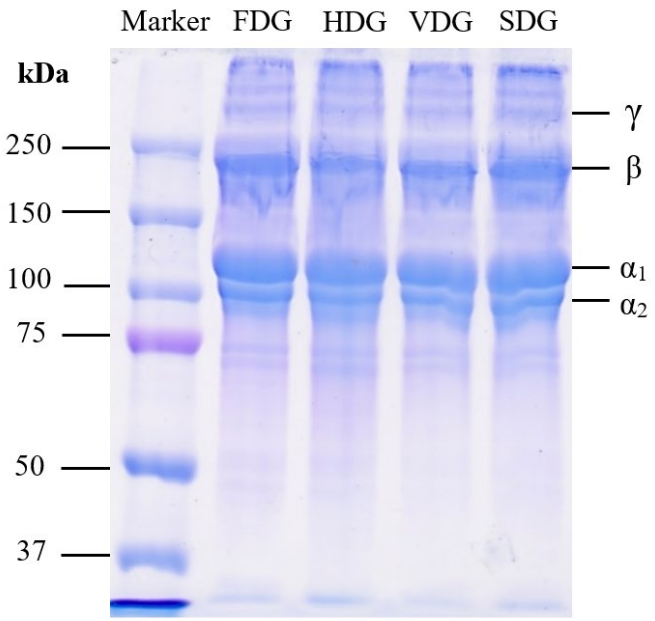
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678 **Fig. 3.**

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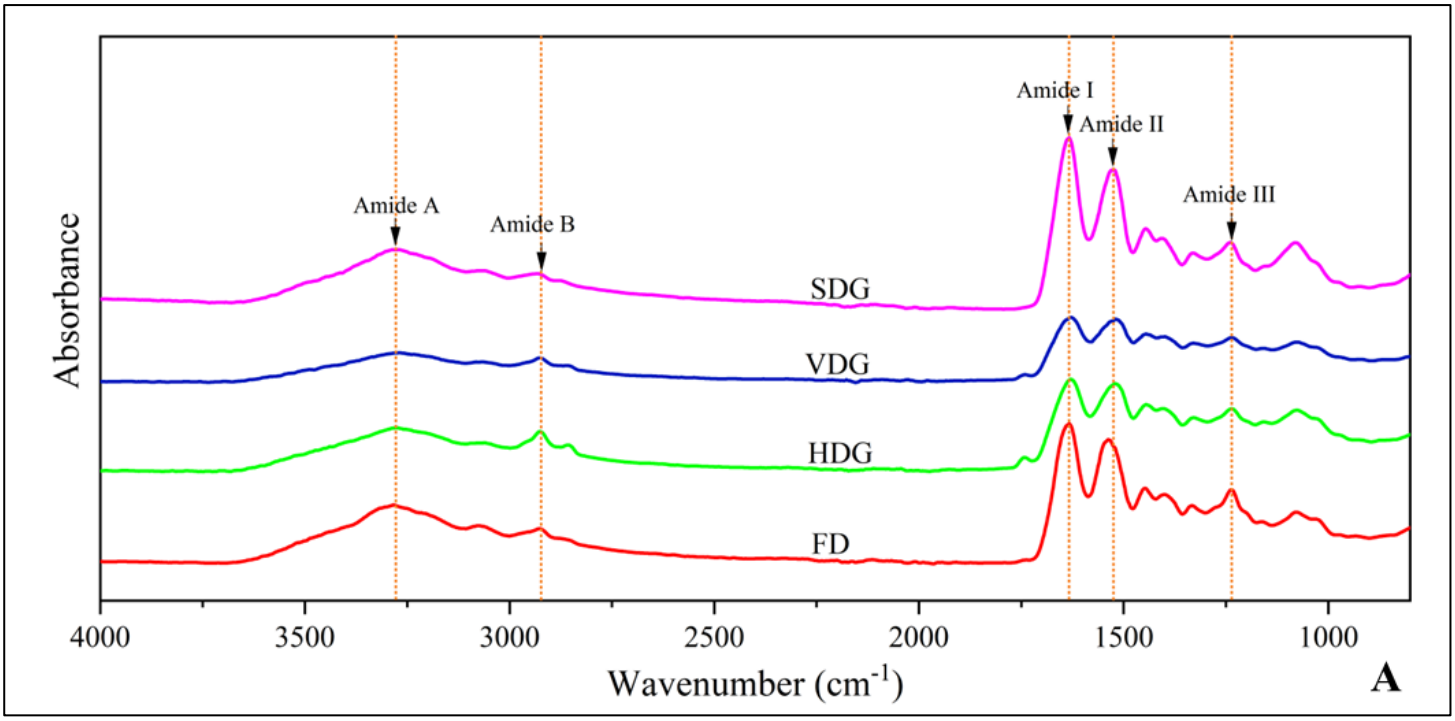


