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## Study on enzymes of industrial interest in digestive viscera: Greater amberjack (Seriola dumerili)

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## ABSTRACT

**Objective:** To investigate the presence of industrial enzymes (trypsin and chymotrypsin) as well as collagenolytic and fibrinolytic activities of the neglected processing waste of greater amberjack *Seriola dumerili* (*S. dumerili*).

**Methods:** The investigations on trypsin, chymotrypsin, collagenolytic and fibrinolytic activities in residues in digestive viscera were performed. The enzymes trypsin and chymotrypsin were physicochemically characterized (including pH- and thermo-stabilities and sensitivity to metal ions and inhibitors). The kinetic parameters were also determined. Collagenolytic and fibrinolytic activities of the crude extract were also investigated.

**Results:** The crude extract of *S. dumerili* showed enzymatic activity of  $(1.4600 \pm 0.0011)$  and  $(0.7100 \pm 0.0079)$  IU/mg protein for trypsin and chymotrypsin, respectively. The optimal temperature and pH was 60 °C and 9 for trypsin and 40 °C and 8 for chymotrypsin. The main inhibitory ions were in the following order: Fe<sup>3+</sup> > Cu<sup>2+</sup> > Zn<sup>2+</sup> > Cd<sup>2+</sup> > Hg<sup>2+</sup> (trypsin) and Hg<sup>2+</sup> > Fe<sup>3+</sup> > Zn<sup>2+</sup> > Cd<sup>2+</sup> > Mn<sup>2+</sup> (chymotrypsin). TLCK and benzamidine were the strongest inhibitors for both enzymes. The crude extract also presented collagenolytic activity [(42.44 ± 0.01) IU/mg] and fibrinolytic activity [(26.70 ± 0.05) IU/mL].

**Conclusions:** The crude extract of *S. dumerili* showed potential for reuse of its residues and to provide biomolecules of industrial interest like trypsin and to be a source of fibrinolytic enzymes.

Solid wastes (carcasses, skin, bones, fins and heads) discarded during fish processing are potential sources of biologically active molecules of industrial interest[1-5]. The proteases recovered from these sources have physicochemical and kinetic properties similar to the mammalian enzymes commonly employed in the production processes of the textile, pharmaceuticals and foods[6]. Moreover, the use of neglected fish viscera contributes to reducing environmental damage. In this context, fish enzymes can minimize production costs and can be a cost-effective alternative to biomolecules for the fishery and aquaculture production chain[7,8].

Trypsin and chymotrypsin are hydrolases that can be obtained from processed fish residues[9]. Digestive serine proteases have already been extracted from the guts of different types of fishes, such as trypsin from the Silver mojarra *Diapterus rhombeus*[7], sardinelle *Sardinella aurita*[10], zebra blenny *Salaria basilisca*[11], barbel *Barbus callensis*[12], pirarucu *Arapaima gigas*[8], crevalle jack *Caranx hippos*[1], tropical gar *Atractosteus tropicus*[13] and Nile tilapia *Oreochromis niloticus*[14]; and chymotrypsin from the cuttlefish *Sepia officinalis*[15], monterey sardine *Sardinops sagax caerulea*[16], tropical gar *A. tropicus*[13] and Nile tilapia *O. niloticus*[9].

Collagenolytic activity is a property also found in discarded solid waste from tropical and neotropical fishes and involves the cleavage of the collagen triple helix[5]. Serinocollagenases from aquatic sources have been reported in a mixture of haddock, herring, ground fish and flounder crude extracts[17] and intestinal viscera of smooth weakfish *Cynoscion leiarchus*[4]. To date, no fibrinolytic proteases have been identified in fish species, and these species constitute alternative sources because of the industrial/biomedical importance of this enzyme as a thrombolytic agent. Thus, this study aimed to

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investigate the presence of enzymes of industrial interest (trypsin and chymotrypsin) as well as the collagenolytic and fibrinolytic activities from the neglected processing waste (digestive viscera) of greater amberjack *Seriola dumerili*.

