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Collagen extraction from rainbow (*Oncorhynchus mykiss*) trout heads and skins

Extracción de colágeno de cabezas y pieles de trucha arcoíris (*Oncorhynchus mykiss*)

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ABSTRACT

Fish processing produces significant amounts of by–products. The disposal of these wastes can lead to environmental and human health problems; however, these by–products can often be transformed into high–value and beneficial products. This study investigated the physicochemical properties of gelatin extracted from the heads and skins of rainbow trout (*Oncorhynchus mykiss*). The yield of gelatin obtained from the heads and skins were 6.17 and 8.46%, respectively. The protein content of gelatin obtained from the skin (84.68%) was greater than that obtained from the head (81.9%) (P<0.05). Both gelatins exhibited slightly acidic pH values (6.42 for heads and 6.20 for skins). FTIR analysis confirmed typical collagen–specific spectral features in both samples. The gelatin obtained from the skin exhibited a lighter and more reddish–yellow color compared to the gelatin obtained from the head (P<0.05). The sensory analysis results indicated that both gelatin samples had a mild odor; however, the gelatin obtained from the head exhibited a easily more perceptible odor compared to that extracted from the skin (P<0.05). These findings suggest that the heads and skins of rainbow trout are appropriate sources for gelatin production and support the sustainable use of wastes from fish processing.

Key words: Gelatin; trout by–products; physicochemical properties; FTIR analysis; sustainability

RESUMEN

El procesamiento del pescado produce cantidades significativas de subproductos. La disposición de estos desechos puede provocar problemas ambientales y de salud humana; sin embargo, estos subproductos a menudo pueden ser transformados en productos mas beneficiosos y de alto valor. Este estudio investigó las propiedades fisicoquímicas de la gelatina obtenida de las cabezas y pieles de la trucha arcoíris (*Oncorhynchus mykiss*). El rendimiento de gelatina obtenida de las cabezas y pieles fue del 6,17 y 8,46 %, respectivamente. El contenido de proteína en la gelatina obtenida de la piel (84,68 %) fue mayor que el de la gelatina obtenida de la cabeza (81,9 %) (P<0,05). Ambas gelatinas exhibieron valores de pH ligeramente ácidos (6,42 para las cabezas y 6,20 para las pieles). El análisis FTIR confirmó las características espectrales típicas específicas del colágeno en ambas muestras. La gelatina obtenida de la piel exhibió un color más claro y más rojizo–amarillo en comparación con la gelatina obtenida de la cabeza (P<0,05). Los resultados del análisis sensorial indicaron que ambas muestras de gelatina tenían un olor suave; sin embargo, la gelatina obtenida de la cabeza exhibió un olor fácilmente más perceptible en comparación con el derivado de la piel (P<0,05). Estos resultados indican que las cabezas y pieles de trucha arco íris son fuentes adecuadas para la producción de gelatina y promueven la utilización sostenible de los subproductos generados durante el procesamiento del pescado.

Palabras clave: Gelatina; subproductos de trucha; propiedades fisicoquímicas; análisis FTIR; sostenibilidad

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INTRODUCTION

Global fish production, especially aquaculture, has shown significant growth in recent years. In parallel with this increase, the fish processing industry is also expanding, and by–products from processed fish are rapidly accumulating [1]. Among these, rainbow trout, which is a high–value and widely consumed aquatic species, produces large amounts of waste during processing. These wastes are generally composed of heads, bones, skins, and other body parts. These by–products, which are often of low economic value, also pose an environmental issue [2, 3].

Lately, investigations into the potential value of fish processing waste has significantly increased. These studies have demonstrated that fish wastes can be transformed into high quality products through biotechnological processes. In particular, collagen, one of the biologically significant compounds that can be obtained from these wastes, has emerged as an important source from an industrial perspective [4, 5, 6, 7, 8, 9, 10, 11, 12, 13].

Gelatin is a biopolymer protein obtained through the partial hydrolysis of collagen, a basic component of animal bone, connective tissue and skin, and has a wide range of uses in many areas, especially in the food, pharmaceutical and photography industries [14, 15]. Gelatin is traditionally sourced from the skin and bones of pigs and cattle. However, due to the rising demand for halal food and concerns about health risks, there has been growing interest in alternative gelatin sources derived from seafood by–products. Fish waste is a highly abundant source of collagen and has the potential to yield high–quality gelatin production. Gelatin obtained from fish is more soluble and has a wider range of applications than gelatin obtained from traditional sources such as pigs and cattle [16, 17, 18].

