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Ciencias Técnicas y Aplicadas
Artículo de Investigación

Extracción de colágeno de desechos de pescado

Extraction of Collagen from Fish Waste

Extração de colagénio de resíduos de peixes

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Resumen

La industria pesquera en Ecuador ha crecido un 12%, generando más residuos como pieles, huesos y escamas de pescado. Si no se gestionan adecuadamente, estos residuos aumentan la carga orgánica causando problemas ambientales. Esta investigación se enfoca en la utilización de dos métodos para extraer colágeno a partir de escamas de pescado. Objetivos: El objetivo es aplicar dos métodos para la extracción eficiente de colágeno a partir de residuos de pescado. Metodología: Se exploraron dos métodos, hidrólisis y desmineralización para obtener colágeno hidrolizado de alta pureza, y desnaturalización de proteínas para obtener colágeno parcialmente hidrolizado. Se caracterizó la materia prima mediante el método de Micro Kjeldahl determinando el contenido de proteínas y humedad de las escamas de diferentes especies de pescado. Resultados: El rendimiento fue de 60% y 39.72%, cumpliendo con los requisitos fisicoquímicos y microbiológicos para el consumo humano, indicando que los procesos utilizados fueron eficientes. Conclusiones: Se analizaron diversos procedimientos de obtención de colágeno, agrupándolos en dos métodos colágeno de alta pureza y parcialmente hidrolizado. El método para colágeno de alta pureza mostró un rendimiento desfavorable del 0.745%, mientras que el método para colágeno parcialmente hidrolizado alcanzó un rendimiento del 15.44%.

Palabras Claves: Extracción; colágeno; pescado.

Abstract

The fishing industry in Ecuador has grown by 12%, generating more waste such as fish skins, bones, and scales. If not properly managed, this waste increases organic load, causing environmental problems. This research focuses on using two methods to extract collagen from fish scales.Objectives: The goal is to apply two methods for efficient collagen extraction from fish waste. Methodology: Two methods were explored: hydrolysis and demineralization, to obtain high-purity hydrolyzed collagen and denatured proteins to get partially hydrolyzed collagen. The raw material was characterized using the Micro Kjeldahl method to determine the protein and moisture content of scales from different fish species.Results: The yield was 60% and 39.72%, meeting physicochemical and microbiological requirements for human consumption, indicating that the processes used were efficient. Conclusions: Various collagen extraction procedures were analyzed, grouped into two methods: high-purity collagen and partially hydrolyzed collagen. The high-purity collagen method

showed an unfavorable yield of 0.745%, while the partially hydrolyzed collagen method achieved a yield of 15.44%.

Keywords: Extraction; collagen; fish.

Resumo

A indústria pesqueira no Equador cresceu 12%, gerando mais resíduos como peles, ossos e escamas de peixe. Se não forem geridos adequadamente, estes resíduos aumentam a carga orgânica, causando problemas ambientais. Esta investigação centra-se na utilização de dois métodos para a extração de colagénio das escamas de peixe. Objetivos: O objetivo é aplicar dois métodos para a extração eficiente de colagénio dos resíduos de peixe. Metodologia: Foram explorados dois métodos: hidrólise e desmineralização, para obter colagénio hidrolisado de elevada pureza e proteínas desnaturadas para obter colagénio parcialmente hidrolisado. The raw material was characterized using the Micro Kjeldahl method to determine the protein and moisture content of scales from different fish species.Results: The yield was 60% and 39.72%, meeting physicochemical and microbiological requirements for human consumption, indicating that the processes used were eficiente. Conclusões: Foram analisados vários procedimentos de extração de colagénio, agrupados em dois métodos: colagénio de elevada pureza e colagénio parcialmente hidrolisado. O método do colagénio de elevada pureza apresentou um rendimento desfavorável de 0,745%, enquanto o método do colagénio parcialmente hidrolisado obteve um rendimento de 15,44%.

Palavras-chave: Extração; colagénio; peixe.

Introduction

Climate change is one of the most critical issues today, exacerbated by the generation of solid waste. The fishing industry produces millions of tons of waste during filleting and cleaning processes, often discarded without proper management, leading to pest proliferation (National Fisheries Chamber, 2021).

