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Fish Protein Hydrolysate and Gelatin from Tuna Processing By-Products Using Freeze-Drying and Oven-Drying Methods

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ABSTRACT

Tuna processing industries generate a significant amount of by-products, including dark muscle and skin, which are potential sources of functional proteins such as fish protein hydrolysate (FPH) and gelatin. This study aimed to characterize FPH from tuna dark muscle and gelatin from tuna skin, while evaluating the effects of freeze-drying (FD) and oven-drying (OV) on their physicochemical and antioxidant properties. The results indicated that the yield and proximate composition of FPH-FD and FPH-OV did not differ significantly (p>0.05). In contrast, antioxidant activity showed a significant effect (p<0.05), with FPH-OV exhibiting a stronger radical-scavenging capacity than FPH-FD, as indicated by its lower IC_{50} value, namely 152.75 μg/mL and 647.68 μg/mL, respectively. Unlike FPH, the drying methods for gelatin significantly influenced moisture content, ash content, gel strength, and antioxidant activity (p<0.05). The freeze-dried gelatin had higher gel strength (399.7 g) than oven-dried gelatin (372.7 g), although both values exceeded the typical high-Bloom range of 200–300 g. Under the tested laboratory conditions, oven-drying at 60 °C produced FPH with yield and proximate composition comparable to those of freeze-drying and with higher antioxidant activity, whereas it increased moisture and ash contents of gelatin and reduced its gel strength.

Keywords: antioxidant, fish protein hydrolysate, freeze-drying, gelatin, oven-drying

INTRODUCTION

The tuna processing industry generates a substantial volume of by-products, including bones, skin, and heads, which constitute approximately 50–70% of the total fish weight (Yin *et al*., 2025). However, the utilization of these materials remains limited to low-value products, such as animal feed, or they are discarded as environmental waste (Marinou *et al*., 2025; Nguyen *et al*., 2022; Vazquez *et al*., 2024). In fact, these by-products serve as a rich source of functional proteins, abundant in essential amino acids and antioxidant compounds *(*Bui *et al*., 2021; Gao *et al*., 2021), offering great potential for conversion into fish protein hydrolysates (FPH) and gelatin. Such conversion efforts have become a crucial strategy for enhancing economic value. Nevertheless, a major challenge currently faced is maintaining the physicochemical stability and bio-active activity of the products during the dehydration process, which often represents a critical stage in preserving the final quality of these functional food ingredients. Various studies have been conducted to develop FPH and gelatin from fishery by-products across different fish species and specific drying methodologies. For instance, Abd El-Rady *et al*. (2023) produced FPH from mixture of minced fish tuna by-products using oven-drying at 60 °C for 24 h, while other researchers investigated tuna-derived FPH without employing a drying stage (Rachman *et al*., 2023; Vásquez *et al*., 2022). The production of FPH utilizing either spray-drying or freeze-drying techniques has been reported across several fish species, including bighead carp (*Aristichthys nobilis*) skin (Dong *et al*., 2022), *Priacanthus hamrur* skin (Baraiya *et al*., 2025), grass carp (*Ctenopharyngodon idella*) scales (Yang *et al*., 2025), and Tra catfish (*Pangasius hypophthalmus*) (Nguyen *et al*., 2022). Regarding gelatin recovery, the application of freeze-drying has been documented by Yin *et al*. (2025), whereas oven-drying methods have been utilized in studies by Osiriphun *et al*. (2022) and Tayel *et al*. (2024).

However, despite these advancements, the optimization of drying methods for FPH and gelatin remains a major challenge. Most existing studies rely heavily on freeze-drying to maintain protein quality, yet its application at an industrial scale is rarely

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studied due to prohibitive operational and investment costs (Dong *et al*., 2022). Furthermore, while spray drying offers a faster alternative for FPH production, the process largely necessitates the addition of carrier agents such as maltodextrin to prevent stickiness, which consequently leads to a significant reduction in protein purity when compared to the high purity products obtained through freeze-drying (Fawzya *et al*., 2020). To date, there are a few data available comparing the effectiveness of low temperature oven-drying as a filler free alternative to freeze-drying, particularly concerning its ability to preserve the antioxidant properties and physicochemical characteristics of both FPH and gelatin simultaneously.

The novelty of this research lies in the systematic evaluation of low temperature oven-drying for recovering functional proteins from tuna by-products without the use of additives. Unlike previous works that focus on a single bioproduct, this study provides a direct comparative analysis of the yield, proximate composition, and antioxidant activity of FPH and gelatin, together with the amino acid profile of FPH, under two contrasting drying mechanisms (sublimation and evaporation). This approach offers a new insight into whether moderate thermal processing can serve as a technically feasible alternative to freeze-drying with respect to the physicochemical and bioactive properties measured in the products.

This study provides laboratory-scale data on the effects of freeze-drying and low-temperature oven-drying on FPH and gelatin from tuna by-products, which can serve as a basis for further evaluation of drying strategies, including energy, cost, and scale-up analyses, for the downstream processing of tuna by-products into functional food ingredients. The scope of the present study is limited to measured laboratory outcomes (yield, proximate composition, antioxidant activity, amino acid profile of FPH, gel strength, and Fourier transform infrared (FTIR) spectra of gelatin); energy consumption, cost, throughput, shelf-life, and scale-up were not evaluated.

MATERIALS AND METHOD

Materials

The raw materials, consisting of frozen dark muscle and skin of tuna (*Thunnus albacares*), were obtained from PT Benteng Laut Sejahtera, Bitung, North Sulawesi, Indonesia. The samples were transported in a frozen state using a refrigerated container and subsequently transferred to the laboratory of the Center for Applied Testing of Marine and Fisheries Product (BBP3KP) using a cool box via a 2-hour road transport.

