Arch Biol Sci. 2024;76(1):83-90 https://doi.org/10.2298/ABS240104004P

Molecular weights and optimum temperature and pH for pepsin activity of three sciaenid finfish species from the Gulf of California

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Received: January 4, 2024; **Revised**: January 18, 2024; **Accepted**: January 19, 2024; **Published online**: January 30, 2024

Abstract: By-products from finfish processing from fisheries and aquaculture are often discarded. However, the enzymatic content of viscera has potential biotechnological and industrial applications. Such is the case for the sciaenids *Cynoscion othonopterus, Cynoscion xanthulus*, and *Cynoscion parvipinnis*, which are food and game fishes from the Gulf of California and whose viscera are commonly discarded after fish dressing. In this study, optimum temperature and pH for activity, as well as molecular weights of pepsin from the stomach of *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* were evaluated for the first time. Pepsin molecular weights were 30, 32.1, and 32.3 kDa, respectively. The highest activity of pepsin against hemoglobin was recorded between 40 and 45°C for *C. othonopterus* and *C. xanthulus* and at 40°C for *C. parvipinnis*. The optimum pH was 2.0 for the three sciaenids. Biochemical characteristics were comparable to pepsins from other marine and freshwater fish species, so they could likely be used in some processes using this enzyme, like collagen extraction, fish silage production, or fish processing, among others.

Keywords: pepsin, enzymatic activity, sciaenids, *Cynoscion othonopterus*, *C. xanthulus*, *C. parvipinnis*

INTRODUCTION

According to "The State of the World Fisheries and Aquaculture", published by the Food and Agriculture Organization of the United Nations [1], the total world fisheries and aquaculture production in 2020 reached 177.8 million tons (live weight equivalent), of which finfish represented 76% of the total production of aquatic animals, 39% comprised marine finfish and 33% freshwater finfish. Waste generated from their processing is considerable, but some recovered by-products can have several potential uses, particularly the viscera, a desired remnant for their enzymatic content with potential biotechnological and industrial applications [2,3]. Among them, pepsin is the main gastric acidic protease in the stomach of fish; this acid/aspartyl proteinase (EC 3.4.23) is produced by the chief cells in the gastric mucosa and secreted as pepsinogen, an inactive proenzyme that becomes active when exposed to hydrochloric acid in the gastric juice, resulting in the proteolytic removal of an N-terminal pro-peptide residue. Once cleaved, pepsin becomes active [4]. This endopeptidase performs the preliminary hydrolysis of proteins in the stomach under acidic conditions, producing smaller peptides by cleaving peptide bonds at the amino-terminal side of the cyclic amino acid residues, tyrosine, phenylalanine, and tryptophan [5].

In fish, there are different types of stomach pepsins with unique structures and enzymatic properties [6]; for instance, two pepsins, I and II, have been reported in the stomach of orange roughy, *Hoplostethus atlanticus*, with molecular weights of 33.5 and 34.5 kDa, respectively. For chum salmon *Oncorhynchus keta*, pepsins I and II, also isolated from the stomach, were 32 and 27 kDa, respectively. For other species like the albacore tuna *Thunnus alalunga*, a single pepsin of 32.7 kDa was reported, and pepsinogen was 39.9 kDa [7], whereas for the yellowfin seabream *Sparus latus*, up to four pepsinogens (I, II, III, and IV) of 36, 32, 32, and 34 kDa, and four pepsins of approximately 30 kDa were present in the stomach [8]. Therefore, the characterization of pepsin and/or pepsin isozymes by electrophoretic techniques in fishes, together with the

assessment of the pH and temperature where activity is maximum to efficiently perform its proteolytic activity, is of great interest for industrial and biotechnological applications [9,10]. Pepsin is used, for example, for collagen extraction [2,11] employed in the cosmetics and pharmaceutical industries [12], to produce fish silage [13,14,15], or in the processing of fish, such as descaling [13] or deskinning [16]. Additional applications also include the medical field, as an aid in the regulation of digestion and treating disorders like dyspepsia, gastralgia, vomiting, infant diarrhea, apepsia, and gastric ulcers, or as an appetite and digestibility enhancer, and as dental antiseptic [3,17,18,19]. Moreover, the sustainable utilization of viscera and other discarded waste products also represents an additional source of income for fishermen and aquaculturists [20,21].