Various organizations worldwide aim to promote sustainable development in the aquaculture sector, ensuring that waste generated during processing is not ignored. Therefore, research is encouraged in various knowledge areas to improve the management, treatment, and reuse of this type of waste (FAO, 2020).

In Ecuador, the fishing industry has become one of the most prominent in Latin America, growing by 12%. This development includes fish skins, bones, and scales. These by-products are often discarded without proper treatment, increasing organic load and causing significant environmental problems for the country (El Universo, 2021). Efficient management is essential to mitigate their environmental impact and represents an opportunity to convert them into value-added products like collagen (Evangelista, 2019).

Collagen is an essential and abundant protein in the human body, constituting about 30% of the total body content and being the main component of connective tissues. In recent years, it has gained attention in the global market due to its multiple health benefits, such as improving skin, joint health, and bone strengthening, and in the industrial field for its use in biomaterials, cosmetics, and food. The growing demand for this essential component has driven the need to develop increasingly efficient extraction methods, with one of the most interesting being its extraction from fish by-products, offering a sustainable solution for both the fishing industry and consumers of this product (Valero D., 2019).

Studies show that collagen can be extracted from the scales of two fish species, Pagrus major and Oreochromis niloticus, through demineralization processes, acid extraction, and recovery with the enzyme pepsin (Ikoma et al., 2003). Scientific literature demonstrates that collagen content can be influenced by the extraction method used and various factors such as species, cultivation location, diet type, and growth medium temperature. Authors like Agut et al. (2008) have determined that there is a significant collagen source in fish species developed in a warm environment (Torres et al., 2008). These qualities have allowed the exploration of different methods for collagen extraction from fish waste. Works by Ramos (2018), López, and Almeida (2018) present their research on the implementation of solid-liquid extraction techniques in varieties of red and brown fish, obtaining a protein yield of 60%, complemented by methods to improve yield through enzyme use, reaching 39.72%. Alzamora Ruiz and Silva (2019) propose a method to obtain collagen from grouper scales, resulting in hydrolyzed collagen that meets optimal physicochemical and microbiological requirements for human consumption (X115, 2020).

Research conducted on collagen content in different fish species using chemical treatments showed that this method could obtain high-purity collagen (Pati Adhikari and Dhara, 2010). Other authors achieved a 5% yield using an acid solution extraction method from species like catla and labeo rohita

(Minh Okazaki and Osako, 2014). Scales of the Chile fish or synodontidae yielded 0.43 to 1.5% (Bhagwat and Dandge, 2016) and 9.79% based on wet weight from carp, while Quintero and Zapata (2017) characterized the dry raw material and identified that the collagen percentage in the sample was 6.54%, obtaining a recovery of 96.2% of the total processed collagen.

Methods involving protein denaturation allow obtaining partially hydrolyzed collagen (Ramos, 2018) through solid-liquid extractions specifically from red and brown fish species, characterized by protein quantification using the Kjeldahl method, with a 60% yield based on the physicochemical composition of scales performed by Quintero and Zapata (2017).

López and Almeida (2018) studied the influence of pepsin on collagen yield, with results showing a non-significant variation related to protein yield. In their best trials, they achieved a 39.72% total yield. Alzamora Ruiz and Silva (2019) obtained collagen from grouper scales using the solid-liquid extraction method, performing physicochemical, microbiological, and sensory tests that met the requirements for human consumption. For sensory analyses, the product was mixed with orange juice.

Currently, no process allows the industrial production of collagen from fish waste. Therefore, contributing from scientific research will propose treatments for waste generated by the fishing industry. In this context, the research objective is to obtain hydrolyzed collagen from fish scales.

Methodology

The study characterized the raw material, specifically fish scales collected in the markets of Riobamba city, Chimborazo province. The protein and moisture content in the scales was determined.

Characterization of fish waste

Protein Analysis

The standard method used to determine total nitrogen was Kjeldahl-Willfart-Gunning, which considers fundamental stages such as digesting the waste treated with concentrated sulfuric acid and copper sulfate to oxidize organic matter into CO2 and water and transform nitrogen into ammonia from proteins and amino acids.

Organic matter $+H2SO4(CONC)$ Catalyst/Heat $>CO2(g)+H2O(g)+SO2(g)+(NH4)2SO4$

Distillation

For sample digestion, an excess of 40% m/v sodium hydroxide was added, decomposing ammonium sulfate into ammonia, which was distilled by steam drag.

