Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(6): 269 – 286 (2025)
www.ejabf.journals.ekb.eg

Effect of Treatment with Ultrasonic Pulses on the Functional Properties and Antioxidant Capacity of Dogfish (*Galeorhinus galeus)* Viscera Proteins

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ARTICLE INFO

Article History:
Received: July 20, 2025
Accepted: Oct. 15, 2025
Online: Nov. 8, 2025

Keywords:
Degree of hydrolysis,
Protein hydrolysates,
Ultrasound,
Antioxidant,
Techno-functional,
Byproducts

ABSTRACT

The valorization of fish by-products represents a sustainable strategy for obtaining high-value functional ingredients. The viscera of *Galeorhinus galeus* were evaluated as a source of protein hydrolysates with nutritional and bioactive potential. The objective was to determine the impact of ultrasonic pretreatment on proximate composition, antioxidant activity, and techno-functional properties of enzymatic hydrolysates. Viscera were subjected to autohydrolysis with and without ultrasonic pulses. Proximate composition was analyzed, antioxidant activity was assessed by DPPH and FRAP assays, and the degree of hydrolysis (DH) was quantified. Protein degradation was evaluated by SDS-PAGE, while foaming and emulsifying capacities were determined as indicators of functional performance. Ultrasound significantly improved protein solubilization, increasing DH to 28.4% compared with untreated controls. Antioxidant activity was enhanced, with ultrasound-treated hydrolysates reaching up to 97.8% radical inhibition at acidic pH and moderate hydrolysis times. Electrophoretic analysis confirmed progressive fragmentation into low-molecular-weight peptides, consistent with the observed increase in DH. Techno-functional evaluation showed a marked improvement in foaming capacity (from 24% to 97%), whereas emulsifying capacity decreased (<25%) due to disruption of amphipathic structures. These findings demonstrate that ultrasound-assisted hydrolysis enhances the nutritional, antioxidant, and techno-functional properties of shark viscera proteins. This approach provides a sustainable alternative for generating protein ingredients with potential applications in food formulations and nutraceutical development.

INTRODUCTION

Fishing activity involves more than just capturing marine products; it also includes a series of small-scale post-harvest processes carried out by fishermen to

optimize commercialization. However, these practices generate byproducts and organic waste that are often inadequately managed, resulting in indiscriminate disposal and significant environmental degradation (**Castro *et al.*, 2020)**. The rapid degradation of fish muscle tissue exacerbates this situation. In the absence of a cold chain, microbial and enzymatic processes such as proteolysis and lipid oxidation accelerate, generating a significant increase in waste production over a short period of time **(Luo *et al.*, 2025)**. The dogfish (*Galeorhinus galeus*) processing industry produces large amounts of organic waste, primarily viscera, accounting for up to 60% of the organism's total biomass **(Mowbray *et al.*, 1988)**. These materials have traditionally been considered waste, but they contain protein fractions of high nutritional and functional value, particularly for their ability to release bioactive peptides with antihypertensive and antioxidant activity **(Je *et al.*, 2015; Ahmed *et al.*, 2022)**, This circumstance underscores the need to implement technological strategies that can transform these by-products into high-value ingredients.

Historically, alkaline extraction has predominated among conventional methods due to its simple operation **(Sun *et al.*, 2020)**. However, this method is limited by its low yield and extraction efficiency compared to more recent technologies **(Preece *et al.*, 2017; Karlsen *et al.*, 2022)**. One of the most promising emerging tools in this field is ultrasound technology. Based on the principle of acoustic cavitation, this technology facilitates cell disruption and improves mass transport. These effects contribute to increased efficiency in extracting bioactive compounds **(Higuera-Barraza *et al.*, 2016; Chemat *et al.*, 2017)**. Ultrasound is considered safe, cost-effective, and ecologically advantageous due to its reduced solvent usage, making it an attractive alternative to traditional methods. Additionally, ultrasound has been shown to significantly increase enzyme activity and bioactive hydrolysate production **(Higuera-Barraza *et al.*, 2016; Cadena-Cadena *et al.*, 2024)**.