The enzymatic hydrolysis for FPH production was performed using Alcalase (50,000 U/g), produced by Xi'an Best Bio-Tech Co., Ltd (China). The chemicals used for gelatin production consisted of 100% acetic acid (Emsure, Germany) and sodium hydroxide (Emsure, Germany). Analytical grade reagents, including sodium hydroxide, chloroform, trichloroacetic acid, boric acid (Emsure), hydrochloric acid 32% (Emplura, Germany), Kjeldahl tablets, sodium thiosulfate pentahydrate (Merck, Germany), and 2,2-diphenyl-1-picrylhydrazyl (Himedia, India), were utilized for chemical analyses. The tools used include centrifuge (Universal 320R, Hettich Asia Pacific Pte. Ltd., Germany), water bath shaker (WB 1024, FOSS Analytical Co. Ltd., China), furnace (MT1100-40-B2, Magma Therm, Turkey), spectrophotometer UV-Visible (Cintra 1010, GBC Scientific Equipment Pty. Ltd., Australia), digital scales (MS204TS, Mettler Toledo GmbH, Switzerland), oven (Oven 125 BD, IKA, China), freeze dryer (Cryodos 1382, Telstar, Spain), Kjeldahl distillation unit (VAP 450, Gerhardt GmbH & Co, Germany) and Soxhlet extraction system (ST 243 Soxtec™, FOSS Analytical Co. Ltd., China).

Production of tuna black muscle protein hydrolysate

The production of FPH was conducted following the methods of Bui *et al*. (2021) and Mutamimah *et al*. (2018) with slight modifications. The process involved a two-stage optimization approach. In the first stage, the optimal hydrolysis time was determined by measuring the degree of hydrolysis (DH) from liquid FPH samples collected hourly over a 6 h period. In the second stage, the concentration of Alcalase (0.1; 0.2; and 0.3% w/w) was evaluated based on yield, proximate composition, and antioxidant activity to establish the optimal production parameters. The selected optimal condition was further subjected to drying treatments to compare freeze-drying and oven-drying methods.

The minced tuna dark muscle was homogenized with distilled water at a ratio of 1:1 (w/v). The mixture was preheated to 65 °C before the addition of Alcalase at the specified concentrations (0.1; 0.2; and 0.3% w/w). Hydrolysis was performed in a water bath shaker at 65 °C for 1 to 6 h. Following the reaction, enzyme inactivation was achieved by heating the mixture to 90 °C for 15 min. After cooling, the samples were centrifuged at 7,800 × *g* for 15 min at 4 °C to separate the supernatant from the residue. The supernatant was dried using a freeze dryer at −35±2 °C, 0.154±0.05 mbar for 96 h. For the oven-drying treatment, the supernatant was dried at 60 °C for 24 h. The resulting FPH was then analyzed for yield, proximate composition (moisture, ash, lipid, and protein contents), antioxidant activity, and amino acid profile, as described in the following sections. The DH was determined separately on the liquid hydrolysates during the optimization stage.

Extraction of gelatin from the tuna skin

The extraction of fish gelatin was conducted according to the method described by previous studies Osiriphun *et al*. (2022) and Yin *et al*. (2025) with slight modifications. Initially, the fish skin samples were thoroughly washed under running tap water to remove impurities. The cleaned skin was then subjected to an alkaline treatment by immersion in 0.1 mol/L NaOH at a skin to solution ratio of 1:5 (w/v) for 2 h at 25 °C to eliminate non-collagenous proteins and fats. Following the treatment, the skin was rinsed with running water until a neutral pH was achieved. For further purification and to facilitate complete swelling, the alkaline-treated skin was soaked in 0.2 mol/L acetic acid (1:5 w/v) for 12 h at 25 °C. After another neutral rinsing step, the gelatin was extracted using distilled water at a 1:1 (w/v) ratio in a water bath at 60 °C for 12 h. The resulting gelatin solution was filtered through a double layered white cotton cloth. The filtrate was dried using two different methods: (1) oven-drying at 60 °C for 48 h, and (2) freeze-drying at −35±2 °C for 96 h. Finally, the dried gelatin sheets were pulverized into a powder with a particle size of 0.5 mm (30 mesh). The resulting gelatin was then analyzed for yield, proximate composition, antioxidant activity, gel strength, and functional groups by FTIR spectroscopy, as described in the following sections.

Analysis of the FPH and gelatin yield

The yield of FPH and gelatin was determined by comparing the final weight of the dried product to the initial weight of the raw material, following the method described by Sayuti *et al*. (2023) and Yuniarti *et al*. (2024). A higher yield percentage indicates a more efficient recovery of the output product from the given input during the processing stages. The yield was calculated using Equation 1.

$$\text{Yield (\%)} = \frac{W_p}{W_i} \times 100 \quad \text{..... (1)}$$

W_p= the weight of the final dried product (g) and W_i= the initial weight of the raw material (g) used in the process.

Proximate analysis

The proximate composition of the raw materials (tuna dark muscle and skin), as well as the resulting FPH and gelatin, was determined following the official methods of AOAC (2016). The analysis included moisture content (oven-drying gravimetric method; AOAC 977.12), ash content (AOAC 938.08), total protein content determined by the Kjeldahl method (AOAC 940.25), and lipid content measured using Soxhlet extraction (AOAC 948.15).

Measurement of degree of hydrolysis (DH)

The DH was determined by measuring the ratio of 10% trichloroacetic acid (TCA)-soluble nitrogen to the total nitrogen in the sample (Hoyle and Merritt, 1994). To obtain the TCA-soluble fraction, aliquots of the liquid hydrolysate were mixed with an equal volume of 20% TCA to achieve a final concentration of 10% TCA. The mixtures were then centrifuged at 7,800 × *g* for 15 min to separate the soluble and insoluble fractions. The nitrogen content of the resulting supernatants and the total nitrogen of the initial raw material were analyzed using Kjeldahl method (AOAC, 2005). The DH was calculated using Equation 2.

$$\text{DH (\%)} = \frac{\text{10\% TCA soluble N in sample}}{\text{Total N in sample}} \times 100 \quad \text{..... (2)}$$

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, according to the method of Yuniarti *et al*. (2024) with slight modifications. A 3 mL aliquot of the sample solution at varying concentrations (100, 200, 300, 400, and 500 *μ*g/mL) was mixed with 1 mL of 0.1 mM DPPH solution. The mixture was homogenized using a vortex mixer and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Methanol was used as the negative control, while ascorbic acid (2–10 *μ*g/mL) served as the positive control. The DPPH radical scavenging activity was calculated using Equation 3. To evaluate the antioxidant potency, the IC_{50} value (the concentration required to inhibit 50% of DPPH radicals) was determined through linear regression analysis by plotting the scavenging percentage against the sample concentration (Rachman *et al*., 2023).

$$\text{DPPH Inhibition (\%)} = \frac{\text{Abs control-Abs sample}}{\text{Abs control}} \times 100 \quad \text{..... (3)}$$

Abs control= the absorbance of the DPPH solution without sample and Abs sample= the absorbance of the DPPH solution after reaction with the sample.