Bromocresol green and methyl red were used as indicators, and an alcoholic boric acid solution was used in an Erlenmeyer flask to collect the distilled ammonia.

$$NH_3+H_3BO_3 \rightleftarrows NH_4+BO_2^-+H2O$$

For titration, a standardized hydrochloric acid solution was used to titrate the ammonium borate formed.

$$BO2^-+H^++H_2O \rightleftarrows H_3BO_3$$

Moisture Determination

Moisture determination was carried out by indirect methods, separating water from the waste and drying it at temperatures above 100°C. It was important to know the water mass contained in the sample taken for analysis using the following formula.

$$m_{H2O}=malimento\ (inicial)-m_{\text{malimento}}\ (seco)$$

$$\%\text{Humidity}=m_{H2O}/m_{muestra}$$

In the first stage to determine protein content, the sample was subjected to digestion using concentrated sulfuric acid and copper sulfate as a catalyst to oxidize organic matter into CO2 and water, transforming nitrogen into ammonia. Subsequently, the sample was distilled, adding sodium hydroxide, decomposing ammonium sulfate into ammonia, which was then distilled by steam drag. The distilled ammonia was collected in an alcoholic boric acid solution and titrated using standardized hydrochloric acid. For moisture determination, a RADWAG moisture analyzer was used. This method requires drying the sample at temperatures above 100°C and calculating the water mass contained in it, allowing the percentage of moisture to be obtained.

Two main methods for collagen extraction were compared: The first based on hydrolysis and demineralization, and the second through protein denaturation.

The first method, based on the work of Quintero and Zapata (2017), included several steps: the scales were initially washed with water and a 200 ppm sodium hypochlorite solution. Then, alkaline hydrolysis was performed using a 0.4M NaOH solution, keeping the sample at 25°C for 3 hours. Subsequently, the sample was demineralized with a 0.5M EDTA solution adjusted to pH 8 with

agitation for 48 hours. Then, acid hydrolysis was carried out using a 0.7M acetic acid solution at 11°C for 18 hours, concluding with collagen precipitation with a 12% NaCl solution, followed by filtration. The second method, based on Ramos' study (2018), consists of washing the scales with purified water and acetic acid, then drying them at a temperature of 50-60°C for 8 hours. The sample was then crushed to obtain a 2 mm size. Subsequently, extraction was performed using the solid-liquid method at 90°C with a 90:10 ratio of water to scales, finally packaging the obtained product and refrigerating it at 13°C for 12 hours.

To select the most suitable extraction method, the collagen yields obtained were evaluated, and the necessary reagents and equipment for the process were considered. The protein denaturation method (Method 2) was determined to be the most effective due to its higher yield. Once the method was selected, the process was optimized to improve the organoleptic properties of the final product by adding additional steps such as sieving the dry flakes to remove impurities, evaporating the product before drying to concentrate it, and drying to obtain a powder. Additionally, a grinding step was implemented to reduce particle size and facilitate solubility for consumption and packaging. To further enhance the product's characteristics, additives were added to reduce odor and improve preservation.

RESULTS

Characterization of the raw material

The raw material analyses were performed by the Laboratory of Chemical and Microbiological Analytical Services (SAQMIC).

Table 1. Protein content in the raw material.

Parameters	Unit	Method	Result
Nitrógeno	%	INEN 16	5.8
Proteína (factor 5.55)	%	INEN 16	32.19

By:

Tabla 2. Moisture content in the raw material.

Parameters	Unit	Method	Result
Humedad	%	INEN 1953	12,775

By:

The protein analysis results were from a sample consisting of a mix of fish scales, randomly collected from markets in the city of Riobamba. According to scientific literature, protein content can vary depending on the fish species (López and Almeida 2018, pp. 2) and their development conditions (Pati, Adhikari, and Dhara 2010, pp. 3742). Characterization of scales conducted by (Quintero and Zapata 2017, pp. 113) showed 67.96% protein content in tilapia scales with a margin of error of 0.19 and a moisture content of 15.18% with a margin of error of 0.27. Analyses by authors like (Santos 2020, pp. 32) show a protein content of 16.23% with a margin of error of 0.11 and a moisture content of 62.79%. On the other hand, studies by (López and Almeida 2018, pp. 32) had results of 38.95% protein with a margin of error of 0.28 and a moisture content of 8.51%.