## 2. Materials and methods

## 2.1. Materials

Tris (hydroxymethyl) was purchased from Sigma (St. Louis, MO, USA). Glycine was acquired from Amersham Biosciences. Hydrochloric acid (HCl) was obtained from Merck. The spectrophotometer used was Bio-Rad Smartspec<sup>™</sup> 3000. The centrifuges were product of BioAgency Bio-Spin.

#### 2.2. Use of digestive viscera and process of extraction

The digestive viscera of greater amberjack *S. dumerili* were kindly provided by the fishermen's colony of Ponta Verde, Maceió, Alagoas, Brazil after the evisceration process by fishermen as discarded products. Samples of 300 g intestine were collected, packaged in plastic containers, kept on ice and transported to the Laboratory of Enzymology, Center of Biological Sciences, Federal University of Pernambuco, Recife, Pernambuco, Brazil. The enzymatic extraction was performed according to the method described by Oliveira *et al.*[4]. The ratio of viscera to extraction buffer (0.05 mol/L Tris-HCl, pH 7.5, containing 5 mmol/L CaCl<sub>2</sub>) was 1:5 (w/v). The intestinal waste was homogenized for 5 min by a homogenizer with speed adjustment to 10000–12000 r/min (4 °C) (IKA RW 20D S32, China). The homogenate was then centrifuged (Sorvall Superspeed Centrifuge RC-6, North Carolina, USA) at 10492 r/min for 30 min at 4 °C. The final supernatant was defined as the crude extract and stored at –25 °C.

#### 2.3. Trypsin and chymotrypsin activity and protein determination

Protease activity was measured using Na-benzoyl-DL-arginine-pnitroanilide (BApNA) and Succinyl-DL-phenylalanine-p-nitroanilide (Suc-Phe-p-Nan) dissolved in aminomethane and dimethyl sulfoxide (DMSO) as specific substrate for trypsin and chymotrypsin, respectively. The final concentration used was 8 mmol/L. The substrate  $(30 \ \mu L)$  was incubated in wells of microplate with the enzyme  $(30 \ \mu L)$ µL) and 140 µL of 0.05 mol/L Tris-HCl buffer (pH 7.5) containing 5 mmol/L CaCl2. The release of p-nitroaniline was measured as an increase in absorbance at 405 nm in a microplate reader. The test for controls was performed without enzyme. One unit (IU) of enzyme activity is considered as the amount of enzyme able to produce 1 µmol of p-nitroaniline per minute[9]. The specific activity, calculated as the ratio between the protease activity (IU/mL) and the total protein in the sample (mg/mL), was expressed in IU/mg. The protein concentration of all tissue extracts was determined according to method of Smith et al.[18], using bovine serum albumin (BSA) as a standard.

## 2.4. Michaelis-Menten kinetic assay (K<sub>m</sub> and V<sub>max</sub>)

The substrates used in the kinetic tests were BApNA and Suc-Phe-p-Nan, with final concentrations varying from 0 to 4.5 mmol/ L and dissolved in DMSO. The reaction was performed in triplicate in microplates and consisted of a mixture of crude extract (30 µL), 0.5 mol/L Tris–HCl buffer (140 µL, pH 7.5) and substrate (30 µL). The release of p-nitroaniline was monitored by a microplate reader at 405 nm and the enzymatic activity was calculated as described in section 2.3. The activity values obtained for each substrate concentration were plotted on a Michaelis–Menten graph using the MicroCal<sup>TM</sup> Origin<sup>®</sup> Version 8.0 (MicroCal, Northampton, MA, USA)[8].

#### 2.5. Physicochemical properties

#### 2.5.1. Optimum temperature and thermal stability

The effect of temperature on the enzyme activity and stability was evaluated at temperatures ranging from 25 to 80 °C. For optimal temperatures, the assays were carried out by incubating the crude extract with the substrates, 8 mmol/L BApNA or Suc-Phe-p-Nan, in a water bath. To test thermal stability, the enzyme was incubated in a water bath for 30 min and the remaining activity was then measured at 25 °C. The activity was calculated as the ratio between the enzymatic activity at the end of each incubation run and that at the beginning, and expressed as percentage (%)[7].