Ultrasound-assisted extraction (UAE) has successfully isolated proteins from various raw materials, including pork liver **(Zou *et al.*, 2018)**, extract proteins from abalone viscera **(Wu *et al.*, 2021)**, isolated chickpea proteins **(Kang *et al.*, 2022)**, modified the functional and biological properties of legume proteins **(Loushigam *et al.*, 2023)**, and separate proteins from egg whites **(Alizadeh *et al.*, 2025)**. During PU, the physical and chemical changes induced by microbubble collapse can alter the three-dimensional structure of proteins, influencing their subsequent functionality **(Tiruneh *et al.*, 2025)**.

Previous research has shown that ultrasonic treatment enhances the antioxidant properties of watermelon protein hydrolysates **(Wen *et al.*, 2019)**. Similarly, analysis of changes in the surface hydrophobicity of chickpeas revealed that ultrasonic treatment

significantly improved digestibility **(Kang *et al.*, 2022)**. Regarding the quality of salted *Culter alburnus* fish, increased ultrasound power was found to intensify structural degradation of muscle fibers and significantly modify myofibrillar protein conformation **(Dongyin *et al.*, 2023)**.

The purpose of this study was to extract bioactive peptides from dogfish viscera using ultrasound. The effect of ultrasound on antioxidant activity was analyzed. Given the limited number of studies in this area, the results obtained could be relevant for both the comprehensive utilization of dogfish by-products and the development of functional ingredients aimed at enhancing nutrient bioavailability.

MATERIALS AND METHODS

Raw material

The specimens were collected in Bahía de Lobos, Sonora (27°21′06″N; 110°27′14″W) and transported to the Ultrasonic Pulse Laboratory of the National Technological Institute of Mexico/Yaqui Valley Technological Institute under an ice-product-ice system within a maximum of three hours. In the laboratory, the organisms were eviscerated, and the viscera were packed in polyethylene bags and subsequently stored by cryopreservation at -80°C to preserve their physicochemical integrity until analysis.

Processing of viscera

The viscera were washed with a 3:1 (v/v) ratio of a 50 mM citric acid buffer solution (pH 4.0) containing 20 mM NaCl and stored at 4°C for 10 minutes **(Maza *et al.*, 2007)**. The samples were rinsed at the same temperature for 30 minutes. After this process, the samples were stored at −85°C until further use. To prepare the working solution, the viscera were homogenized in a 20 mM citric acid buffer solution containing 60 mM NaCl (pH 5.5) at a 1:1 (w/v) ratio. Homogenization was carried out at 4 °C for two hours. Then, the samples were centrifuged at 9,000×g at 4 °C, and filtered sequentially using Whatman No. 1 and No. 42 paper **(Celis-Guerrero *et al.*, 2004)**. Ultrasonic treatment with an amplitude of 40% was applied for 10 minutes using a VCX750 ultrasonic processor (Vibra Cell, Sonics; Newtown, CT, USA) with 20-second on-and-off pulses **(Cadena-Cadena *et al.*, 2022)**. During treatment, 100mL of the working solution was processed in beakers placed in an ice bath to prevent temperature increases. Finally, the protein concentration and antioxidant activity **(Benzie *et al.*, 1996; Re *et al.*, 1999)**.

Proximal chemical analysis

Proximal chemical analysis was performed to characterize the raw material and evaluate possible variations in the composition of fish viscera. The moisture content (method 950.46), protein (method 981.10), lipids (method 960.39), and ash (method

938.08) were determined in accordance with the procedures established by the **AOAC (2000)**. The carbohydrate content was calculated by difference.