Amino acid profile analysis

Amino acid analysis was performed using high-performance liquid chromatography (HPLC, Shimadzu SCL-10A, CBM 20A, Japan) with fluorescence detection (AOAC, 2016) following pre-column derivatization using o-phthalaldehyde (OPA). A total of 6 mg of protein from each sample was hydrolyzed using 2 mL of 6 N HCl in a nitrogen-sealed

screw-cap tube, then incubated at 110 °C for 24 h. The hydrolysate was subsequently evaporated to dryness using a rotary evaporator (Heidolph Laborota 4003, Germany), reconstituted with 10 mL of 0.01 N HCl, and filtered through a 0.45 μm membrane filter (0.45 μm pore size, MF-Millipore™, Germany) prior to derivatization. The OPA reagent was prepared by mixing 25 mg of OPA, 2 mL of methanol, 0.02 mL of 2-mercaptoethanol, 0.05 mL of Brij-30, and 0.5 mL of 1 M borate buffer (pH 10.4), which was then diluted with borate buffer at a ratio of 1:2 (v/v) prior to use. Subsequently, 5 μL of the sample solution was reacted with 25 μL of OPA reagent for one minute at room temperature to ensure complete derivatization. Following derivatization, 5 μL of the mixture was injected into the HPLC column, and chromatographic separation was carried out for approximately 25 min. The HPLC conditions used for amino acid analysis were as follows: column type, Thermo Scientific ODS-2 Hypersil; eluent flow rate, 1 mL/min; and detector, fluorescence.

Determination of gel strength

The gel strength of the tuna skin gelatin was determined following the standard method of SNI 8812.1:2019 (BSN, 2019) and Yin *et al*. (2025). A 6.67% (w/v) gelatin solution was prepared by dissolving 7.5 g of gelatin in 105 mL of distilled water. The mixture was hydrated at 25 °C and subsequently incubated at 65 °C with periodic stirring for 20 min to ensure complete dissolution. The homogenized solution was then poured into a 10 mL Bloom jar (or standardized container) and allowed to cool at 25±2 °C for 30 min. To facilitate gelation, the samples were incubated at 4 °C for 12 h. The gel strength was measured using a texture analyzer (TMS Pro, Food Technology Corporation, USA). The Bloom jar was positioned centrally beneath the plunger, and the probe pierced the gel surface to a depth of 4 mm at a constant speed of 0.5 mm/s.

Determination of FTIR

Functional group characterization of gelatin extracted from tuna fish skin was performed using Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu Prestige-21, Japan). Prior to analysis, samples were ground to a uniform and homogeneous consistency, and subsequently scanned over a wavenumber range of 500 to 4,000 cm^{-1} (Prasetyo *et al*., 2025).

Data analysis

The degree of hydrolysis (DH) was measured in duplicate (n= 2), whereas yield, proximate composition, DPPH radical scavenging activity, and gel strength were measured in triplicate (n= 3). The results were expressed as mean ± standard deviation. Statistical analysis was conducted using IBM SPSS Statistics version 27. The DH data were analyzed using a two-factor analysis of variance (ANOVA) to evaluate the interaction effects between hydrolysis time and Alcalase enzyme concentration, followed by Duncan's Multiple Range Test (DMRT). The data for proximate composition, yield, antioxidant activity, and gel strength were analyzed using one-way ANOVA, with post-hoc comparisons performed using Duncan's test. The effect of drying methods (freeze-drying and oven-drying) on the measured parameters was analyzed using an independent samples t-test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Composition of tuna black muscle and skin

As shown in Table 1, the black muscle of tuna (*T. albacares*) used as a raw material for FPH production was composed of 75.16% moisture, 22.89% protein, 2.37% lipid and 1.16% ash. Additionally, the tuna skin used for gelatin extraction exhibited a proximate composition of 59.26% moisture, 34.85% protein, 8.52% lipid and 1.27% ash. Based on the results, the proximate composition of the tuna dark muscle in this study did not differ significantly from the literature reported by Bui *et al*. (2021). Similarly, the composition of the tuna skin was generally consistent with the findings of Gallego *et al*. (2026).

Table 1. The proximate composition of tuna black muscle and tuna skin

Parameters	Tuna Black Muscle		Tuna Skins		
	Thunnus albacares	*Sarda orientalis*[a]	*Thunnus albacares*	*Thunnus albacares*[b]	*Thunnus albacares*[c]
Moisture (%)	75.16±0.51	67.7±0.1	59.26±1.26	69.18±0.06	47.4±0.2
Protein (%)	22.89±0.42	24.0±0.1	34.85±1.55	26.77±1.08	34.0±2.0
Lipid (%)	2.37±0.20	0.52±0.2	8.52±0.94	1.69±0.29	8.5±0.7
Ash (%)	1.16±0.03	2.15±0.4	1.27±0.03	0.20±0.05	9.8±0.9

Note: [a]Bui *et al*. (2021); [b]Rachman *et al*. (2023); [c]Gallego *et al*. (2026). Data are presented as mean ± standard deviation (n= 3)

The protein content in tuna skin was higher than that in the dark muscle. This difference can be attributed to the higher moisture content in the dark muscle, which dilutes the proportion of protein. In contrast, tuna skin contains a lower moisture level and is rich in structural proteins, particularly collagen, which contributes to its relatively higher protein content (Jaziri *et al*., 2022). These characteristics confirm that the skins are an excellent substrate for gelatin extraction, while the dark muscle serves as a high-protein base for FPH production.

Optimization of FPH processing

Degree of hydrolysis

The first stage of FPH production optimization was conducted based on the DH to determine the optimal hydrolysis time for yielding high-quality FPH within a reduce processing time. The DH is a key parameter widely used to evaluate the extent of protein breakdown during enzymatic hydrolysis (Witono *et al*., 2020). This parameter reflects the cleavage of peptide bonds, resulting in the formation of smaller peptides and free amino acids (Alahmad *et al*., 2022). Therefore, DH is commonly employed as an initial indicator to assess the efficiency of the hydrolysis process and to determine optimal processing conditions in the production of FPH.