Rainbow trout (*Oncorhynchus mykiss*) is an important freshwater fish species both economically and ecologically, and holds a significant place in aquaculture. Due to its rapid growth rate, high protein content, and excellent taste, rainbow trout has become an attractive option for both consumers and producers. Rainbow trout accounted for 744,000 tons of total aquaculture production in 2021. The leading exporting countries are Türkiye, Chile, and Norway [19]. According to the 2023 data, trout production in Türkiye, which was 134.174 thousand tons in 2021, has exceeded 200 thousand tons [20]. However, this level of production results in the generation of a significant amount of fish by–products during processing. It has been determined that about 35% of the total amount of fish is wasted [19, 21].

Several studies have been conducted on gelatin production from rainbow trout skins [10, 22, 23]. However, based on the available literature, no research have been reported on gelatin production from rainbow trout heads. Therefore, this study aims to assess the efficiency and quality of gelatin extracted from rainbow trout heads and skins, as well as to explore the potential of converting trout processing waste into valuable products. In this context, gelatin was extracted from raw materials, and its chemical composition, pH value, color, turbidity, FTIR spectroscopy, and sensory properties were analyzed. The study seeks to contribute to the sustainable utilization of fish processing waste by transforming it into high–value products.

MATERIALS AND METHODS

Raw materials

Rainbow trout, weighing an average of 713.285 ± 197 g, were obtained from domestic markets and transported to the laboratory. The fish samples were placed in ice boxes and transferred to the laboratory within 1 hour (h). On the same day (d), the heads and skins of the fish were manually removed using appropriate knives. The weight measurements were conducted using a KERN CB 6K 1N digital scale (Germany). The fish heads and skins were washed with cold water, placed into plastic bags, and stored in a deep freezer at -18 ± 2°C (Arçelik 5287 NFI, Türkiye).

Extraction of gelatin from fish heads and skins

Gelatin was extracted from fish heads and skins using a slightly modified version of the method described by Boran and Regenstein [24]. Initially, frozen fish heads and skins were thawed under running tap water. Subsequently, both materials were manually cut into small pieces and processed separately in a household food processor (Arçelik, Robomaster, Türkiye) until a homogeneous paste was obtained. The resulting paste was mixed with drinking water at a paste–to–water ratio of 1:3 and agitated for 15 min to remove water–soluble proteins.The mixture was then filtered through a nylon sieve to separate the liquid from the solid components. The remaining solid mass was washed with a 0.55 N NaOH solution for 30 min, with the NaOH solution replaced every 3 h.

After these treatments, the material was rinsed with distilled water and added to a 0.1 N hydrochloric acid (HCl) solution at a 1:5 (w/v) ration, then left at 25 ± 1 C for 1.5 h. Following the acidic and alkaline washings, the materials were washed three times with pure water at a ratio of 5:1 (w/v) and filtered through two layers of muslin cloth, followed by manual squeezing.

Finally, the materials were subjected to extraction in a water bath at 50°C for 3 h at a ratio of 4:1 (water:head), until a gelatin solution was obtained. The solution was then filtered again using a double layer of muslin cloth and subsequently dried in an oven at 60°C for approximately 72 h. Once dried, the gelatin was carefully removed from the containers and ground to a fine powder using a household blender. The resulting gelatin was placed in plastic storage containers and stored in desiccators to protect it from moisture [5]. These procedures were applied in the same manner for both fish heads and skins.

Gelatin yield determination

Gelatin yield was determined as the ratio of the dry weight of the obtained gelatin to the weight of the unprocessed raw material (fish heads or skins). This calculation was performed using the following formula:

$$Gelatin\ \ yield\ \left(\%\right) = \frac{Dry\ \ gelatin\ \ weight}{Raw\ \ \ sample\ \ weight} \times 100$$