The Gulf of California in Mexico is a very productive area, with commercial fisheries of sciaenids like the Gulf corvina *C. othonopterus*, the orange mouth corvina *C. xanthulus*, and the shortfin corvina *C. parvipinnis*, with great potential for aquaculture. By capture volume, *C. othonopterus* is the leading species, with a fisheries quota of 4,880 tons for 2022, established by the National Institute of Fisheries and Aquaculture (INAPESCA) in the Official Journal of the Federation [22]. Catches of *C. xanthulus* and *C. parvipinnis* can be considerable since both are food and game fish. Unfortunately, fisheries record-keeping for these two species is unavailable [23,24]. Advancing the knowledge of the biochemical characteristics of the digestive enzymes from these sciaenids can contribute to the understanding of their digestive capacities and may also contribute to the formulation of balanced feeds to develop their culture. Of no lesser importance, it is a prerequisite for using fish waste to produce valuable products with potential industrial applications. Thus, the objective of the present study was to determine the molecular weight, as well as the optimum temperature and pH for the activity of pepsin of *C. othonopterus, C. xanthulus*, and *C. parvipinnis*.

MATERIALS AND METHODS

Ethics statement

The fish in this study were not killed for scientific purposes but for food; fishermen caught them as a regulated activity in the community of Kino Bay, Sonora, Mexico. Nonetheless, the study was approved by the University of Sonora Research Ethics Committee, in compliance with the Official Mexican Norm (NOM-062-ZOO-1999) on the Technical Specifications for the Production, Care, and Use of Laboratory Animals so that part of the specimens could be used for scientific purposes.

Collection of specimens

Wild *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* caught using seine nets in October 2021 around the Kino Bay area (latitude 28°48'59.99"N, longitude 111°55'59.99"W), Sonora, Mexico, were acquired from fishermen upon landing. Specimens were placed in an ice-filled cooler (≈ 4°C) and immediately transported to the Department of Scientific and Technological Research of the University of Sonora (DICTUS); they were identified at the species level using the FAO's Western-Central Pacific species identification guide for fish [25]. A total of 9 specimens of *C. othonopterus*, 12 of *C. xanthulus*, and 10 of *C. parvipinnis*, with an empty gastrointestinal tract (GIT) or viscera, were selected and weighed. The average individual weights by species were 906.4 ± 41.6, 835.3 ± 30.8, and 813.5 ± 37.2 g, respectively. Fish were then dissected, and the stomach was stored in labeled, resealable bags at -82°C until further analyses.

Preparation of enzyme extracts

Stomach samples were transferred from -82°C to -4°C and allowed to thaw for 12 h. Then, a 0.5 g sample was dissected from the stomach and homogenized (Model T10, IKA® Works Inc., Wilmington, NC, USA) in 2.5 mL of 10 mM Tris-HCl buffer solution of pH 7.5 at 4°C. The homogenates were centrifuged (Heraeus Fresco 21, Thermo Fisher Scientific, Dreieich, Germany) at 14,000 *g* for 20 min at 4°C; the precipitates were discarded, and the supernatants were recovered and centrifuged one more time under the same conditions, to make sure they were clear of debris. After centrifugation, the supernatants or crude extracts containing the enzyme were stored at -20°C for 18 h until further analysis.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