DH was analyzed using a two-factor ANOVA to evaluate the effects of hydrolysis time and Alcalase enzyme concentration, as well as their interaction. DH was used as a preliminary parameter to determine the optimal conditions for FPH production. The results showed that hydrolysis time, enzyme concentration, and their interaction significantly influenced DH values (p<0.05). The DH values increased as the hydrolysis time progressed from 1 to 6 h (Figure 1), as the enzyme had more time to cleave complex protein chains into smaller peptides. Similarly, increasing the Alcalase concentration from 0.1% to 0.3% consistently produced higher DH values at nearly every time point, since a greater amount of enzyme provided more active sites available to catalyze peptide bond cleavage, thereby accelerating the hydrolysis reaction (Alahmad *et al*., 2022). The significant interaction between time and enzyme concentration indicates that the effect of hydrolysis time on DH was not uniform across enzyme concentrations; at the highest concentration (0.3%), DH increased more rapidly and reached a higher maximum value within a shorter time compared to the lower concentrations, before declining after 5 h. This pattern suggests that a higher enzyme concentration accelerates the attainment of maximum hydrolysis but also hastens the point at which excessive hydrolysis begins to occur.

The DH values generated by FPH using 0.1% Alcalase ranged from 23.03% to 62.41% during 1 to 6 h of hydrolysis. In this first treatment, the DH trend continued to increase up to the 6th hour. A similar trend was also observed in the second treatment, namely FPH using 0.2% Alcalase, where the DH values increased from 21.24% to 60.28% from the 1st to the 6th hour. Although the DH value continued to increase up to the 6th hour, statistical analysis indicated that the increase was significantly different (p<0.05) from the DH value at the 5th hour (57.30%). Meanwhile, in the third treatment, namely FPH using 0.3% Alcalase, the increasing trend of DH reached its maximum at the 5th hour and subsequently decreased at the 6th hour.

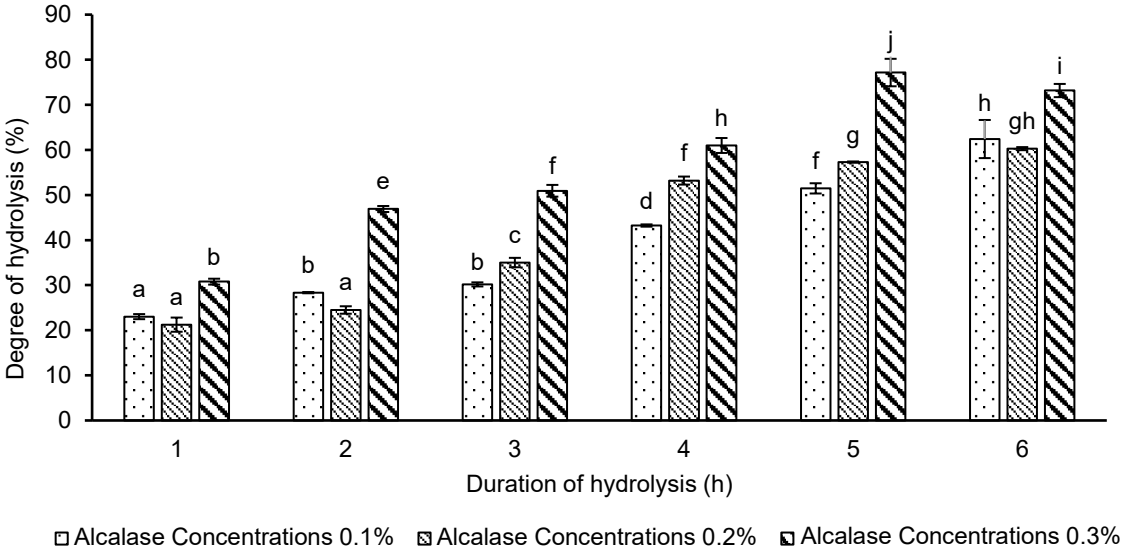

Figure 1. Degree of hydrolysis at different Alcalase concentrations across various hydrolysis durations. Data are expressed as mean ± standard deviation (n= 2). Different superscript letters indicate significant differences (p<0.05)

The DH values of FPH using 0.3% Alcalase increased from 30.78% to 77.16% from the 1st to the 5th hour. As the enzymatic hydrolysis time increased, the enzyme interacted more extensively with the substrate, resulting in a greater cleavage of peptide bonds, which ultimately increased the degree of hydrolysis. However, when the hydrolysis time exceeded 5 h, the process became excessive, causing the peptides formed to be further degraded into free amino acids (Zhou *et al*., 2024). Alcalase at a concentration of 0.3% yielded the highest DH compared to the 0.1% and 0.2% treatments. Based on the value of the DH, a hydrolysis time of 5 h is the optimum time for FPH production.

Effects of enzyme concentration

The second stage of FPH production optimization aimed to investigate the effect of the enzyme concentration on the characteristics of FPH. The determination of optimal FPH was based on multiple indicators, including yield, proximate composition, and antioxidant activity. These parameters served as comprehensive evaluation criteria, ensuring that the FPH was assessed not only for its production yield but also for its bioactive quality.

Yield and proximate composition

The yield and proximate composition of the FPH produced using various Alcalase concentrations are summarized in Table 2. The concentration of Alcalase significantly affected to yield, moisture, ash, and protein content of the FPH (p<0.05). However, the lipid content did not show a significant difference across all treatments (p>0.05). The yield of FPH significantly increased from 7.45 to 9.59% as the Alcalase concentration increased from 0.1 to 0.3%, in line with previous reports by Alahmad *et al*. (2022) and Chiodza and Goosen (2024).

The protein content reached its highest level (81.81±0.97%) in the 0.3% Alcalase treatment. However, this value was not significantly different (p>0.05) compared to the FPH produced with 0.2% Alcalase. The enzymatic hydrolysis process effectively concentrates protein by removing insoluble fractions and lipids. Conversely, the lipid content in all samples remained very low (<0.50%) and still complies with the standard established by the Protein Advisory Group of FAO, which recommends that fish protein hydrolysate intended for human consumption should not exceed 0.5% (Kristinsson & Rasco, 2000). The centrifugation process and the removal of the sludge layer during preparation effectively reduced the fat content. Low lipid content is crucial for the stability of FPH because it prevents lipid oxidation during storage (Kristinsson & Rasco, 2000).