Proximate analysis

The proximate analysis of the raw samples (heads and skins) and the extracted gelatin was performed by determining moisture, ash,

and protein content, following the AOAC [25] methods. Moisture content was measured by drying the samples in an oven (FN–500, Nuve, Türkiye) at 105 ± 2°C until a constant weight was achieved. The ash content was measured by subjecting the samples to incineration in a muffle furnace (M 1813 model, Elektro–Mag, Türkiye) at 550°C for 4 h. The protein content was calculated by measuring the total nitrogen content using the Kjeldahl method. This process was carried out using a Kjeldahl digestion and distillation apparatus (Gerhardt Vap–40, Germany). The protein content of the raw materials was calculated by multiplying the nitrogen values by a conversion factor of 6.25, while the protein content of the gelatin was determined using a conversion factor of 5.55 [26]. The crude fat content was determined using the Soxhlet extraction method with an SER 148 (Velp Scientifica, Italy). Each analysis was carried out in triplicate.

pH

The pH value of the gelatin was measured by preparing a 6.67% (w/w) gelatin solution. The pH measurements were taken at room temperature using a Thermo Scientific Orion 3–Star Benchtop model pH meter (Thermo Scientific, USA), with five independent measurements taken for each sample.

FTIR spectroscopy analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the chemical components of the samples. The analyses were carried out using a Thermo Scientific Nicolet IS5 FT–IR spectrometer (Thermo Nicolet Corporation, USA) within the spectral range of 4000–450 cm^{-1}.

Color and turbidity analysis

The color of the gelatin samples was analyzed using a 3nh NR200 portable colorimeter (3nh Ltd., Shenzhen, China). Color measurements were obtained based on the L* (lightness, ranging from 0 for black to 100 for white), a* (redness/greenness), and b* (yellowness/blueness) parameters. Each sample was measured by collecting color data from three distinct points at room temperature. Turbidity analysis was performed using a 6.67% (w/v) gelatin solution. For the preparation of the solution, 6.67 g of gelatin powder was added to distilled water, and the total volume was adjusted to 100 mL with additional distilled water. The mixture was then stirred in a water bath at 60°C for 30 min to ensure complete dissolution and achieve a homogeneous solution. The turbidity of the resulting gelatin solution was determined at a wavelength of 620 nm using a UV–VIS spectrophotometer (UV mini-1240, Shimadzu, Japan) [27].

Sensory evaluation

The sensory evaluation was carried out by a panel of 10 individuals capable of perceiving and describing odors. Initially, 6.67% gelatin solutions were prepared in screw–capped tubes, and the caps of the tubes were loosely closed to allow dissolution in a water bath at 60°C. Once the dissolution process was finished, the samples were presented to the panelists, who were asked to open the screw caps of the test tubes, smell the contents, and describe the odor perceived. Additionally, the panelists were asked to evaluate the intensity of the odor using a six–point scale. This scale was defined based on the perceivability of the odor as follows: 5 = very strong and very unpleasant, 4 = strong and unpleasant, 3 = strong but not unpleasant, 2 = faint but easily perceptible, 1 = very faint and perceptible only with careful attention, 0 = no odor [28].

Statistical analysis

Data analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Differences between gelatin samples obtained from heads and skins were evaluated using the independent samples *t*–test. The statistical significance level was set at $P<0.05$, and the data are presented as mean ± standard deviation (SD). All analyses were conducted in triplicate.

RESULTS AND DISCUSSION

The gelatin extraction from the heads and skins of rainbow trout was conducted following the same protocol. The yields of the obtained gelatins are given in TABLE I. The gelatin yield was expressed as the percentage of dry gelatin obtained per 100 g of raw material. The gelatin yield obtained from trout heads was found to be 6.17%, while the yield from trout skin was 8.46% ($P<0.05$). These yields differ from the results reported for other fish species in the literature. For example, Muyonga *et al.* [28] stated gelatin yields of 2.4 and 16% from the bones and skin of adult Nile perch, respectively. Ninan *et al.* [29] found gelatin yields of 12 and 12.93% from the skin of common carp and rohu, respectively. Elavarasan *et al.* [5] documented a gelatin yield of 1.67% from Tiger Tooth Croaker fish head waste. Additionally, Valcarcel *et al.* [10] determined gelatin yields from the skin of rainbow trouta, seabass and gilthead seabream as 1.56, 6.83 and 1.56%, respectively. These differences can be attributed to the biological characteristics of the fish species used, as well as variations in extraction methods and processing conditions.