#### 2.5.2. Optimum pH and stability

These experiments were carried out in different pH ranges using the buffers: 0.5 mol/L citrate–phosphate (pH 4.0–7.0), 0.1 mol/L Tris–HCl (pH 7.5–8.5) and 0.1 mol/L glycine–NaOH (pH 9.0–12.0), containing 5 mmol/L CaCl<sub>2</sub>, using 8 mmol/L BApNA and Suc-Phe-p-Nan as substrate. For the optimum pH, the crude extract was mixed with 140  $\mu$ L of buffer solutions, then 30  $\mu$ L of substrate was added and incubated for 10 min at 25 °C. The influence of pH on enzyme stability was determined by incubating the enzyme with various buffer solutions at a ratio of 1:1 for 1 h at 25 °C. Then, 30  $\mu$ L aliquots were withdrawn and used to assess the activity of the enzyme at the optimum pH presented using 8 mmol/L substrate. The activity was calculated as the ratio between the enzymatic activity observed at the end of each incubation run and that at the beginning, and expressed as percentage (%)[8].

#### 2.6. Sensitivity to metal ions and inhibitors

For the test for sensitivity to metal ions, samples of crude extract (30  $\mu$ L) were added to a 96-well microtitre plate with 1 mmol/L (30  $\mu$ L) of the ions Mn<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Hg<sup>2+</sup>, Mg<sup>4+</sup>, Al<sup>3+</sup>, Fe<sup>3+</sup> and Pb<sup>2+</sup>. Deionized water was used to prepare the solutions of all metals. After 1 h of incubation, 110  $\mu$ L of 0.05 mol/L Tris-HCl buffer (pH 7.5) with 5 mmol/L CaCl<sub>2</sub>, and 30  $\mu$ L of 8 mmol/L BApNA or Suc-Phe-p-Nan were added. The test for sensitivity to inhibitors was performed by incubating the crude extract (30  $\mu$ L) for 1 h at 25 °C with protease inhibitors (30  $\mu$ L, 8 mmol/L): phenylmethylsulphonyl fluoride (PMSF); N-p-tosyl-L-lysin chloromethyl ketone (TLCK); benzamidine; N-tosyl-L-phenylalaninechloromethyl ketone (TPCK); ethylenediamine tetraacetic acid (EDTA); and β-mercaptoethanol. After incubation, 8 mmol/L BApNA was added and the release of p-nitroaniline was measured as the increase in absorbance at 405 nm[8].

#### 2.7. Collagenolytic activity

The test for collagenolytic activity of the crude extract was performed according to method of Oliveira *et al.*[4], using Azo dye-impregnated collagen (azocoll) as substrate. A reaction mixture containing azocoll (5 mg), 50 mmol/L Tris–HCl buffer (500  $\mu$ L, pH 7.5, contained 5 mmol/L CaCl<sub>2</sub>) and crude extract (500  $\mu$ L) was typically incubated at 55 °C for 30 min with stirring. Thereafter, trichloroacetic acid (200  $\mu$ L) was added and incubated to stop the reaction. After 10 min, the samples were centrifuged at 10492 r/min for 10 min at 4 °C. The sample reading was performed using a spectrophotometer at a wavelength of 595 nm. One enzyme unit was defined as the amount of enzyme required to increase the absorbance of 0.01 at 595 nm.

## 2.8. Fibrinolytic activity

The fibrinolytic activity (FA) was determined using the spectrophotometric method. First, 0.4 mL of 0.72% fibrinogen was placed in a test tube with 0.1 mL of 245 mmol/L phosphate buffer (pH 7.0) and incubated at 37 °C for 5 min. Then, 0.1 mL of a 20

IU/mL thrombin solution was added. The solution was incubated at 37 °C for 10 min, then 0.1 mL of diluted enzyme solution was added, and incubation continued at 37 °C. At 60 min, 0.7 mL of 0.2 mol/L trichloroacetic acid (TCA) was added, and mixed. The reaction mixture was centrifuged at 11 730 r/min for 10 min. Then, 1 mL of the supernatant was collected and the absorbance at 275 nm was measured. In this assay, 1 unit (fibrin degradation unit, FU) was defined as the increase of 0.01 in absorbance per minute at 275 nm[19].