The molecular weight of pepsin was determined by a method employing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [26]. Samples were prepared by mixing equal volumes of crude extract with a solution of 95% 2× Laemmli Sample Buffer (Bio-Rad®, Hercules, CA, USA) and 5% 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA); 15 µL aliquots were loaded into 12% polyacrylamide gels and resolved at 110 V for 3 h at 10°C in a vertical electrophoresis device (Mini-Protean Tetra Cell, Bio-Rad®, Hercules, CA, USA). Gels were rinsed in distilled water and stained overnight in gentle agitation with QC colloidal Coomassie stain (Catalog #161-0803, Bio-Rad®, Hercules, CA, USA) and then destained by rinsing in distilled water. Pepsin molecular weight was estimated by comparison with an internal molecular weight standard (Precision Plus Protein Standard Dual Color, Bio-Rad®, Hercules, CA, USA) with protein markers of 10 to 250 kDa and pepsin (P-6887) from porcine gastric mucosa (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference molecular marker. The gels were documented in a calibrated densitometer (Model GS-900, Bio-Rad®, Hercules, CA, USA) for identification of the bands using the ImageLab 5.0 software (Bio-Rad®, Hercules, CA, USA).

Zymography

Detection of the enzyme was carried out by native PAGE [27]. After electrophoresis, the gels were submerged for 15 min in a 0.1 M HCl solution to reduce the pH to 2.0 and allow the enzyme to become active, incubated for 30 min at 4°C in a solution with 0.25% Hb (Hemoglobin, H2625, Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M glycine (Gly) HCl pH 2, followed by an additional incubation for 90 min in fresh Hb solution at 37°C. Subsequently, the gels were washed in distilled water and fixed in 12% trichloroacetic acid (TCA) solution for 15 min. Finally, the gels were stained overnight in gentle agitation with QC colloidal Coomassie stain and destained by rinsing in distilled water. Pepsin was visualized by the contrast of a clear white band in a dark blue background. To confirm the effectiveness of the enzyme's reaction against Hb in the chromogenic substrate, a band with pepsin (P-6887) from porcine gastric mucosa was also run. Additional gels were also run using pepstatin A (77170, Sigma-Aldrich, St. Louis, MO, USA), a pepsin inhibitor that prevents the binding of the enzyme to the substrate. Gels included replicate lanes of the same sample with and without the inhibitor and then followed the same zymography technique for further confirmation of bands corresponding to pepsin.

Enzyme activity

The activity of pepsin was determined in quadruplicate samples from different specimens of each species; each of the four samples and the blanks were analyzed in duplicate. Pepsin activity was analyzed according to the procedure previously described [28] with modifications, where 1.0 mL of 0.5% Hb diluted in 0.1 M Gly.HCl pH 2 with 200 µL of enzyme extract were incubated at 35°C for 30 min. The reaction was stopped with 0.5 mL of 20% TCA, stored at 4°C for 20 min, and centrifuged at 14,000 *g* for 5 min at 4°C. Then, absorbance was read at 260 nm in a UV/Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, Madison, WI, USA). The specific activity of pepsin was estimated as units of pepsin activity per mg of protein as follows:

$$(U\ mg^{-1}) = (\text{units of pepsin } mL^{-1}) \div \text{protein concentration } (mg\ mL^{-1}).$$

The optimum temperature and pH for the enzymatic activity of pepsin were analyzed in quadruplicate samples from different experimental organisms, while their blanks were analyzed in duplicate, adjusting the incubation temperature to 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70°C, while the 0.1 M Gly.HCl buffer was set at pH 2.0. The pH values evaluated were 2, 3, 4, 5, and 6, adjusted with HCl, and the temperature set at 35°C, a value within the optimal range observed in the temperature test for all species.

Statistical analysis

The molecular weights of pepsins from *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* were analyzed by descriptive statistics, and enzymatic activity in their stomachs at different temperatures and at different pH was analyzed by one-way analysis of variance (ANOVA) using a significance level of $P \leq 0.05$. When statistically significant differences were detected, Tukey's

HSD test was used for separation of means. Before one-way ANOVA, normality and homoscedasticity of data were verified through Shapiro-Wilk's and Bartlett's tests, respectively. All statistical procedures were performed using the Statistical Analysis System (SAS Institute Inc. 2013, Software Release 9.4, Cary, NC, USA) software package.