The proximate composition of rainbow trout head, skin, and the gelatin obtained from these by–products, along with the yield of the gelatin, are presented in TABLE I. The protein content of fish heads was found to be approximately 21.36%, while the protein content in the skin was approximately 23.52%. The protein content in the heads was lower than in the skins, and additionally, the heads exhibited higher ash and moisture content compared to the skins. The fat content in the skins was higher than in the heads, which is likely related to the accumulation of subcutaneous fat in the fish. The fish heads also contained a higher ash content

TABLE I
Proximate compositions and yield of raw and gelatin extracted from rainbow Trout heads and skins

Component	Head	Skin	Head Gelatin	Skin Gelatin
Moisture (%)	59.98 ± 2.35^{A}	55.81 ± 2.21^{A}	9.86 ± 0.46^{a}	7.85 ± 0.23^{b}
Protein (%)	21.36 ± 1.41^{A}	23.52 ± 1.78^{A}	81.9 ± 2.26^{a}	84.68 ± 2.63^{b}
Fat (%)	5.49 ± 0.91^{A}	7.68 ± 2.53^{B}	3.58 ± 0.42^{a}	3.85 ± 0.81^{a}
Ash (%)	4.74 ± 0.18^{A}	1.24 ± 0.03^{B}	0.53 ± 0.43^{a}	0.45 ± 0.2^{b}
Yield (%)	11.29 ± 1.34^{A}	9.53 ± 0.83^{A}	6.17 ± 0.70^{a}	8.46 ± 0.64^{b}

The data are means ± SD. Different uppercase letters (A–B) in the same row indicate a significant difference ($P<0.05$) for fish head and skin, while different lowercase letters (a–b) indicate a significant difference ($P<0.05$) for gelatin obtained from the head and skin

than the skins. The proximate composition of fish heads and skins varies significantly among different fish species. These results are consistent with the proximate composition of Atlantic salmon processing by–products [30] and rainbow trout heads [31]. These findings indicate that fish heads and skins possess distinct biochemical compositions and suggest that both by–products hold potential as valuable resources for the food industry.

The proximate composition of gelatin was shown to differ based on the type of raw material used. Gelatin extracted from fish heads exhibited a higher moisture content than gelatin obtained from fish skins ($P<0.05$).

The quality of gelatin is largely dependent on its protein content. According to the results of the study, the protein content of gelatin extracted from rainbow trout heads was 81.9%, while the protein content of gelatin extracted from the skins was 84.68%. The lipid content in the skins was higher than that in the heads, likely due to the increased accumulation of subcutaneous lipids in the fish; the fat content of gelatin from fish heads was measured at 3.58%, while that of gelatin from fish skins was 3.38%. Ash content was higher in gelatin obtained from fish heads (0.53%) compared to gelatin extracted from fish skins (0.45%). These findings indicate that gelatin extracted from fish skins has lower ash content and higher protein content ($P<0.05$). Elavarasan *et al.* [5] reported that gelatin extracted from the tiger tooth croaker fish head had a protein content of 83% and a moisture content of 7.83%. Similar results were also reported by Ninan *et al.* [29] and Rosmawati *et al.* [32].

The pH value of gelatin plays an important role in determining its applications. In this study, the pH values of the collagen obtained from rainbow trout heads and skins were determined to be measured as 6.42 ± 0.06 and 6.20 ± 0.10, respectively. The observed difference can be attributed to the distinct biochemical compositions of these two tissues. These results indicate that the pH of the gelatin obtained through acidic and alkaline treatments is generally slightly acidic in nature. Adnan *et al.* [33] reported a pH range of 6.11–6.38 for gelatin isolated from by–products of various freshwater fish species, including striped catfish, catfish, and tilapia, which is consistent with the present findings. Similarly, Elavarasan *et al.* [5] reported comparable findings for gelatin extracted from the head waste of tiger tooth croaker. Cheow *et al.* [34] reported pH values ranging from 3.35 to 4.87 for gelatin extracted from the skins of tropical fish species, *Johnius dussumieri* and *Decapterus macrosoma*. These values are significantly lower than those observed for gelatin extracted from rainbow trout heads and skins.

FTIR is a fast and effective spectroscopic technique used to identify functional groups in materials and assist in their characterization. This method enables the identification of functional groups and the examination of molecular structures, as each functional group vibrates at a specific frequency, which appears as characteristic peaks in the FTIR spectrum [35]. The FTIR spectra of gelatin extracted from fish heads and skins of rainbow trout (*Oncorhynchus mykiss*) are presented in FIG. 1 and TABLE II.