Method 1. High purity collagen extraction.

Table 3. Results for method 1

Test	Repetition	Scale Weight	Duration H+	Collagen Weight	Yield %	Average	Standard Deviation
1	1	49,23 g	12 h	0,214 g	0,435	0,571	0,190339851
	2	50,26 g	12 h	0,246 g	0,489		
	3	50,36 g	12 h	0,397 g	0,788		
2	4	49,78 g	18 h	0,055 g	0,110	0,252	0,191793054
	5	49,86 g	18 h	0,087 g	0,174		
	6	50,21 g	18 h	0,236 g	0,470		
3	7	51,23 g	24 h	0,361 g	0,705	0,566	0,275622065
	8	50,62 g	24 h	0,126 g	0,249		
	9	49,91 g	24 h	0,372 g	0,745		

By: Bastidas, Fernanda, 2022

The results described in the table are from a sample of scales, considering the results of (Minh, Okazaki, and Osako 2014), who achieved yields of 1.43 to 1.5% due to the fish's development conditions. However, authors like (Pati, Adhikari, and Dhara 2010) mentioned yields of 5% using an acid solution in their experiments, and (Bhagwat and Dandge 2016) obtained yields of 9.79% based on wet weight. The yield obtained was calculated based on the dry weight of the product. The yields achieved by the previously cited authors were higher due to the suitable extraction conditions for the type of scale.

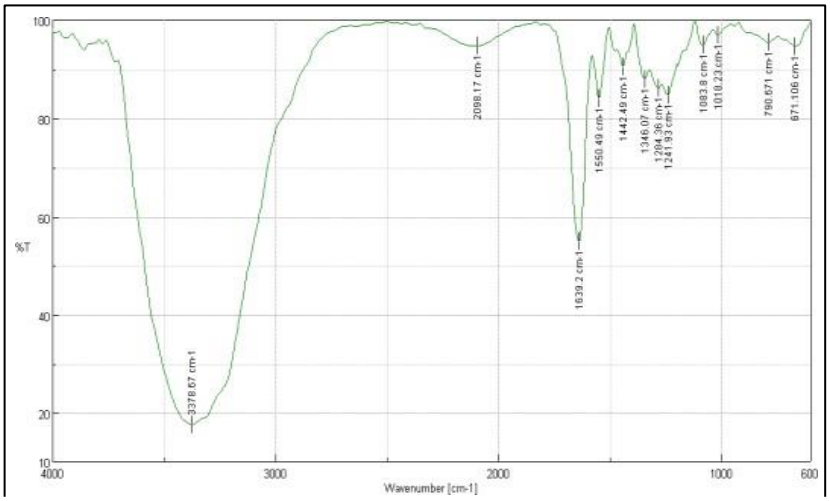

Figure 1. Spectrum of collagen obtained by method 1

Table 4. Wavelength for collagen obtained by method 1

Wave number [cm-1]	% Transmittance
3378.67	17.569
2098.17	94.7109
1639.2	54.8235
1550.49	83.9694
1442.49	90.4906
1346.07	88.0472
1284.36	86.1328
1241.93	84.7881

1083.8	94.9701
1018.23	96.8922
790.671	95.4403
671.106	94.603

The overlap of spectra conducted by (Vitale et al. 2019, pp. 175) indicates a medium intensity band at 1337 cm-1 associated with C-N stretches. At 1240 cm-1, there is the characteristic Amide III band corresponding to N-H deformations associated with tertiary amines and cyclic amines. Bands of low intensity between 1100 and 1005 cm-1 are associated with C-O-H, C-O, and C-O-C deformations of carbohydrate residues and out-of-plane torsions of carboxylic acids. Considering the wave number range table (Shurvell 2006, pp. 9), the wave number 3378.67 cm-1 is associated with OH bonds due to the hydroxyproline content (Pati, Adhikari, and Dhara 2010, pp. 3740). The range 1640 to 1580 indicates the presence of NH3 functional groups in amino acids and NH2 in primary amines. The medium intensity band at 1639.2 cm-1 is within this range. The FTIR analysis by (Pati, Adhikari, and Dhara 2010) confirms this with a peak at 1643.05 cm-1 in the Amide I region. The most relevant peaks are at 3378.67 cm-1 for Amide A, 1639.39 cm-1 for Amide I, 1442.49 cm-1 for Amide II, and 1241.93 cm-1 for Amide III, matching the FTIR analysis of collagen obtained from tilapia scales by (El-Rashidy et al. 2015, pp. 4).