### 2.9. Statistical analysis

All values were presented as mean  $\pm$  SD. Data were statistically analyzed for normal distribution by Shapiro–Wilk and Kolmogorov–Smirnov tests and homogeneity of variances by Levene's test. One-way ANOVA followed by Tukey's test was used for normally distributed data, whereas Kruskal–Wallis ANOVA would be used in case of non-normally distributed data. Differences between groups were accepted as significant at a confidence level of 95% (P < 0.05).

## **3. Results**

The crude extract of greater amberjack *S. dumerili* presented total protein of  $(3.29 \pm 0.01)$  mg/mL and enzymatic activity of  $(1.4600 \pm 0.0011)$  and  $(0.7100 \pm 0.0079)$  IU/mg protein for trypsin and chymotrypsin, respectively. The specific activity of

chymotrypsin was lower than trypsin at species studied.

Kinetic parameters of BApNA and Suc-Phe-p-Nan hydrolysis were examined in the present study. The Michaelis-Menten ( $K_m$ ) constant for the crude extract was (0.35 ± 0.09) and (0.34 ± 0.07) mmol/L for trypsin and chymotrypsin, respectively. The  $V_{max}$  was found to be (391.07 ± 12.80) and (108.08 ± 2.56) IU/mg for trypsin and chymotrypsin, respectively.

The effect of temperature on trypsin activity is illustrated in Figure 1A. The optimum temperature for trypsin was 60 °C, and the activity of trypsin remained stable at 25–60 °C (Figure 1B). The optimum temperature for chymotrypsin was 40 °C (Figure 1C), and the activity of chymotrypsin remained stable at 25–45 °C (Figure 1D). The optimum pH for trypsin activity was 9.0 (Figure 2A), and the activity maintained its stability in the pH range of 6.0 to 12.0 (Figure 2B). The optimum pH for chymotrypsin was 8.0 (Figure 2C), remaining stable between 6.5 and 8.5 (Figure 2D).

The effect of the metal ions and inhibitors on enzyme activity was evaluated and presented in Table 1. In this work, the trypsin and chymotrypsin were inhibited by the following ions in decreasing order:  $Fe^{3+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Hg^{2+} > Pb^{2+} > Mg^{4+} > Al^{3+} > Mn^{2+};$  and  $Hg^{2+} > Fe^{3+} > Zn^{2+} > Cd^{2+} > Mn^{2+} > Cu^{2+} > Al^{3+} > Pb^{2+} > Mg^{4+}$  respectively. The trypsin inhibitors (TLCK and benzamidine) were those that exerted the greatest inhibitory effect on the activity of both enzymes (trypsin and chymotrypsin). The crude intestinal extract analyzed showed collagenolytic and fibrinolytic activity of (42.44  $\pm$  0.01) IU/mg and (26.70  $\pm$  0.05) IU/mL, respectively.



**Figure 1.** The thermal stability and effect of temperature on the activity of serine proteases extracted from greater amberjack *S. dumerili*. A: Optimum temperature for trypsin activity in a range of 25–80 °C; B: Thermal stability of trypsin after 1 h incubation at temperature in the range of 25–80 °C; C: Optimum temperature for chymotrypsin activity in a range of 25–80 °C; D: Thermal stability of chymotrypsin after 1 h of incubation in the temperature range of 25–80 °C.