Antioxidant activity

The antioxidant activity of the FPH was characterized by its ability to scavenge DPPH radicals, as shown by the percentage of inhibition in Figure 2. The scavenging activity of all FPH samples increased as the concentration rose from 100 to 500 μg/mL. Vitamin C was used as a positive control and reached 84.8% inhibition at 10 μg/mL. Although the antioxidant potency of FPH is lower than vitamin C, the results confirm that the enzymatic hydrolysis of tuna dark muscle successfully releases bioactive peptides with radical-scavenging properties. Radical scavenging DPPH can also be expressed with IC_{50} value, representing the concentration required to scavenge 50% of DPPH free radicals. The IC_{50} values of the FPH produced with different Alcalase concentrations are presented in Table 3. The statistical analysis showed that the enzyme concentration significantly influenced the antioxidant potency of the resulting hydrolysates (p<0.05).

The IC_{50} value significantly decreased from 754.24±4 μg/mL to 647.68±27 μg/mL as the Alcalase concentration increased from 0.1% to 0.3%. A lower IC_{50} value indicates higher antioxidant activity (Mutamimah *et al*., 2018). The 0.3% Alcalase treatment produced the most potent antioxidant activity among all samples. However, this value was not significantly different (p>0.05) compared to the FPH produced with 0.2% Alcalase. Based on the comprehensive evaluation of DH, yield, proximate composition, and antioxidant activity, the 0.3% Alcalase treatment produced the most favorable characteristics. At 5 h of hydrolysis, 0.3% Alcalase gave the highest DH (77.16%), and it also resulted in the highest yield (9.59%), the highest protein content (81.81%), the lowest ash content (5.40%), and the lowest IC_{50} value (647.68 μg/mL).

Table 2. Yield and proximate composition of fish protein hydrolysate (FPH)

Parameters	FPH with 0.1% Alcalase	FPH with 0.2% Alcalase	FPH with 0.3% Alcalase
Yield (%)	7.45±0.21^{c}	8.65±0.17^{b}	9.59±0.11^{a}
Protein (%)	78.07±1.91^{b}	80.53±1.14^{ab}	81.81±0.97^{a}
Ash (%)	7.13±0.15^{a}	6.32±0.25^{ab}	5.40±0.79^{b}
Moisture (%)	6.17±0.14^{b}	7.38±0.19^{a}	7.08±0.50^{a}
Lipid (%)	0.40±0.09	0.39±0.06	0.26±0.03

Note: Data are expressed as mean ± standard deviation of triplicates. Different letters in the same row indicate significant differences at p<0.05

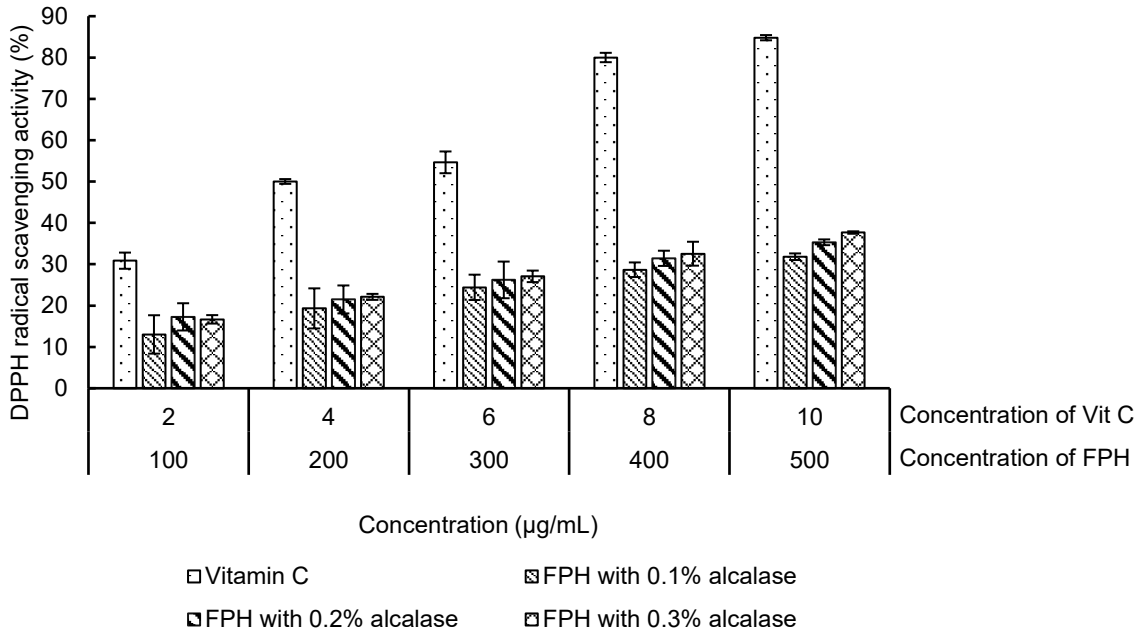

Figure 2. DPPH radical scavenging activity of vitamin C, FPH with 0.1% Alcalase, FPH with 0.2% Alcalase, and FPH with 0,3% Alcalase. Data are expressed as mean ± standard deviation (n= 3)

Table 3. IC_{50} values of fish protein hydrolysate

Samples	IC_{50} (*µ*g/mL)
FPH with 0.1% Alcalase	754.24±4[b]
FPH with 0.2% Alcalase	688.95±29[a]
FPH with 0.3% Alcalase	647.68±27[a]

Note: Data are presented as mean ± standard deviation (n= 3). Different superscript letters indicate significant differences (p<0.05)

Although protein content, ash content, and antioxidant activity of the FPH produced with 0.3% Alcalase were not significantly different (p>0.05) from those produced with 0.2% Alcalase, the yield was significantly higher (p<0.05). Therefore, hydrolysis using 0.3% Alcalase for 5 h was selected as the optimal condition for FPH production and was used in the subsequent drying treatments.

Effect of drying methods on characteristics of FPH

The drying process is a critical step that determines the final quality of fish protein hydrolysates. The comparison between freeze-drying and oven-drying (60 °C) on the yield, proximate composition, and antioxidant activity is summarized in Table 4. The yield and proximate components, including protein, lipid, and ash content, did not show significant differences between the two drying methods. Both methods produced FPH with high protein content (>80%) and low lipid levels (<0.3%).