RESULTS

Molecular weights and zymography

Mean molecular weights±standard error of the mean (SEM) of pepsin from *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* (Fig. 1) were 30±0.28, 32.1±0.23, and 32.3±0.23 kDa, respectively. Zymography of the crude extracts from these sciaenids showed a white band against the blue background that corresponded with the activity of pepsin against Hb in the chromogenic substrate, and the band of each species was matched to the blue bands observed on the SDS-PAGE gels.

Enzyme activity

Statistical differences (P<0.0001) were observed in the activity of pepsin for all three species at different temperatures and different pH. For *C. othonopterus*, no significant differences in the activity of pepsin were detected between 25 and 60°C, with values ranging from 2.60 (at 60°C) up to 2.79 U/mg protein (at 40°C), whereas temperatures below 20°C or above 65°C showed statistically lower activity values, below 2.06 U/mg protein (Table 1). A similar trend was observed for *C. parvipinnis*, where enzymatic activity was significantly higher between 25 and 60°C, with values ranging between 4.12 (at 60°C) and 4.65 U/mg protein (at 40°C), compared to the rest of the evaluated temperatures with enzymatic activities ranging from 2.63-3.16 U/mg protein. In the case of *C. xanthulus*, significantly higher enzymatic activities were recorded between 35 and 50°C, ranging from 4.13 to 4.39 U/mg protein, whereas significantly lower activities were recorded at 10, 60, 65, and 70°C, with values ranging between 2.70 and 2.88 U/mg protein (Table 1).

On the other hand, pepsin activity for *C. othonopterus* was highest at pH 2.0 with 2.58 U/mg protein,

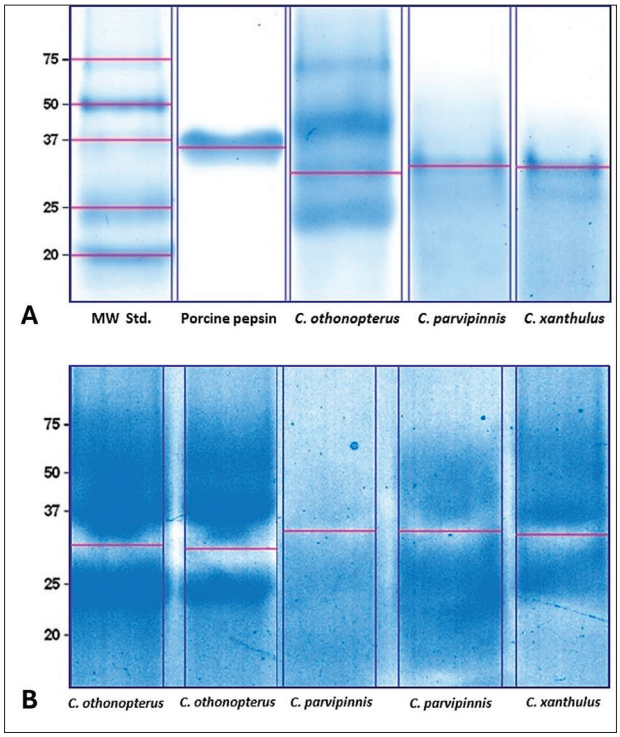

Fig. 1A – SDS-PAGE gel of extracts from sciaenids: lane 1 – molecular weight standard (MW Std.); lane 2 – porcine pepsin; lane 3 – pepsin from *C. othonopterus* of 30.0 kDa; lane 4 – pepsin from *C. parvipinnis* of 32.3 kDa; lane 5 – pepsin from *C. xanthulus* of 32.1 kDa. **B** – Zymogram of extracts from sciaenids with pepsin reaction against hemoglobin: Lanes 1 and 2 – *C. othonopterus*; lanes 3 and 4 – *C. parvipinnis*; lane 5 – *C. xanthulus*.