Autohydrolysis conditions

To determine the optimal conditions for producing low-molecular-weight peptides with potential antioxidant activity through autohydrolysis, the effects of different hydrolysis times (0.5, 1, 2, 4, 6, and 12 hours) were evaluated. At the end of each reaction time, the proteases were inactivated by adding distilled water at 95°C and maintaining the mixture at this temperature with constant stirring for 15 minutes. The samples were then cooled to room temperature and homogenized for two minutes while maintaining the temperature below 10°C **(Wilde *et al.*, 1996)**.

Foaming property

The foaming capacity was determined according to the method described by **Wilde *et al.* (1996)**, with slight modifications. Twenty milliliters of each hydrolysate were used which was homogenized at 1800 × g for 1 minute. The volume of foam generated was measured in a 100mL graduated cylinder, and the foaming capacity was calculated as the ratio between the final volume after shaking and the initial volume of the sample **(Wilde *et al.*, 1996)**.

Emulsifying property

Each emulsion was prepared by homogenizing 50mL of each hydrolysate at 1800 × g for 2 minutes, gradually adding 50mL of soybean oil while continuing to stir for an additional minute **(Villamil *et al.*, 2017)**. Subsequently, 25mL aliquots were taken and transferred to 25mL graduated cylinders, allowing them to stand at 25°C for 15 minutes. Finally, the volume of the separated aqueous phase was recorded to calculate the stability of the emulsion **(Sathivel *et al.*, 2006)**.

$$ES\ (\%) = \left({Separate\ volume}/{Total\ volume}\right) \times 100 \qquad \text{Eq. 1}$$

Degree of Hydrolysis

The O-phthalaldehyde (OPA) methodology was used to calculate the degree of hydrolysis. This method is based on the formation of a colored compound that is detectable at 340nm due to the reaction of amino groups with the OPA compound in the presence of a thiol group. In this reaction, it is necessary to know the milliequivalents (meq) of Ser-NH2 released, which can be calculated as follows:

$$Ser - NH_2 = \left[\frac{Abs\ Sample - Abs\ blank}{Abs\ standard - Abs\ blank}\right](0.96516)(0.1)\left(\frac{100}{XP}\right) \quad \text{Eq. 2}$$

Where, $Ser - NH_2 = mEq\ Ser - NH_2/g\ protein$; X is the mass of the reaction mixture in g; P is the percentage of protein in the sample; and 0.1 is the volume of the sample in liters (L), 0.9516 is concentration constant (in meqv/L) of the Serine standard solution **(Nielsen *et al.*, 2001)**. Next, hydrolysis (h) was calculated as follows:

$$h = \frac{(Ser{-}NH_2)(\beta)}{\alpha} \quad \text{Eq. 3}$$

Where, β and α applied to fish viscera are 0.40 and 1.0, respectively **(Sánchez-Sanchez *et al.*, 2014)**. Finally, the DH was calculated as follows:

$$DH = \left({}^{h}/_{h\ tot}\right) * 100 \quad \text{Eq 4}$$

Where, htot used for fish viscera is 8.6 **(Nielsen *et al.*, 2001)**. The degree of hydrolysis is expressed as the total percentage of hydrolyzed protein.

SDS-polyacrylamide gel electrophoresis

The presence of proteins and their degradation by hydrolysis were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions based on the method of **Laemmli (1970)**. Twelve percent polyacrylamide gels were used and 20µL of sample was loaded using 100 V for protein separation. The gels were stained with Coomassie Blue R-250 and destained in a methanol:water:acetic acid solution (5:4:1, v/v/v). These conditions were used to analyze the effect of ultrasonic pulse pretreatment on globulin hydrolysates.

Determination of antioxidant activity

These tests are based on the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) free radicals by antioxidant compounds over a 30-minute reaction time **(Müller *et al.*, 2011; Gulcin *et al.*, 2023)**.

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's comparison of means at a 95% confidence level were used to determine significant differences in properties such as foaming, emulsifying, and antioxidant activity across different hydrolysis times.