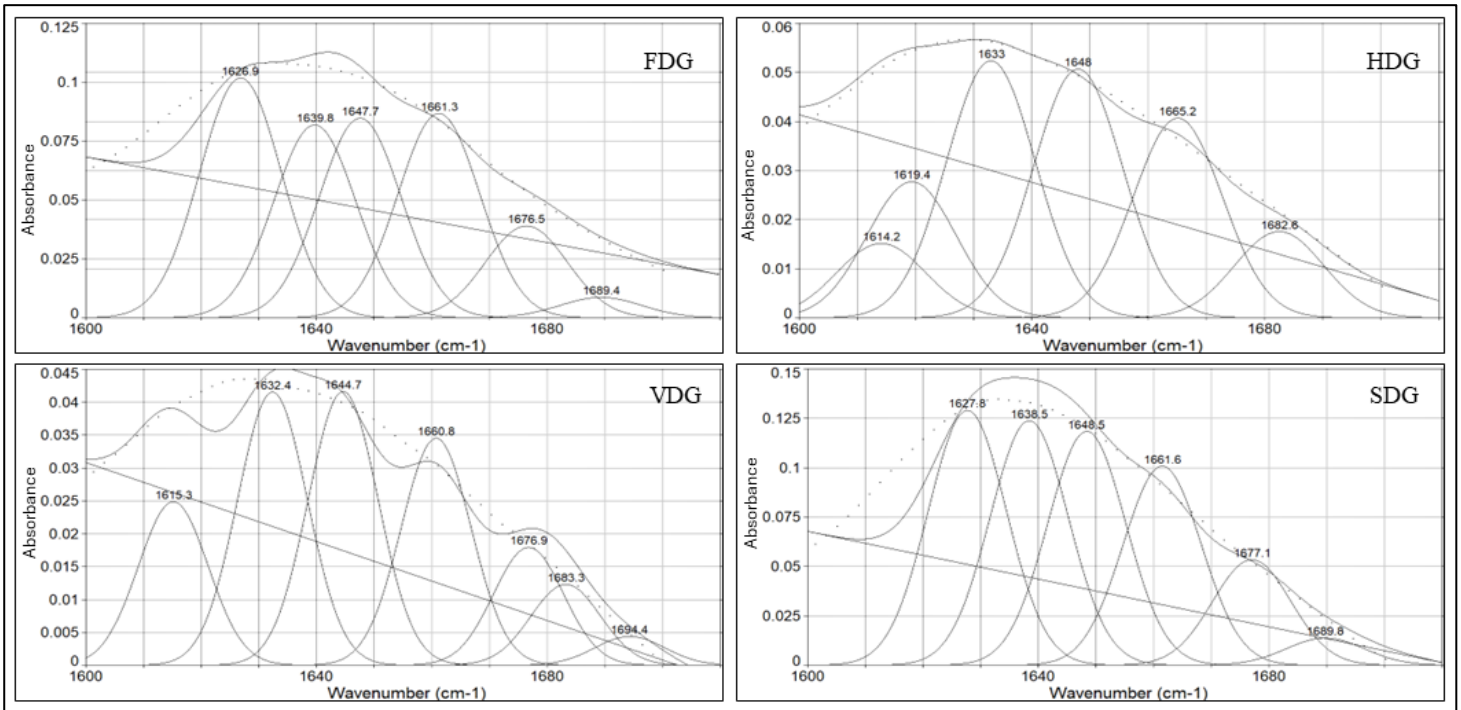
680

681 **Fig. 4**.

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Secondary structural changes in Amide I region: 1700-1600 cm^{-1}

Samples	Secondary structural composition (%)*			
	α-helix	β-sheets	β-turn	Random coil
FDG	-	57.43	21.53	21.03
HDG	-	55.25	19.93	24.83
VDG	-	54.53	21.97	23.44
SDG	-	59.35	18.70	21.96

B

684

685 **Fig. 5**.