**Figure 2.** Effect of pH on the activity and stability of serine proteases extracted from greater amberjack *S. dumerili*. A: Optimal pH for the activity of trypsin, using different buffers in the pH range from 4.5 to 12.0, expressed as percentage of the maximum obtained in 0.05 mol/L Tris-HCl buffer; B: pH stability of trypsin after incubation for 1 h in the pH range 4.0 to 12.0; C: The optimum pH for the activity of chymotrypsin using different buffers in the pH range from 4.5 to 12.0, expressed as percentage of the maximum obtained in 0.05 mol/L Tris-HCl buffer; D: pH stability of chymotrypsin after incubation for 1 h in the pH range 4.0 to 12.0.

### Table 1

Effect of ions and	inhibitors on the	he residual	activity	(%) of	trypsin	and
chymotrypsin from	greater amberjac	k Seriola di	umerili.			

#### 4. Discussion

	5		
Ions and inhibitors	S. dumerili		
	BApNA	Suc-Phe-p-Nan	
Metal ions <sup>*</sup> (1 mmol/L)			
Mn <sup>2+</sup>	$100.98^{a}$	21.49 <sup>b</sup>	
$Zn^{2+}$	55.20 <sup>b</sup>	17.05 <sup>b</sup>	
Fe <sup>3+</sup>	41.71 <sup>b</sup>	2.14 <sup>b</sup>	
Cu <sup>2+</sup>	55.14 <sup>a</sup>	22.39 <sup>b</sup>	
Hg <sup>2+</sup>	65.61 <sup>b</sup>	$0.00^{b}$	
Mg <sup>4+</sup>	78.91 <sup>a</sup>	100.62 <sup>a</sup>	
Cd <sup>2+</sup>	59.20 <sup>b</sup>	19.41 <sup>b</sup>	
Al <sup>3+</sup>	79.69 <sup>b</sup>	25.45 <sup>b</sup>	
Pb <sup>2+</sup>	69.74 <sup>b</sup>	52.14 <sup>b</sup>	
Inhibitor <sup>*</sup> (8 mmol/L)			
PMSF	62.18 <sup>b,ab</sup>	68.04 <sup>a,a</sup>	
ТРСК	54.03 <sup>b,a</sup>	87.56 <sup>a,a</sup>	
TLCK	26.71 <sup>b,c</sup>	26.28 <sup>b,b</sup>	
Benzamidine	19.11 <sup>b,c</sup>	48.30 <sup>b,b</sup>	
β-Mercaptoethanol	43.40 <sup>b,c</sup>	66.84 <sup>b,ab</sup>	
EDTA	45.02 <sup>b,bc</sup>	67.82 <sup>b,ab</sup>	

\*: In the controls, determinations were performed without ions or inhibitors. The activities of controls were 1.11 IU/mg (trypsin) and 0.54 IU/mg (chymotrypsin). The data followed by different letters are significantly different. In the values for inhibitors, the first superscript letter (before coma) represents comparison with control group that was considered as 100% (a). The second superscript letter is related to comparison between inhibitors. The initial concentration used for the assay with inhibitors was 8 mmol/L. The final concentration used for the assay with ions was 1 mmol/L. Different superscript letters represent statistical differences (P < 0.05).

The activity of the trypsin from crude extract tested in the present study was higher than that from crude extract of Japanese sea bass *Lateolabrax japonicus* (0.30 IU/mg)[<sup>20</sup>], zebra blenny *Salaria basilisca* (0.12 IU/mg)[<sup>11</sup>], pirarucu *A. gigas* (0.37 IU/mg[8], tropical gar *A. tropicus* (0.000006 IU/mg)[<sup>13</sup>] and hybrid catfish (*Clarias microcephalus x Clarias gariepinus*) (0.18 IU/mg)[<sup>21</sup>].

The chymotrypsin activity obtained in this study was higher than that reported for *A. tropicus* (0.0012 IU/mg)[13]. Cuenca-Soria *et al.*[22] observed the chymotrypsin activity of 0.52 and 0.8 IU/mg for the Mayan cichlid *Cichlasoma urophthalmus*. Zhou *et al.*[23] reported that chymotrypsin of marine fish that acclimated to cold regions has higher catalytic activities and *S. dumerili* is a species that acclimated to cold water. Falcón-Hidalgo *et al.*[24] reported increasing activity of trypsin and chymotrypsin of Cuban *Limia vittata* and Cuban gambusia *Gambusia punctate* during the development of the species.