RESULTS

1. Proximal chemical analysis

The results of the proximate chemical analysis are shown in Table (1). These results indicate that the viscera of dogfish that were not treated with ultrasonic pulses showed significant differences when these pulses were applied. Treatment with pulses increased protein by approximately 6% and ash by 0.74%, while lipids, carbohydrates and moisture decreased by 0.61%, 0.27% and 5.85%, respectively. The protein content increased from 68.8% in the control to 75%, while the lipid content decreased from 18.5 to 13.1%.

Table 1. Proximal chemical analysis

Sample	Protein (%)	Lipids (%)	Moisture (%)	Ash (%)	Carbohydrates (%)
Dogfish (without pulses)	17.72±2.71	4.76±0.71	74.25±3.4	1.38±0.71	1.89±0.17
Dogfish (with pulses)	23.71±1.74	4.15±0.54	68.4±4.2	2.12±0.42	1.62±0.12

2. Antioxidant activity and degree of hydrolysis

Applying ultrasonic pulses modified the antioxidant capacity of the hydrolysates obtained by autolysis in the viscera of *Galeorhinus galeus*. The lowest DPPH radical inhibition values were recorded after 90 and 120 minutes of hydrolysis at pH 7.5, indicating a decrease in antioxidant activity. In the FRAP assay, samples without ultrasonic pretreatment exhibited minimal reducing capacity, achieving a maximum value of 52.84 ± 2.5 after 60 minutes of incubation. Decreases in activity were detected at 30 and 120 minutes. In contrast, ultrasonically treated hydrolysates showed greater than 10% increases in inhibition compared to the control (81.55 ± 2.23%), at both pH 4 and 7.5 (Table 2). Depending on the reaction time, the degree of hydrolysis (DH) showed that antioxidant activity increased four- to sixfold in the samples with and without ultrasound. DH increased from 15.32% in the control samples to 28.45% in the ultrasound-treated samples. The electrophoretic profile (SDS-PAGE, Fig. 1A) showed that the control

sample had intense high-molecular-weight bands. In treatments with 10, 15, and 20% DH, progressive attenuation of these bands and the appearance of smaller fragments were observed. As %DH increased, the bands became more diffuse and migrated toward the low-molecular-weight region. However, combining an optimal ultrasonic frequency of ~350–400 kHz with a prolonged time of 100–120 minutes favored an increase in %DH and modified the molecular profile of the hydrolysates (Fig. 1B).

Table 2. DPPH and FRAP antioxidant capacity of hydrolysates with and without ultrasonic pulse treatment as a function of DH

		Time (min)	DH (%)	% of inhibition DPPH	% of inhibition FRAP
Dogfish (without pulses)	pH 7.5	*30*	*3.88*	*51.52±5.5*	*25.20*
		60	*6.71*	*48.15±3.4*	*52.84±2.5*
		90	*10.88*	*52.30±7.2*	*45.24±3.3*
		120	*15.32*	*53.34±2.5*	*14.87±0.98*
	pH 4	*30*	*5.87*	*60.84±3.4*	*31.45±1.27*
		60	*12.09*	*67.91±3.1*	*65.87±4.25*
		90	*16.24*	*65.23±2.5*	*60.22±1.88*
		120	*19.12*	*65.23±4.7*	*32.74±4.87*
Dogfish (with pulses)	pH 7.5	*30*	*5.52*	*69.30±3.3*	*62.44±0.70*
		60	*10.27*	*78.51±2.8*	*66.61±0.66*
		90	*15.66*	*78.51±2.4*	*42.25±2.29*
		120	*21.25*	*84.17±2.2*	*33.16±1.88*
	pH 4	*30*	*8.92*	*87.09±1.9*	*87.24±1.33*
		60	*16.60*	*97.81±4.6*	*89.88±1.99*
		90	*21.32*	*97.27±3.3*	*84.14±0.88*
		120	*28.45*	*92.36±2.2*	*74.19±0.54*