A major finding emerged in the antioxidant activity of the hydrolysates. Surprisingly, oven-drying at 60 °C significantly enhanced the antioxidant potency compared to freeze-drying. The IC_{50} value of oven-dried FPH (152.75±2.77 *µ*g/mL) was much lower than that of freeze-dried FPH (688.95±29 *µ*g/mL), indicating a much stronger radical scavenging capacity. Similarly, at a concentration of 1 mg/mL, oven-dried FPH achieved an inhibition of 84.17%, while freeze-dried FPH only reached 44.90%.

The significant increase in antioxidant activity during oven-drying is likely attributed to the Maillard reaction. The moderate heat (60 °C) applied during oven-drying promotes the reaction between the amino groups of the peptides and the residual reducing sugars in the hydrolysate. According to El Hosry *et al*. (2025), Maillard reaction products (MRPs), such as melanoidins, possess potent antioxidant properties and can act synergistically with existing bioactive peptides to enhance radical scavenging activity (Fu *et al*., 2019). This phenomenon is visually supported by the physical appearance of the samples, where oven-dried FPH exhibited a distinct browning effect compared to the white appearance of the freeze-dried FPH (Figure 3). Such color transformation is a hallmark of the Maillard reaction, which typically results in the browning of food products during thermal processing (El Hosry *et al*., 2025).

Table 4. Comparison of physicochemical and antioxidant activity of fish protein hydrolysate (FPH) under different drying treatments

Parameters	Freeze-dried FPH	Oven-dried FPH
Yield (%)	9.59±0.10	9.47±0.19
Protein (%)	81.81±0.97	81.07±0.07
Ash (%)	5.40±0.79	5.54±1.31
Moisture (%)	7.08±0.50	7.66±0.11
Lipid (%)	0.26±0.03	0.23±0.02
[1]Inhibition of DPPH (%)	44.90±0.57[b]	84.17±0.51[a]
IC_{50} (μg/mL)	647.68±27[b]	152.75±2.77[a]

Note: [1]The concentration for FPH was 1 mg/mL. Data are presented as mean ± standard deviation (n= 3). Different letters in the same row indicate significant differences at $p<0.05$



A B

Figure 3. Physical appearance of FPH obtained via freeze-drying (A) and oven-drying (B)

Amino acid composition of FPH obtained by freeze-drying and oven-drying

The amino acid composition of FPH produced by freeze-drying (FPH-FD) and oven-drying (FPH-OV) is presented in Table 5. Both products contained a complete range of amino acids, with glutamate, lysine, aspartic acid, and alanine as the most abundant, and included the essential amino acids histidine, threonine, valine, isoleucine, leucine, phenylalanine, lysine, and tryptophan. Total amino acids were 66.46% in FPH-FD and 59.18% in FPH-OV. Most individual amino acids were present at similar or slightly lower levels in FPH-OV, whereas glycine, histidine, and leucine were slightly higher. The largest differences were observed for tryptophan (1.82% vs 0.32%), threonine (3.96% vs 2.77%), and aspartic acid (6.34% vs 5.07%). The lower tryptophan value in FPH-OV should be interpreted cautiously, because Tryptophan is known as the amino acid most susceptible to heat and oxidation due to its complex ring structure; its degradation during heating has been shown to yield indole-derived compounds as well as Maillard products that possess antioxidant properties (Bellmaine *et al*., 2020). As amino acids are vital bioactive components, particularly the essential amino acids which are crucial for functional food applications, the retention of these compounds highlights the nutritional quality of both FPH products.

In terms of antioxidant activity, the presence of specific amino acids plays a pivotal role. Hydrophobic amino acids, such as leucine, valine, glycine, arginine, isoleucine and phenylalanine, are known to enhance radical scavenging by increasing the solubility of peptides in lipid-rich environments, allowing for better interaction with free radicals. Meanwhile, hydrophilic amino acids contribute to the overall stability and electron-donating capacity of the peptides (Sheng *et al*., 2022).

The relationship between these amino acids and the Maillard reaction explains the increase antioxidant potency of FPH-OV. The type and concentration of amino acids significantly influence the rate and formation of MRPs. While amino acids like alanine and glycine are less reactive and contribute to the structural stability of the protein, others like lysine and arginine are highly reactive toward reducing sugars due to their primary amino groups (El Hosry *et al*., 2025). In the present data, lysine and arginine were lower in FPH-OV (6.01% and 4.08%) than in FPH-FD (6.61% and 4.22%), corresponding to reductions of about 9% and 3%, respectively, while total amino acids were about 11% lower.

Table 5. The amino acid composition of fish protein hydrolysate (FPH) dried using freeze-drying and oven-drying

Parameters	Freeze-dried FPH (%)	Oven-dried FPH (%)
Essential amino acids (EAA)		
Tryptophane[ab]	1.82±0.001	0.32±0.001
Lysine[a]	6.61±001	6.01±01
Histidine[ac]	4.13±002	4.64±0.02
Threonine[ac]	3.96±0.008	2.77±0.003
Valine[ab]	2.75±0.01	2.60±0.001
Phenylalanine[ab]	2.38±0.02	1.79±0.004
Isoleucine[ab]	2.40±0.02	2.03±0.005
Leucine[ab]	4.80±0.06	5.17±0.009
∑ Essential amino acids (EAA)	28.85±0.12	25.35±0.06
Non-Essential amino acids (NEAA)		
Glycine[b]	5.16±0.01	5.82±0.001
Glutamate[c]	10.94±0.004	9.80±0.015
Arginine[b]	4.22±0.004	4.08±0.006
Alanine[b]	6.21±0.003	5.11±0.01
Tyrosine	1.96±0.007	1.42±0.005
Aspartic Acid[c]	6.34±0.001	5.07±0.009
Serine[c]	2.78±0.02	2.60±0.004
∑ Non-Essential amino acids (NEAA)	37.61±0.03	33.93±0.05
Total amino acids (TAA)	66.46±0.09	59.28±0.05
EAA/TAA ratio (%)	43.41	42.75

Note: [a]essential amino acids, [b]hydrophobic amino acids, and [c]hydrophilic amino acids. Data are presented as mean ± standard deviation (n= 2)

These differences are compatible with, but do not by themselves demonstrate, the participation of reactive amino acids in the Maillard reaction, as part of the variation may also arise from analytical factors such as acid hydrolysis and derivatization. The browning of FPH-OV (Figure 3) and its significantly lower IC_{50} value support the formation of MRPs such as melanoidins, which may act synergistically with the hydrophobic and hydrophilic amino acids and peptides present to enhance radical scavenging activity.