One of the characteristics of fish proteases is to have a higher affinity to the specific substrate (lower  $K_m$ ) when compared to the enzymes from other sources, such as bovine trypsin; this is mainly due to differences in the region of substrate breakdown[6]. The  $K_m$  is used to assess the affinity of the tested enzyme to the substrate and constitutes one of the characteristics observed by the industry during the prospection process for novel commercial enzymes. The results for trypsin showed similar values to that of alkaline trypsin from silver mojarra *D. rhombeus*[7], pirarucu *A. gigas*[8] and crevalle jack *C. hippos*[1]; while chymotrypsin values diverged from that reported by Castillo-Yañez *et al.*[25].

The effect of temperature on the activity of trypsin in this work was

similar to that of Brownstripe red snapper *Lutjanus vitta*<sup>[26]</sup> and zebra blenny *Salaria basilisca*<sup>[11]</sup>. The loss of activity presumably was due to heat treatment. Another similar result was reported for pirarucu *A. gigas*<sup>[8]</sup>, with optimal temperature at 65 °C. Results of optimum temperature for chymotrypsin from Monterey sardine *Sardinops sagax caerulea*<sup>[25]</sup> are in accordance with that found in the present work. Guerrero-Zárate *et al.*<sup>[13]</sup> reported optimum temperature of 60 °C for alkaline proteases (trypsin and chymotrypsin) in juveniles of tropical gar *A. tropicus*. For affecting the folding of proteins due to the breaking of disulfide bonds, the temperature influences the enzymatic activity<sup>[6]</sup>. Because of less disulfide bonds, when compared to trypsin, chymotrypsin becomes more sensitive to the temperature. The chymotrypsin activity of fish species presents low tolerance to temperature variations<sup>[23]</sup>.

Optimum pH of trypsin is consistent with that reported by Ben Khaled et al.[10], Freitas-Junior et al.[8], Ktari et al.[11] and Sila et al.[12] for the fish species sardinelle S. aurita, pirarucu A. gigas, zebra blenny S. basilisca and barbel B. callensis, respectively, indicating an optimum pH range between 8.0 and 10. In this study, peak activity of trypsin was found in the pH 9.0. Similar reports have showed the optimum activity of enzymatic crude extracts from Brownstripe red snapper Lutjanus vitta[26], Japanese sea bass L. japonicus[20] and pirarucu A. gigas[8] in the range from 8.5 to 10.0. These results are consistent with those reported for trypsin from fish species in literature[6]. Trypsin stability to pH variation was similar to the findings reported by Silva et al.[7] for silver mojarra D. rhombeus remaining stable in a range of alkaline pH (8.5-11.0) and being unstable in pH below 8.5 and showing negligible activity at pH 4.5, while Cai et al.[20] reported recovery of activity for Japanese sea bass L. japonicus in the pH range from 7.0 to 11.0.

Optimum pH of chymotrypsin was similar to the reports for Monterey sardine *S. sagax caeruleus*<sup>[25]</sup>, showing a relatively high activity in the pH range of 8.0–10.0. Here, the optimum pH range (7.5–9.0) found for chymotrypsin indicated denaturation when subjected to extensive acidic pH range (below 5.0) or for long time to high alkaline pH ranges (> 12.0). In the stability test, chymotrypsin was more sensitive to changes in pH than trypsin. Sudden changes in pH lead to changes in charge and consequently in the conformational structure of the protein. Strong acidic or alkaline solutions cause irreversible denaturation of the enzyme, leading to the loss or inactivation of the activity[7].

The results obtained for the optimum temperature and pH and the recovery of the activity for both enzymes (trypsin and chymotrypsin) when subjected to thermal stress and pH fluctuations are in agreement with parameters considered by the industry in several industrial sectors. In addition, the alkaline proteases derived from fish processing residues present biotechnological potential for textile, food and biomedical applications[6,23].