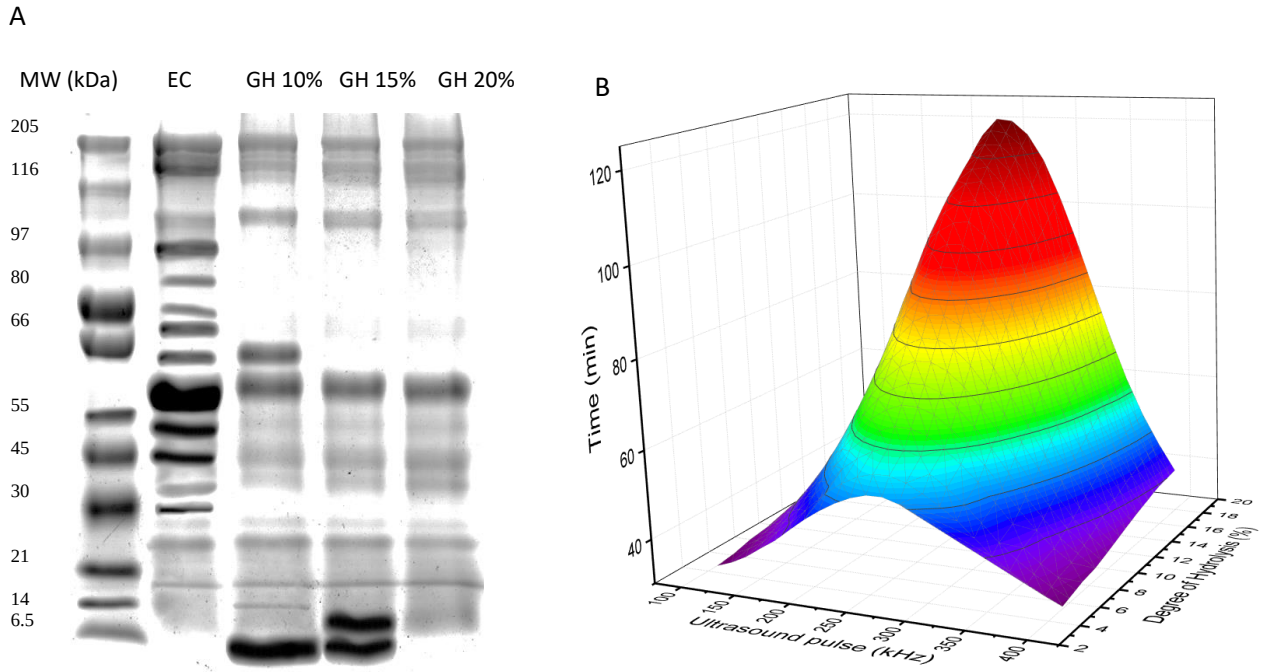

Fig. 1. Effect of ultrasonic pulses on viscera proteins of *Galeorhinus galeus*: (A) SDS-PAGE pattern at different degrees of hydrolysis; (B) Response surface of the degree of hydrolysis as a function of time and ultrasound frequency

3. Foaming and emulsifying properties

The evaluation of the foaming capacity of protein hydrolysates obtained from the viscera of *Galeorhinus galeus* revealed an increase that persisted throughout the enzymatic autohydrolysis process. An initial foaming value of 24% was recorded in the early stages of the process. After one hour of hydrolysis, the foaming capacity increased to 44%, continuing to rise significantly until reaching a maximum value of 97% after six hours (Figs. 2A, 1A). The emulsifying capacity of the hydrolysates exhibited opposite behavior to that observed in foam formation. As hydrolysis time increased, emulsification capacity decreased. Moderate emulsification values were observed during the first hours of the reaction. However, after five to seven hours of hydrolysis, the emulsifying capacity decreased to levels below 25% (Fig. 2B). The results are summarized in Table (3).