Table 1. Activity of pepsin from *C. othonopterus*, *C. parvipinnis*, and *C. xanthulus* at different temperatures

	Pepsin activity (U/mg protein)		
Temperature (°C)	***C. othonopterus***	***C. parvipinnis***	***C. xanthulus***
10.0	1.95^{b}±0.11	2.77^{b}±0.22	2.88^{ef}±0.05
15.0	2.00^{b}±0.09	2.96^{b}±0.20	3.13^{de}±0.03
20	2.06^{b}±0.08	3.16^{b}±0.17	3.17^{d}±0.03
25	2.62^{a}±0.05	4.24^{a}±0.12	3.91^{c}±0.05
30	2.57^{a}±0.07	4.32^{a}±0.10	4.06^{bc}±0.09
35	2.59^{a}±0.06	4.34^{a}±0.12	4.13^{abc}±0.08
40	2.79^{a}±0.05	4.65^{a}±0.16	4.38^{a}±0.05
45	2.77^{a}±0.05	4.55^{a}±0.12	4.39^{a}±0.04
50	2.70^{a}±0.04	4.50^{a}±0.09	4.29^{ab}±0.03
55	2.61^{a}±0.04	4.29^{a}±0.11	4.06^{bc}±0.01
60	2.60^{a}±0.04	4.12^{a}±0.17	2.88^{ef}±0.05
65	1.76^{b}±0.07	2.69^{b}±0.19	2.83^{f}±0.06
70	1.73^{b}±0.10	2.63^{b}±0.22	2.70^{f}±0.07
ANOVA P>F	<0.0001	<0.0001	<0.0001

Values are the means±SEM of four replicates per species; each quadruplicate sample and its blank were analyzed in duplicate. Means with different superscripts within the same column are significantly different (P≤0.05).

decreasing significantly (P<0.0001) at pH 3.0 (2.14 U/mg protein), and even more at pH above 4.0 (Table 2). Similarly, for *C. parvipinnis* and *C. xanthulus*, activity was significantly higher (P<0.0001) also at pH 2.0, with 4.34 and 4.13 U/mg protein, respectively, decreasing significantly at pH 3.0 (3.36 and 3.23 U/mg protein, respectively), with the lowest values also recorded at pH above 4.0 for both species (Table 2).

Table 2. Activity of pepsin from *C. othonopterus*, *C. parvipinnis*, and *C. xanthulus* at different pH

	Pepsin activity (U/mg protein)		
pH	***C. othonopterus***	***C. parvipinnis***	***C. xanthulus***
2.0	2.58^{a}±0.06	4.34^{a}±0.12	4.13^{a}±0.08
3.0	2.14^{b}±0.10	3.36^{b}±0.16	3.23^{b}±0.04
4.0	1.01^{c}±0.02	1.72^{c}±0.03	1.79^{c}±0.06
5.0	1.02^{c}±0.02	1.75^{c}±0.02	1.57^{cd}±0.02
6.0	1.16^{c}±0.02	2.07^{c}±0.02	1.55^{d}±0.03
ANOVA P>F	<0.0001	<0.0001	<0.0001

Values are the means±SEM of four replicates per species; each quadruplicate sample and its blank were analyzed in duplicate. Means with different superscripts within the same column are significantly different (P≤0.05).

DISCUSSION

The potential use of enzymes from the gastrointestinal tracts of fish obtained from either fisheries or aquaculture represents not only a sustainable but also a profitable alternative for these unused remnants. To do so, characterization of the enzymes present in the GIT, such as the determination of molecular weight and optimum temperature and pH for their activity, becomes crucial for further applications. Molecular weights and optimum activities for pepsin and pepsin isozymes have been described for several fish species, especially those with a well-defined stomach. Molecular weights have been reported for the smooth-hound shark *Mustelus mustelus* (35.0 kDa) [29], Atlantic cod *Gadus morhua* (pepsin I, 35.5 kDa, pepsin II (a and b) 34 kDa) [30], Monterey sardine *Sardinops sagax caerulea* pepsin (29 kDa) [31], and albacore tuna *Thunnus alalunga* (32.7 kDa) [7]. The four isozymes of pepsin (P-I, P-II, P-III, and P-IV) from the sea bream *Sparus latus* [8] and the three isozymes (P-I, P-II, and P-III) of the freshwater European eel *Anguilla anguilla* [32] were all approximately 30 kDa. Our results indicated that pepsins from *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* were 30, 32.1, and 32.3 kDa, respectively. There are not many reports describing molecular weights of pepsins from other sciaenids in the literature, although the observed values are comparable to those of the aforementioned species, confirming that pepsins from these sciaenids were consistent with values reported for unrelated marine and freshwater fish species.