The results of *in vitro* exposure to metal ions for trypsin are in accordance with those obtained for zebra blenny *Salaria basilisca*[11]. Costa *et al.*[1] observed inhibition on trypsin activity from crevalle jack *C. hippos* after incubation with  $Cd^{2+}$ ,  $Al^{3+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Pb^{2+}$  and  $Hg^{2+}$  at 1 mmol/L, revealing high sensitivity of the enzyme to metal ions. Silva *et al.*[7] reported reduction in trypsin activity of silver mojarra *D. rhombeus* when it was incubated in the presence of Fe<sup>2+</sup>,  $Cd^{2+}$ ,  $Cu^{2+}$  and  $Al^{3+}$  in a proportion of 20% to 35%, while ions  $Hg^{2+}$  and  $Zn^{2+}$  inhibited about 53.11% and 71.23%, respectively. According to these reports, the Pb<sup>2+</sup> ions completely inhibited the enzymatic activity. The chymotrypsin was susceptible to various metal ions, such as  $Mg^{2+}$  and inactivated by the ions Fe<sup>2+</sup>,  $Mn^{2+}$ ,  $Cu^{2+}$  and  $Zn^{2+}$  as reported by Yang *et al.*[27] for crucian carp *C. auratus.* In the present work, the ion  $Hg^{2+}$  completely inhibited the activity of chymotrypsin from *S. dumerili*.

The results of inhibition on trypsin by benzamidine and TLCK are similar to those reported by Silva et al.[7], Freitas Junior et al.[8], Costa et al.[1] and França et al.[3]. When subjected to a potent serine protease inhibitor (PMSF), activity of 62.18% was detected for trypsin of S. dumerili. Cuenca-Soria et al.[22] reported a high degree of inhibition on alkaline proteases (trypsin and chymotrypsin) from extracts of C. urophthalmus by specific inhibitors such as TPCK, TLCK, and EDTA (greater than 80%) and PMSF (60%). When using the specific inhibitor of chymotrypsin (TPCK), residual trypsin activity of 54.03% was detected for S. dumerili. Chymotrypsin has sensitivity to certain natural and synthetic specific inhibitors, which may result in partial decrease or complete loss of enzymatic activity. In this study, chymotrypsin activity was poorly influenced by specific inhibitors, when compared with the other inhibitors tested, especially when subjected to trypsin inhibitors, TLCK and benzamidine.

High collagenolytic activity was reported for intestinal crude extract of smooth weakfish *Cynoscion leiarchus* (72.5 IU/mg)[4], and Daboor *et al.*[17] reported the collagenolytic activity (11.63 IU/mg) using a mixture of haddock, herring, flounder and ground fish crude extracts. Souchet and Laplante[28] also detected the collagenolytic activity (13.3 IU/mg) in byproducts of snow crab *Chionoecetes opilio*. Currently, much of the collagenase used in the market is of microbial origin. The fibrinolytic activity found in the extracts of greater amberjack *S. dumerili* indicates the biotechnological potential of these neglected digestive wastes. Thrombolytic agents are used for thrombosis of the cardiac valve[29], in the enzymatic debridement of the pleural cavity[30], cancer therapy[31] and pathophysiological action in the nervous system[32]. To our knowledge, no record of fibrinolytic agents from fish processing residues has been reported to date; the present study was the first report.

The rapid processing and high catalytic efficiency of proteases from fish residues, such as the gut, make this material an alternative and promising source in the supply of biomolecules with viable applications in the industrial market, mainly because they have desirable physicochemical properties in the productive chains of food, textile and pharmacological products. In this work, internal viscera of greater amberjack *S. dumerili* provided enzymes with resistance to high temperature and maintenance of its functionality in a wide range of pH. Thus, this material can be used as an alternative source after technological purification of the appropriate enzyme to the needs of each industrial sector, reducing costs, adding value to the fishery byproducts and contributing to the reduction of environmental impact.

#### **Conflict of interest statement**

We declare that we have no conflict of interest.

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