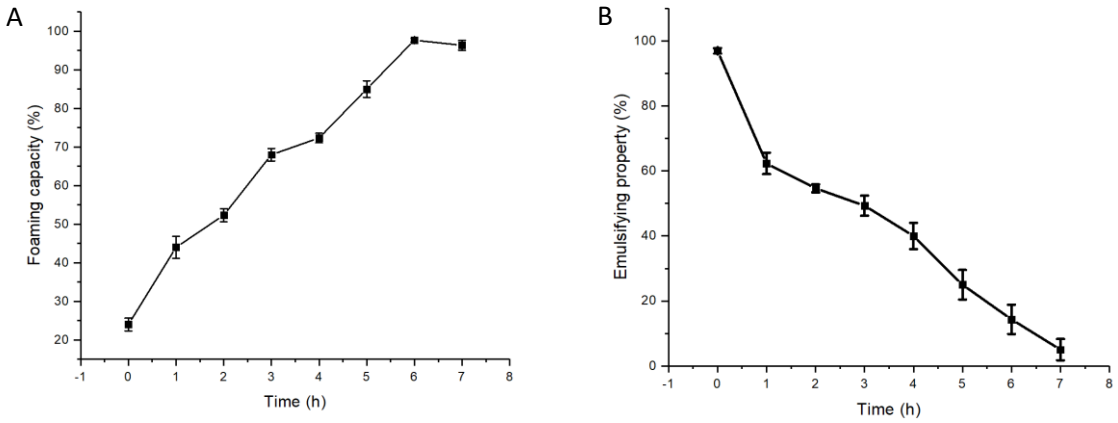

Fig. 2. A. Foaming capacity; B. Emulsifying capacity

Table 2. Effect of hydrolysis time on the structural characteristics of dogfish protein

Hydrolysis time	Structural changes	Foaming capacity	Emulsifying capacity	Antioxidant activity
0 h (native protein)	High molecular mass, compact structure, well-defined amphipathic domains	Low (24%), slow migration to the air-water interface	High, stable micelles with lipids	Moderate, partially hidden functional groups
1–3 h (hydrolysis moderate)	Partial reduction in molecular size, increased flexibility, exposure of hydrophobic regions	Medium-high (44–70%), greater deployment in the interface	Medium, slight loss of stabilizing capacity	High, increased exposure to thiols and amines
4–6 h (high hydrolysis)	Small peptides (<10 kDa), high surface hydrophilicity, loss of amphipathic domains	Very high (80–97%), rapid adsorption and rearrangement	Low (<25%), weak interfacial films	High, maximum availability of reactive groups
>6 h (excessive hydrolysis)	Extreme fragmentation, loss of secondary structure, hydrophilic predominance	Slight reduction due to excessive fragmentation	Very low, inability to stabilize oil droplets	High, but with lower affinity for interfaces

DISCUSSION

Applying ultrasonic pulses (UP) to the viscera of *Galeorhinus galeus* altered the proximal composition, biofunctional properties, and technological properties of the obtained hydrolysates. Proximal analysis revealed an increase in protein content (from 17.72% to 23.71%), accompanied by an increase in the ash fraction (from 1.38% to 2.12%). Meanwhile, lipids, carbohydrates, and moisture content decreased. These results can be explained by high-frequency ultrasound's ability to induce cavitation. This phenomenon promotes the rupture of cell membranes and the release of soluble protein fractions while causing the loss of retained water and the destabilization of lipids **(Higuera-Barraza *et al.*, 2016; Kalla-Bertholdt *et al.*, 2021)**. The observed decrease in moisture content (5.85%) is consistent with the process of cavitation-induced dehydration, which, in turn, has been shown to increase the relative concentration of protein and minerals **(Asaithambi *et al.*, 2025)**. The reduction in lipids (−0.61%) and carbohydrates (−0.27%) could be associated with oxidation phenomena and the breakdown of non-protein components, which are less represented compared to the increase in the nitrogen fraction **(Weiss *et al.*, 2025)**.