To evaluate whether oven-drying selectively affected the essential amino acid fraction, the ratio of essential amino acids to total amino acids (EAA/TAA) was calculated. The total essential amino acids identified in FPH-FD and FPH-OV were 28.85% and 25.35%, respectively, with EAA/TAA ratios of 43.41% and 42.75%. Although the absolute content of essential amino acids was 3.55 percentage points lower in FPH-OV, the EAA/TAA ratio differed by only 0.66 percentage points between the two products. This finding indicates that oven-drying did not reduce the essential amino acid fraction more than the non-essential fraction; both fractions decreased to a similar extent, so the relative protein quality was maintained even though the total quantity declined. More importantly, the EAA/TAA ratios of both FPH products exceeded the minimum threshold of 40% set by FAO (2007) as an indicator of high-quality protein (Darabi *et al*., 2022), confirming that both FPH-FD and FPH-OV meet the internationally recommended protein quality criteria for supporting human nutritional requirements.

Effect of drying methods on characteristics of gelatin

The characteristics of tuna skin gelatin were conducted based on different drying methods. The first treatment involved oven-drying (OV) at 60 °C, and the second treatment involved freeze-drying (FD). The extraction of gelatin from tuna skin resulted in high-purity protein products, as shown in Table 6. The drying method significantly influenced the physical and chemical properties of the resulting gelatin.

Yield and proximate composition of gelatin

The yield of gelatin extracted from tuna skin using freeze-drying and oven-drying methods showed no significant difference (p>0.05). As shown in Table 6, the yield obtained from freeze-drying was 11.1%, while oven-drying resulted in a yield of 10.1%. These values are comparable to those reported by Gallego *et al*. (2026) for the same sample type, but lower than those reported by Yin *et al*. (2025), who found 18.42%. The total protein content of the extracted gelatin also showed no significant difference (p>0.05) between freeze-drying and oven-drying. The protein content was 96.95% for freeze-dried gelatin and 97.64% for oven-dried gelatin. These values are higher than those reported for similar samples by Yin *et al*. (2025) (83.39%) and Gallego *et al*. (2026) (85.1%), but are comparable to the 96.77% reported by Osiriphun *et al*. (2022) for tilapia skin gelatin. In contrast, the drying method significantly affected moisture, ash, and fat contents (p<0.05). Freeze-drying resulted in lower moisture and ash contents compared to oven-drying.

Table 6. Comparison of physicochemical and antioxidant activity of gelatin under different drying treatments

Parameters	Freeze-dried Gelatin	Oven-dried Gelatin
Yield (%)	11.10±0.80	10.10±0.38
Protein (%)	96.95±0.27	97.64±1.51
Ash (%)	0.80±0.02^{b}	2.22±0.01^{a}
Moisture (%)	3.71±0.15^{b}	6.65±0.19^{a}
Lipid (%)	1.98±0.04^{a}	1.27±0.03^{b}
Gel strength (g)	399.7±4^{a}	372.7±3^{b}
[1]Inhibition of DPPH (%)	16.39±1.07^{b}	27.34±0.97^{a}

Note: [1]The concentration for gelatin was 4 mg/mL. Data are presented as mean ± standard deviation (n= 3). Different superscript letters within the same row indicate significant differences (p<0.05)

The moisture and ash contents of freeze-dried gelatin were 3.71% and 0.80%, respectively, whereas oven-dried gelatin exhibited values of 6.65% and 2.22%. These values are lower than those reported by Yin *et al*. (2025) (moisture 14.62% and ash 1.11%) and Tayel *et al*. (2024) (moisture 7.9% and ash 1.5%). According to the standards established by the Gelatin Manufacturers of Europe (GME) and the European Pharmacopoeia, the acceptable limits for edible gelatin are ≤15% for moisture content and ≤2% for ash content (GME, 2024). The moisture content of both gelatins was below the 15% limit, and the ash content of freeze-dried gelatin (0.80%) was below the 2% limit. In contrast, the ash content of oven-dried gelatin (2.22%) slightly exceeded the 2% limit (FAO/WHO JECFA, 1970; GME, 2024). Only moisture, ash, protein, and lipid contents were evaluated in this study; other purity and microbiological criteria for edible gelatin were not assessed, so compliance with edible gelatin standards cannot be concluded from these data.

The fat content of gelatin obtained by freeze-drying (1.98%) was higher than that obtained by oven-drying (1.27%). These values are higher than those reported by Yin *et al*. (2025) where the fat content ranged from 0.38% to 0.62%. This difference may be attributed to the defatting step applied by Yin *et al*. (2025), in which hexane was used to remove lipids and reduce the fat content of the resulting gelatin, whereas no defatting step was included in the present study.

Gel strength

Gel strength is an important functional property of gelatin, reflecting, reflecting the ability of the protein to form a stable and firm triple-helix network. As shown in Table 6, the gel strength of the tuna skin gelatin was significantly affected by the drying method (p<0.05), with values recorded at 399.7 g for the freeze-dried sample and 372.7 g for the oven-dried sample. These results are considerably higher than those reported by Yin *et al.* (2025), who found gel strength values ranging from 218 to 271 g across several tuna species, including frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), longtail tuna (*Thunnus tonggol*), and yellowfin tuna (*T. albacares*). Furthermore, the values obtained in this study also exceed the 339 g gel strength reported by Tayel *et al.* (2024) for sole fish skin gelatin. Both samples had gel strength values (372.7–399.7 g) that exceed the 200–300 g range generally classified as "High Bloom" gelatin (Mohammadnezhad & Farmani, 2022). Gel strength values depend on the measurement method and instrument calibration, and gel strength alone does not define overall gelatin quality. These results indicate that the gelatin from tuna skin retained strong gelling capacity despite the reduction observed after oven-drying; its suitability for specific applications should be assessed against application-specific specifications.