Zymography, as proposed by the method herein employed [27], allowed the identification of the band corresponding to pepsin on SDS-PAGE gels, showing one white band against a blue background for each species (Fig. 2). At this time, using the protocols described, we were able to confirm only one band with pepsin activity, and no isozymes were identified, although the possibility of their presence is not excluded, as for several species two, three, and up two four isozymes have been reported. Isozymes are different molecular forms of the enzyme, coded by different but related genes, resulting in different primary structures but they catalyze the same overall reaction. They are distinguished by their specific electrophoretic mobility and are usually of comparable size [33]. Conversely, isoforms are protein variants resulting from genetic differences but coded by the same gene or gene family; the resulting forms may originate from alternative splicing, variable promoters, or other post-transcriptional modifications. Although isoforms differ in structure, they may catalyze the same reaction, and some may have other unique functions [34,35]. One consideration that may explain the absence of additional isozymes or isoforms under these conditions is the charge of the proteins; the extent to which their carboxyl and amino groups become ionized depends on the pH. The enzyme and its substrate should not both be positively or negatively charged at a given ambient pH, or they will repulse each other [36]. Thus, the zymographic technique described here, where pepsin and Hb interact in 0.1 M Gly.HCl at pH 2, produced only one activity band; nevertheless, at different pH, additional bands might be detected.

In marine finfish, pepsins have been shown to have optimum activity between 37 and 50°C when measured within an optimum pH range, around 2.0 to 3.5. For example, for *G. morhua*, temperature stability ranges of the isozymes at pH 3 were below 37°C for pepsin I and below 40°C for pepsin II a and b [30]. For *S. sagax caerulea*, the optimum temperature was 45°C at pH 3.0, but temperature stability was observed in the range of

30-50°C, while above 55°C activity stopped in ≤ 10 min [31]. Pepsin optimal temperature in *T. alalunga* was 50°C when Hb was used as a substrate [7]. For pepsin of the polar cod *Boreogadus saida* [37] and pepsin P-III of the eel *A. anguilla* [32], optimum temperatures recorded were 37°C and 35°C, respectively; also, for this eel, pepsins' P-I and P-II optimum temperature was 40°C. Our study showed that pepsins of *C. othonopterus* and *C. parvipinnis* showed optimum stability in a wide range of temperatures, from 25 to 60°C, without significant statistical differences, whereas *C. xanthulus* showed significantly higher activity and stability between 35 and 50°C, closer in range to the values of the abovementioned species. Numerically, 40-45°C was the temperature range with higher activity values for C. othonopterus and C. xanthulus, whereas for C. parvipinnis, 40°C was the highest value recorded for activity. Temperature is a predominant parameter in enzymatic reactions as it governs the catalytic efficiency and thermostability of enzymes. For *C. othonopterus* and *C. parvipinnis*, pepsin activity dropped drastically at 65°C, and for *C. xanthulus* at 60°C, presumably due to thermal denaturation of the protein. It was also evident that, quantitatively, pepsin activity was lower in *C. othonopterus*, ranging from 1.73-2.79 U/mg of protein, while in *C, parvipinnis* and *C. xanthulus*, the activity ranged from 2.63-4.65 and 2.70-4.39 U/mg of protein, respectively.