The antioxidant capacity and degree of hydrolysis obtained in this study show a synergistic effect between ultrasonic pulse (UP) treatment and the natural enzymatic degradation process. The DPPH and FRAP results showed that the samples subjected to ultrasound had significantly higher values, more than 10% higher than the control group, which is consistent with previous research on marine and plant proteins subjected to ultrasound **(Chemat *et al.*, 2017; Kang *et al.*, 2022)**. This increase in antioxidant capacity can be attributed to the mechanical and cavitational action of ultrasonic waves, which promote the breakdown of non-covalent bonds and the exposure of functional groups with redox potential, such as thiols, amines, and aromatic structures **(Zhu *et al.*, 2022)**. It was observed that ultrasound-treated samples achieved a maximum inhibition of 97.81% under acidic conditions and at intermediate reaction times. This finding suggests that pH conditions, combined with ultrasonic energy, enhance the release of bioactive peptide sequences capable of donating electrons or hydrogen to neutralize free radicals. Similar results have been reported in studies with soy and lupin proteins, where ultrasound prior to enzymatic hydrolysis significantly increased the antioxidant capacity of the hydrolysates **(Fadimu *et al.*, 2021)**. The degree of hydrolysis (DH) increased significantly in the treated samples, with values ranging from 15.32 to 28.45%, demonstrating that the PUs favored the accessibility of enzymes to peptide bonds. This phenomenon is directly related to the ability of ultrasound to induce partial denaturation of proteins and expose sites susceptible to enzymatic action **(Rahman *et al.*, 2021)**. The

resulting protein fragmentation generates smaller peptides with a more pronounced bioactive profile. This mechanism has been widely documented in milk, fish, and legume proteins, where ultrasonic treatment prior to hydrolysis increases both the speed and efficiency of the enzymatic process **(Qian *et al.*, 2023)**.

Analysis by SDS-PAGE electrophoresis corroborated these observations, showing the progressive fading of high molecular weight bands and the appearance of low molecular weight peptides, particularly in the range corresponding to a DH of 10 to 20%. This pattern confirms the release of smaller bioactive sequences, consistent with the findings of **Cadena-Cadena *et al.* (2022)**. The greater mobility of these fragments through biological interfaces, coupled with the abundance of exposed functional groups, explains the increase in antioxidant activity observed **(Zhu *et al.*, 2022)**. In addition, the reduction in molecular size facilitates their incorporation into food matrices and potential controlled release systems, expanding their technological applications. Another relevant aspect was the influence of treatment parameters. The combination of high ultrasonic frequencies (350-400 kHz) with prolonged times (100-120 minutes) led to a more dispersed hydrolysis pattern, with a shift toward low molecular weight regions. While this intensive fragmentation promotes the formation of small peptides, it can also compromise the functional and structural stability of bioactive sequences, which translates into a possible decrease in their antioxidant capacity over longer periods of time. This coincides with reports from ultrasound studies applied to egg and soy proteins, where it was observed that excessive hydrolysis reduces the stability of peptide fractions and their ability to interact with free radicals. **(Gul *et al.*, 2023; Tawalbeh *et al.*, 2023)**. In contrast, intermediate conditions (60-90 minutes) proved more effective in maximizing antioxidant activity, suggesting the existence of an optimal balance between enzymatic exposure and preservation of functional structures. This balance has also been identified in plant proteins treated with ultrasound prior to enzymatic hydrolysis, where moderate treatment intervals achieved a favorable balance between peptide size and antioxidant bioactivity **(Fadimu *et al.*, 2021)**. This reinforces the hypothesis that ultrasound acts as a modulator of the hydrolysis process, allowing the fragmentation profile to be adjusted according to the frequency and time conditions applied.