The FTIR spectra of gelatin samples obtained from trout heads and skins show generally similar characteristics. The small differences observed in the spectra may be attributed to slight chemical variations between the samples. However, both samples exhibit similar bands and collagen–specific features,

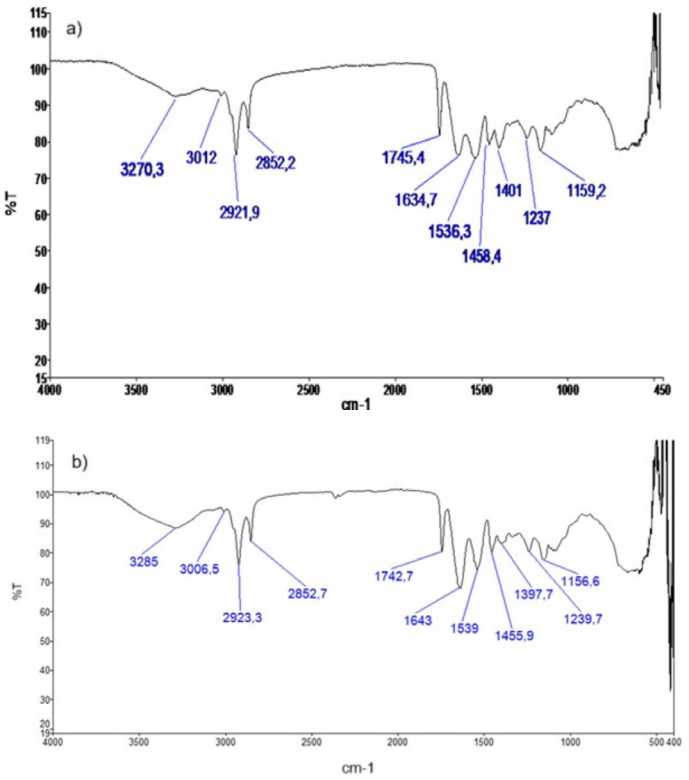

FIGURE 1. Fourier Transform Infrared (FTIR) Spectra of Gelatin Extracted from Fish Heads (a) and Skins (b)

TABLE II
Fourier Transform InfraRed (FTIR) spectra of gelatin extracted from fish heads and skin

Wavenumber (cm^{-1})		Assignment
Head	Skin	
3270.3	3285	N–H stretching
3012	3006.5	Aliphatic C–H stretching
2921.9 – 2852.2	2923.3 – 2852.7	Aliphatic C–H stretching
1745.4	1742.7	C=O stretching
1634.7	1643	Amid I band, Collagen's α–helix and β–pleated sheet structure
1535.6	1539	The Amide II band, C–N stretching and N–H bending
1458.4	1455.9	C–H bending: Aliphatic group
1401	1397.7	C–H bending, Aliphatic hydrogen bending, alkyl groups presence
1237 and 1159.2	1239.7 and1156.6	The Amide III band, N–H bending and C–N bands

indicating that the fundamental protein compositions of both types of gelatin are very similar. The major peaks observed in the spectral analysis of gelatin samples obtained from rainbow trout heads and skins were at wavenumbers of 3270.3–3285 cm^{-1} (Amide A), 1634.7–1643 cm^{-1} (Amide I), 1535.6–1539 cm^{-1} (Amide II), and 1237–1156.6 cm^{-1} (Amide III. These bands correspond to N–H stretching (Amide A), C=O stretching (Amide I), N–H bending and

C–N stretching (Amide II), and C–N stretching and N–H bending (Amide III), respectively. These findings confirm the presence of polypeptide chains and suggest that the type I collagen–like protein structure is preserved in the gelatin samples [36]. Similar spectral patterns and peaks were also obtained by Sila *et al.* [37] and Hassan *et al.* [38] in their analysis of European eel skin and fish gelatins. This similarity indicates that gelatin extracted from different fish species shares common structural features and functional groups.

Color and turbidity values do not influence the practical characteristics of gelatin, but they play an important role in consumer acceptance and potential applications of gelatin in the food industry [39]. The color and turbidity values of the gelatin obtained from rainbow trout head and skin are given in TABLE III.