As for the optimum pH, all three sciaenids showed significantly higher activity at 2.0; it was significantly reduced at pH 3.0, and at pH 4.0 and above, activity was less than 40% of that recorded at the optimum value. Thus, our results agree with previous studies confirming that pepsin of carnivorous fish has higher activity in pH values commonly ranging from 2.0 to 3.5. An optimum pH value of 2.0 has also been reported for *Thunnus alalunga* [7], the longtail tuna *T. tonggol*, and skipjack tuna *Katsuwonus pelamis* [38]. For *S. sagax caerulea*, activity was optimum at pH 2.5, with close to 90% of maximum activity recorded at pH 2, 3, and 3.5 [31], whereas the optimum pH was 3.5 for pepsin P-I and 2.5 for P-II and P-III in *A. anguilla* [32]. Some structural amino acids are positively charged at optimum pH while others are negatively charged. These charges in the amino acid residues at the binding site are extremely important for the substrate to bind to the active site through electrostatic interactions, forming temporary bonds, and are also very important in the amino acid residues at the catalytic site for the enzymatic reaction to occur. Consequently, pH conditions outside the physiologically optimum interval will strongly affect the structure and interaction of enzymes with their substrate, which decreases or nullifies their activity [39].

As with the temperature evaluation, the pH evaluation showed quantitatively lower activity of pepsin from *C. othonopterus*, ranging from 1.01-2.58 U/mg protein, whereas in *C, parvipinnis* and *C. xanthulus* the activity ranged from 1.72-4.34 and 1.55-4.13 U/mg of protein, respectively. Enzyme activity is related to enzyme kinetics [40], i.e., the speed of the enzymatic reaction and the factors influencing the reaction [3]. The smaller the constant value (K_m) is, which is the substrate concentration needed to reach half the speed of the maximum speed (V_{max}), suggests that the enzyme-substrate complex is better as the enzyme has a high affinity for the substrate [40]. Consequently, because an enzyme has different V_{max} and K_m values with substrate concentrations specific for a particular temperature and pH in one species, differences in enzymatic activities can be expected among species.

Overall, pepsins from *C. othonopterus*, *C. parvipinnis*, and *C. xanthulus* are comparable with pepsins from other marine and freshwater fish species in their biochemical characteristics; hence, one could assume they could be used for similar applications of other fish pepsins. Further characterization, isolation, purification, and sequencing of pepsins from these sciaenids is still pending.

CONCLUSIONS

The molecular weights of pepsins from *C. othonopterus*, *C. xanthulus*, and *C. parvipinnis* were 30.0, 32.1, and 32.3 kDa, respectively. Under the experimental conditions of this study, the highest activity of pepsin was recorded between 40-45°C for *C. othonopterus* and *C. xanthulus*, and at 40°C for *C. parvipinnis*. Moreover, *C. othonopterus* and *C. parvipinnis* showed optimum stability in a wide range of temperatures from 25 to 60°C, whereas *C. xanthulus* showed stability from 35 to 50°C. The optimum pH was 2.0 for the three sciaenids. All data ranged within values already reported for other marine and freshwater fish species, suggesting that the pepsins of these sciaenids may have similar biotechnological applications.

Funding: The authors received no specific funding for this work.

Acknowledgments: The authors thank Dr. Ana G. Villalba-Villalba and M.Sc. Lorena Bringas-Alvarado for their assistance with electrophoresis. The mention of trademarks or proprietary products does not constitute an endorsement of the product and does not imply its approval to the exclusion of other products that may also be suitable.

Author contributions: Mayra L. González-Félix and Martin Perez-Velazquez conceived and planned the study. Martin Perez-Velazquez participated in fish collecting. Mayra L. González-Félix and Carlos A. Maldonado-Othón carried out the laboratory analyses. Mayra L. González-Félix took the lead in writing the manuscript. All authors discussed the results and provided critical feedback to the manuscript.

Conflict of interest disclosure: The authors have no conflict of interest related to this work.

Data availability: The data underlying the reported findings have been provided with the submitted article and are available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Perez-Velazquez%20et%20al_Raw%20dataset.xlsx

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