Method 2: Partially hydrolyzed collagen extraction.

Test		Repetition	Scale Weight	Partially Hydrolyzed collagen	Yield	Average	Standard Deviation
1		10	45,41 g	5,43 g	11,95%		
Time	Water	13	48,08 g	4,36 g	9,07%		

30 min	200 ml	17	50,74 g	4,51 g	8,88%	9,97%	0,01717 3867
2		12	48,28 g	2,88 g	5,96%	5,56%	0,00385 8465
Time	Water	19	50,36 g	2,61 g	5,19%		
1 hour	200 ml	21	50,34 g	2,79 g	5,53%		
3		15	47,92 g	5,85 g	12,22%	12,84%	0,00540 2251
Time	Water	16	50,15 g	6,60 g	13,16%		
30 min	250 ml	20	50,65 g	6,66 g	13,15%		
4		11	49,52 g	7,91 g	15,98%	15,44%	0,00520 6732
Time	Water	14	48,24 g	7,43 g	15,39%		
1 hour	250 ml	18	50,05 g	7,48 g	14,94%		

The obtained product has a higher yield in weight compared to method 1. However, the product's appearance reflects a problem in the current method, so it is necessary to correct the organoleptic properties, considering that the product must have a pleasant color, smell, appearance, and taste for the consumer. The appearance of the partially hydrolyzed collagen is due to the skin that remains attached to the scales, and the yellow color is due to the elevated temperature of the scales because of their calcium carbonate content (Ramos 2018). The removal of these occurs largely in the washing of the raw material, but this is not enough to prevent the product from having a dark color. It is necessary to modify the method to improve the color of the product and add additives to reduce the fishy smell.

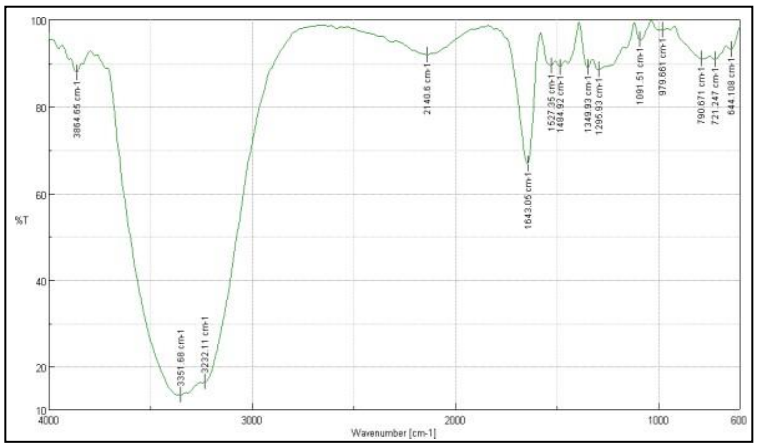

Figure 2. Spectrum of collagen obtained by method 1

Wavenumber [cm-1]	% Transmittance
3864.65	87.8702
3351.68	13.3719
3232.11	16.3941
2140.6	91.9812
1643.05	66.746
1527.35	89.4136
1484.92	89.1221
1349.93	89.0681
1295.93	88.4309
1091.51	95.4267
979.661	97.6433
790.671	90.9284
721.247	90.7674
644.108	93.1852

The location of the FTIR spectrum peaks includes 3351.68 cm-1, a high-intensity band indicating the presence of NH2 functional groups in primary amides (Shurvell 2006, p. 9). At 3232.11 cm-1, there is another peak within the 3700-3100 cm-1 region, suggesting the possible presence of carboxylic

acids, amines, amides, or alkynes (Shurvell 2006, p. 12). At 1643.05 cm-1, there is a medium-high intensity band coinciding with the band obtained by (El-Rashidy et al. 2015, p. 4), within the amide I region with a C=O bond, also corroborated by (Vitale et al. 2019, p. 175), indicating that in proteins, the amide group (C=O) appears around 1650 cm-1. Most collagen contains carbohydrates linked to hydroxylysine and hydroxyproline, which are found in the amide I region and are representative amino acids of collagen, along with nitrogenous functional groups in the amino acid glycine (Ikoma et al. 2003, p. 201).