TABLE III
Color and turbidity values of gelatin extracted from rainbow trout head and skin

Color	Head	Skin
L*	7.73 ± 0.02[a]	7.81 ± 0.01[a]
a	2.05 ± 0.06[a]	2.65 ± 0.07[b]
b	2.03 ± 0.05[a]	2.97 ± 0.04[b]
ΔL*	6.87 ± 0.02[a]	6.94 ± 0.06[a]
ΔE*	7.68 ± 0.05[a]	7.33 ± 0.08[a]
Turbidity		
Absorbans (A_{620})	0.67 ± 0.02[a]	0.62 ± 0.02[a]
Transmittance (%)	21.1 ± 0.3[a]	23.9 ± 0.9[a]

The data are mean ± SD. Different letters in the same row indicate statistically significant differences between groups (P<0.05)

The color analysis reveals significant differences between the gelatin extracted from the head and that extracted from the skin. The color values for the gelatin derived from the head are L = 7.73, a = 2.05, and b = 2.03, indicating a darker and more yellowish tone. In contrast, the gelatin obtained from the skin exhibits color values of L = 7.81, a = 2.65, and b = 2.97, reflecting a lighter, more reddish, and yellowish tone. The ΔL values are 6.87 for the gelatin extracted from the head and 6.94 for that extracted from the skin. Additionally, the ΔE values are 7.68 for the gelatin derived from the head and 7.33 for the gelatin obtained from the skin. The ΔE values being above 5 for both samples indicate that the color differences are visually noticeable.

Gelatin from the head tends to have darker tones with more pronounced red and yellow hues, while the gelatin from the skin is lighter with a more pronounced reddish and yellowish appearance. Similar findings were obtained by Tabarestani *et al.* [23] regarding gelatin production from rainbow trout. However, in the research carried out by Ninan *et al.* [29], it was stated that the gelatin extracted from the skins of common carp and rohu exhibited a pure white appearance. These differences may be linked to variations in the type of raw materials used, extraction conditions, environmental factors, and analysis methods. However, these changes do not affect other functional properties.

Turbidity refers to the cloudiness in a liquid caused by particles blocking light. The turbidity values of gelatin from rainbow trout head and skin were determined to be 0.67 and 0.62 (P<0.05), respectively, indicating high turbidity. The turbidity and dark color of gelatin typically result from contaminant substances that were not eliminated during the extraction process [40]. These substances may include residual proteins, minerals, or other organic compounds left in the gelatin after the extraction process.

The sensory evaluation was carried out to assess consumer preference regarding the odor of gelatin extracted from the heads and skins of rainbow trout. The gelatins obtained from both the fish heads and skins were found to have a subtle but noticeable odor. The average sensory scores for the gelatins from the fish heads and skins were 2.2 and 1.7, respectively. Muyonga *et al.* [28] mentioned that gelatins made from Nile perch bones and skins did not have a fishy odor but instead had a mild, unpleasant smell, with a mean hedonic score ranging from 2 to 2.5. Moreover, a study by Ninan *et al.* [29] discovered that gelatins made from the skins of rohu and common carp had a mild but detectable odor, with mean hedonic scores of 2.3 and 2.4, respectively. Although there is no dominant fish odor in the gelatins, neutralizing the odor completely could increase consumer acceptance and expand its use in the food industry.

CONCLUSIONS

This study investigated the physicochemical properties of gelatin extracted from rainbow trout (*Oncorhynchus mykiss*) heads and skins. The gelatin extracted from the skin had a higher protein content compared to the gelatin from the head. Both gelatin samples exhibited mildly acidic pH values, with the skin extracted gelatin displaying a lighter color. The turbidity of both gelatin samples was high, which may be attributed to residual impurities from the extraction process. FTIR analysis confirmed the presence of collagen–specific spectral features in both samples.

Sensory analysis revealed that the skin extracted gelatin had a milder odor compared to the head extracted gelatin. These findings suggest that both the head and skin of rainbow trout are promising sources of high–quality collagen and can be sustainably utilized. Future research may focus on optimizing extraction methods to improve the yield and quality of gelatin. Gelatin extracted from rainbow trout heads and skins holds significant potential as an alternative and sustainable source of gelatin for applications in the food, pharmaceutical, and cosmetic industries.

Conflict of Interests

The authors declare that there is no conflict of interest.

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