The evidence obtained highlights that peptides released under optimal conditions have improved antioxidant properties, which could have applications in the formulation of functional foods, nutraceutical supplements, and food preservation systems sensitive to lipid and protein oxidation. Furthermore, the results suggest that combining emerging technologies such as ultrasound with conventional enzymatic hydrolysis processes may be an effective strategy for enhancing the added value of fishery by-products, contributing to the comprehensive use of marine resources **(De Aguiar-Saldanha-Pinheiro *et al.*, 2021; Rahaman *et al.*, 2025)**. The findings confirm that ultrasonic treatments not only improve the degree of hydrolysis of marine proteins but also enhance

the generation of bioactive peptides with marked antioxidant activity. However, it is necessary to establish optimal operating windows, as excessive hydrolysis can compromise the functionality of the peptides generated. The evidence obtained opens the possibility of optimizing these processes on a pilot and industrial scale to produce protein hydrolysates with applications in health and the food industry.

A functional analysis of the foaming and emulsifying properties of hydrolysates reveals how structural modifications caused by enzymatic hydrolysis and ultrasound affect the ability of proteins to interact with air-water and oil-water interfaces **(Mirzaee *et al.*, 2024)**. Foam formation increased steadily as hydrolysis progressed, rising from an initial value of 24% for native proteins to a maximum of 97% after six hours of reaction. This increase is directly linked to the progressive reduction in molecular size, the increased flexibility of peptide chains, and the exposure of hydrophobic regions that can quickly orient themselves at the air-water interface **(Song *et al.*, 2021; Mirzaee *et al.*, 2024)**. Small peptides (<10 kDa) generated at advanced stages exhibit high surface mobility and reorganization capacity, explaining the notable increase in foaming capacity **(Chen *et al.*, 2024)**. In contrast, emulsifying capacity showed an opposite pattern, decreasing as hydrolysis time increased. In native proteins, the presence of well-defined amphipathic domains favored the stabilization of lipid micelles; however, excessive fragmentation reduced the integrity of these structures, thereby preventing the formation of stable interfacial films around oil droplets **(Villeneuve *et al.*, 2023)**. Consequently, after five to seven hours of hydrolysis, the emulsifying capacity decreased to value below 25%. The inverse relationship between foaming and emulsification capacity reflects the transition from complete proteins with an amphipathic structure to small, predominantly hydrophilic peptides that are useful for foam generation but inefficient for emulsion stabilization **(Soliman *et al.*, 2023; Weiss *et al.*, 2025)**. This behavior has been documented in species such as *Dosidicus gigas* and *Katsuwonus pelamis* **(Klomklao *et al.*, 2018; Anaya *et al.*, 2020)** and it is caused by several structural factors **(Broyard *et al.*, 2015; Liu *et al.*, 2025)**. Consequently, emulsions formed with highly hydrolyzed hydrolysates are less stable and more prone to coalescence.

The increased protein content and reduced lipid content offer nutritional advantages, while the enhanced antioxidant capacity makes these hydrolysates suitable for use in the development of foods with bioactive properties intended to prevent oxidative damage. A comparative analysis indicates that it is possible to maximize foaming capacity without compromising emulsifying capacity. From a technological standpoint, this duality offers specific applications: hydrolysates with a high degree of hydrolysis are more suitable for products where foam formation is a priority (malts, aerated desserts, egg white substitutes), while those with a moderate degree of hydrolysis are preferable for foods that require stable emulsions (dressings, sauces, protein drinks),

as they retain sufficient amphipathic structures to maintain the stability of the system **(Zhang *et al.*, 2024)**.

CONCLUSION

The application of ultrasonic pulses to the viscera of *Galeorhinus galeus* significantly improved the proximate composition, antioxidant activity, and technological properties of the hydrolysates. The increased protein and lower lipids make it healthier. The increased antioxidant capacity shows that low-molecular-weight bioactive peptides are released, which can prevent oxidative damage. At the technological level, the degree of hydrolysis determined how the liquids behaved: liquids with a lot of hydrolysis formed foam, while liquids with a moderate amount of hydrolysis remained emulsifying. The findings show that using a combination of ultrasound and autolysis is an effective way to make protein ingredients. These ingredients can be used in functional foods. They can be used as foaming agents or emulsion stabilizers.

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