For the analysis of the procedures, an experiment was conducted to obtain two types of collagen. One case involves obtaining high-purity collagen, named as such because non-collagen proteins are removed through basic hydrolysis to release them into the aqueous medium. The sodium hydroxide concentration varied from 0.1M to 1M depending on the fish species from which collagen is to be extracted. It is important to note that extraction conditions, such as time and solution concentration, depend significantly on the environment and how the fish have developed. It was also found that higher collagen yields can be obtained from species developed in a warm environment. The extraction process was carried out with a 0.4M molarity for 3 hours. The demineralization process was standardized in each procedure, with a duration of 48 hours and 0.5M EDTA at pH 8.0 to remove hydroxyapatite and calcium carbonate content. The collagen extraction process was performed with 0.5M acetic acid for 12, 18, and 24 hours, and the product was then separated from the solution and weighed to calculate the yield, which was 0.745% in dry weight, compared to (Minh, Okazaki, and Osako 2014) who achieved a yield of 1.43%. Other authors, such as (Bhagwat and Dandge 2016), (El-Rashidy et al. 2015), and (Pati, Adhikari, and Dhara 2010), obtained collagen solutions with similar assays in carp, tilapia, Labeo rohita, and Catla catla scales, with yields between 5% and 9%, noting that their experiments were conducted using scales from the same fish species.

The second case involved obtaining partially hydrolyzed collagen through a denaturation process, based on Ramos 2018's proposal in "Obtaining and Characterizing Collagen from Red and Brown Scales." The author conducted experiments to obtain a collagen solution and subsequently characterized it through a denaturation process. To complete the analysis and compare it with the first procedure, the sample was dried to calculate its yield, which was 15.44%. However, the product exhibited dark and non-uniform coloration throughout the sample.

To define the collagen extraction method, the yield obtained in each experiment was considered, along with the use of reagents involved in the process and the advantages of each procedure. High-purity collagen is better absorbed by the human body due to its short amino acid chains, which are the specific collagen proteins. However, the yield was not advantageous, and industrial-scale processing would not be beneficial. Partially hydrolyzed collagen, with its long amino acid chains, is more easily absorbed by the human body as few molecules hydrolyze. Studies by (Arquer et al. 2014, p. 35) indicate that ascorbic acid facilitates amino acid absorption, suggesting the potential for collagen products enriched with vitamin C in the future. Additionally, the yield is relatively high compared to the purified collagen extraction method. The downside is the color of the final product, caused by particles like hydroxyapatite and calcium carbonate remaining after scale grinding. Therefore, steps were added to prevent the dark tone in the final product. Phosphate and calcium carbonate turn grayish at high temperatures (Ramos 2018), so grinding is omitted to maintain a clear and uniform tone. The raw material remains in a tray dryer until its moisture is reduced to 45.69% of its initial weight, followed by sieving to remove any foreign particles during the protein denaturation process. The solution is then evaporated in a tray dryer to form sheets, pulverized, and packaged for commercialization.

CONCLUSIONS

The analysis of the procedures for obtaining collagen from fish scales identified two main methods: high-purity collagen and partially hydrolyzed collagen. The high-purity collagen extraction method, due to variations in extraction conditions among different species, resulted in a 0.745% yield, which is not favorable. On the other hand, the partially hydrolyzed collagen method, requiring denaturation at temperatures above 30°C regardless of the species, achieved a 15.44% yield. However, browning was observed in part of the product, possibly due to minerals remaining in the solution during heating. The partially hydrolyzed collagen extraction process included additional steps to produce a powdered product with good organoleptic properties. Removing unwanted components and dehydration were crucial to avoid color changes in the collagen solution. Technical validation through FTIR analysis confirmed the qualitative presence of collagen in the final product, and according to NTE INEN 1961 standards, the results showed 78.25% protein and absence of coliforms, Escherichia coli, Salmonella, and Staphylococcus.

The method for obtaining partially hydrolyzed collagen proved more efficient in terms of yield and final product quality, although strict control of present components is required to avoid unwanted color changes. The technical feasibility of the study was successfully validated, ensuring the quality and safety of the obtained collagen.

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