Antioxidant activity

The antioxidant potential of tuna skin gelatin was evaluated based on its DPPH radical scavenging activity. As shown in Table 6, oven-dried gelatin exhibited a significantly higher antioxidant activity (27.34±0.97%) compared to the freeze-dried sample (16.39±1.07%) at a concentration of 4 mg/mL (p<0.05). The significant difference in antioxidant activity observed in the gelatin samples aligns with the trend found in FPH, which is likely triggered by the occurrence of the Maillard reaction during the drying process. In this reaction, the free amino groups from the gelatin polypeptide chains interact with residual reducing sugars or other carbonyl sources, leading to the formation of melanoidins, compounds known for their potent antioxidant properties. The presence of these melanoidins also induces a browning effect in the final product (El Hosry *et al*., 2025). This phenomenon was visually confirmed by the physical appearance of the samples, where the oven-dried gelatin exhibited a distinct brownish tint, whereas the freeze-dried gelatin remained white (Figure 4).

FTIR spectra of fish gelatin

FTIR analysis was employed to identify the gelatin based on its functional groups. Gelatin is obtained through the extraction of proteins derived from animal collagen. The spectrum indicating collagen denaturation is characterized by changes in the absorption areas of amide A, amide B, amide I, amide II, and amide III (Hidayati *et al*., 2021). Amide A is typically observed within the wavenumber range of 3478–3310 cm^{-1}, while amide B is found between

2935–2915 cm^{-1}. Amide I is detected in the range of 1620-1700 cm^{-1} (Valcarcel *et al*., 2021), amide II between 1560–1335 cm^{-1}, and amide III within 1300–1200 cm^{-1} (Prasetyo *et al*., 2025).

Amide A indicates the vibration of the N-H group due to hydrogen bonding, along with O-H group vibrations. Amide B represents the asymmetric stretching vibration of $-NH_3$ and =C-H groups, with characteristic peaks observed at 2926 and 2854 cm^{-1} in this study (Figure 5). The presence of amide A and B regions signifies the existence of functional groups (C-H, O-H, and N-H) in the tuna skin gelatin samples (Figure 5). Furthermore, absorption in the 1700–1740 cm^{-1} range may partly correspond to the C=O stretching of the amide I group, indicating the presence of C=O groups in the gelatin samples. However, absorption in this region may also originate from the C=O of lipid residues, which has been reported at 1720–1737 cm^{-1} (Valcarcel *et al*., 2021), consistent with the residual lipid content of the gelatin samples (Table 6). The amide II group is identified within the 1560–1335 cm^{-1} range, specifically at 1460 and 1375 cm^{-1}, indicating the presence of C-N and NH_2 groups. Additionally, the amide III group is observed in the 1300–1200 cm^{-1} range, specifically at 1206 cm^{-1}. Amide III generally represents a combination of peaks from N-H vibrations and C-N stretching. The presence of short N-H peptide groups suggests molecular degradation during the fish gelatin extraction procedure (Hidayati *et al*., 2021).



Figure 4. Gelatin physical appearance following freeze-drying (A) and oven-drying (B)

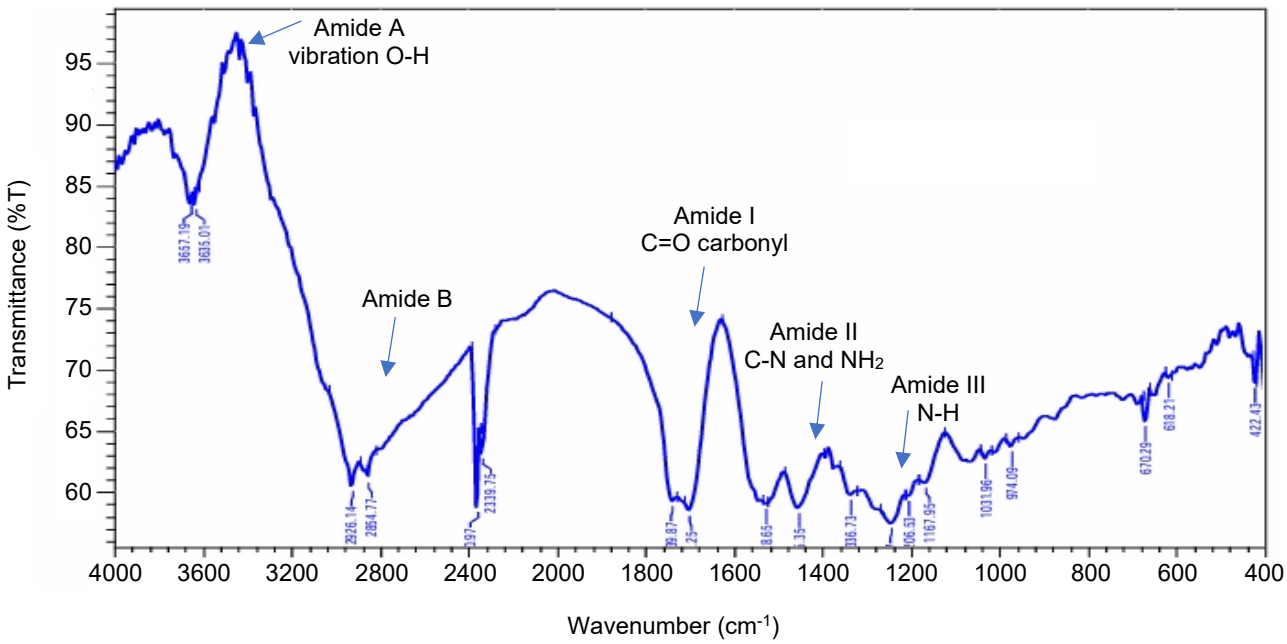

Figure 5. FTIR spectra of tuna fish skin gelatin

CONCLUSION

The results of this study demonstrate that tuna processing by-products, specifically dark muscle and skin, have strong potential as sources of functional proteins in the form of fish protein hydrolysate (FPH) and gelatin. The optimal condition for FPH production was achieved using 0.3% Alcalase concentration with 5 h hydrolysis time. Drying methods significantly influenced the functional properties of the products, particularly antioxidant activity. Oven-drying at 60 °C enhanced the antioxidant capacity of FPH and gelatin, while freeze-drying better preserved gel strength in gelatin. Both gelatins had gel strength above the usual 200–300 g high-Bloom range, but the ash content of oven-dried gelatin exceeded the 2% limit for edible gelatin, and other purity and microbiological criteria were not evaluated. Therefore, within the scope of the measured laboratory outcomes, low-temperature oven-drying is a promising alternative to freeze-drying for FPH, giving comparable yield and proximate composition with higher antioxidant activity, whereas its use for gelatin requires further evaluation, including energy, cost, and scale-up analyses.

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