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Original Article

Characterization of digestive enzymes from captive Brazilian flounder *Paralichthys orbignyanus*

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(With 2 figures)

Abstract

Knowledge of specific enzyme activity, along with animal habits and digestive capacity is essential in formulating an appropriate diet for any species. In this study, we evaluated and characterized the activity of digestive enzymes present in the liver, intestine, and stomach of *Paralichthys orbignyanus*. The effects of pH and temperature on enzyme activity were also evaluated via the use of specific substrates. The use of specific substrates and inhibitors showed strong evidence of the presence of trypsin (BA*p*NA= 0.51 ± 0.2 mU mg^{-1}), chimotrypsin (SA*p*NA= 2.62 ± 1.8 mU mg^{-1}), and aminopeptidases (Leu-*p*-Nan =0.9709 ± 0.83 mU mg^{-1}) in the intestine. Optimum pH for the activity of trypsin, chemotrypsin, leucino aminopeptidase, amilase, and pepsin were 9.5, 9.0, 8.0, 7.5, and 3.5, respectively, while optimum temperatures were 50, 50, 50, 40, and 45 °C, respectively. These results provide additional information regarding the biology of Brazilian flounder and can be used as a basis for further studies regarding fish feeding physiology.

Keywords: trypsin, protease, digestive tract.

Caracterização de enzimas digestivas do linguado brasileiro de cativeiro *Paralichthys orbignyanus*

Resumo

O conhecimento da atividade enzimática é essencial para formular uma correta dieta específica para espécie, além de estarem correlacionadas com o hábito da alimentação e capacidade digestive. Neste estudo determinamos e caracterizamos a atividade enzimática presente no intestino, estômago e fígado do linguado *Paralichthys orbignyanus*. Os efeitos da temperatura e pH sobre a atividade enzimática também foram avaliados utilizando substratos específicos. O uso de substratos e inibidores específicos mostrou uma forte evidência da presença da tripsina (BApNA = 0,51 ± 0,2 mU mg-1), quimotripsina (SAPNA = 2,62 ± 1,8 mU mg-1), e as aminopeptidases (Leu-p-Nan = 0,97 ± 0,83 mU mg-1) no intestino. O pH ótimo observado para a atividade de tripsina, quimotripsina, leucino aminopeptidase, amilase e pepsina foi 9,5, 9,0, 8,0, 7,5 e 3,5, respectivamente. A temperatura ótima observada foi 50, 50, 50, 40 e 45 °C, respectivamente. Estes resultados fornecem informações adicionais sobre a biologia do linguado brasileiro e pode ser usado como base para novos estudos sobre fisiologia alimentar.

Palavras-chave: tripsina, proteases, trato digestório.

1. Introduction

The Brazilian flounder, *Paralichthys orbignyanus* (Valenciennes, 1842) is a native species that inhabits coastal and estuarine areas from Rio de Janeiro – Brazil, to Mar del Plata – Argentina; however, is found in greater abundance in southern Brazil (Bianchini et al., 2010). The flounder fishery industry in the southern region of Brazil has been considered an important resource, however in the last few years has stagnated in capture rates (Brasil, 2010). Thus, aquaculture for this species may be considered as an alternative resource to increase market supply (Sampaio et al., 2008). Reproduction and larviculture of Brazilian flounder is well established (Bianchini et al., 2010), and the effects of salinity on growth are also known (Sampaio et al., 2007); these represent important features to be considered in a new species for coastal and estuarine aquaculture. However, there is a lack of current literature related to the digestive physiology and nutritional requirements of this species.

Feeding is one of the most expensive factors in aquaculture, often representing more than 50% of total costs (Alam et al., 2009). In this sense, it is important to offer well balanced diets that promote maximum fish growth while reducing production costs and nitrogenous residues to the environment (Gatlin, 2010). Relevant information regarding enzymatic activity and the digestive processes from each fish species is essential for developing correct feeding formulations (López-López et al., 2005). Therein, digestive enzyme patterns are correlated to fish feeding habits and digestive capacity (Kuz'mina et al., 2011).

In this study, the activites of digestive enzymes present in the intestine, stomach and liver of Brazilian flounder were characterized.

Trypsin, chymotrypsin, pepsin and aminopeptidase are among the most important digestive enzymes in Brazilian flounder, due to their high proteolytic activities (Parra et al., 2007). Trypsin is considered a key proteolityc and self-activated enzyme, which may also influence the activity of other pancreatic zymogens, and potentially limit growth rate in the cod *Gadus morhua* (Lemieux et al., 1999). Amylase is the primary glucosidase found in fish. Amylase hydrolyses α1 - 4 bonds present in amylose, glycogen, or linear fragments of amylopectin secreted by pancreatic cells (Krogdahl et al., 2005). Amylase activity has been observed in several teleosts (including herbivorous to strictly carnivourous fish) at different portions of the digestive tract, although mainly in the pyloric cecum, liver and pancreas (Deng et al., 2010).

Thus, the aim of this study was to identify and partially characterize digestive enzymes present in the liver, stomach and intestine of Brazilian flounder, thereby providing relevant information regarding the biology and digestive physiology of the species.

2. Material and Methods

2.1. Experimental fish

A total of 41 unsexed juvenile Brazilian flounders (mean weight 112 ± 35 g, 4 months of age) were used in this study. Fish were reared at the Laboratório de Piscicultura Estuarina e Marinha – FURG, according to the protocol of Sampaio et al. (2008). Fish were euthanized with high benzocaine concentration (500 ppm) and complete digestive tracts were immediately dissected on ice. Stomach, liver and intestine were deep-frozen and all enzymatic assays were conducted at the Laboratório de Enzimologia – UFPE. All assay reagents were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Crude extract preparation and determination of total soluble protein

The digestive tracts of 41 juveniles were subdivided into three groups (14, 14 and 13) in case of loss of material. Collected organs (1g/mL) were homogenized in chilled 0.15M NaCl using a tissue homogenizer (IKA RW 20 digital) at 615 rpm for 10 minutes. Crude extracts were obtained from supernatants following centrifugation (10,000 × *g*, 25min, 4 °C) and lipid removal, and then stored at -20 °C for later use. Total soluble protein contents of crude extracts were determined using bovine serum albumin as the standard protein (Bradford, 1976).

2.3. Non-specific enzyme assays

Total protease alkaline activity was assayed through hydrolysis of 1% azocasein dissolved in 0.1M Tris-HCl buffer pH 8.0. Triplicate samples (30 µL) of crude intestine extract were incubated with substrate solution (50 µL) for 60 min at 25 °C in a microtube (Bezerra et al., 2005). The reaction was stopped with the addition of 10% trichloroacetic acid (240 µL) and the mixture was centrifuged at 8,000 × *g* for 5 min. Unhydrolysed substrate was sedimented and the supernatant was recovered and then further mixed (70 µL) with 1M NaOH (130 µL). The absorbance of supernatants was measured in a microtitre plate reader (Bio-Rad 680, Japan) at 450 nm. Changes in absorbance over time were calculated by the differences between reactions stopped at zero (blank controls) and after 10min.

Total acid proteolytic activity was determined (triplicate) according to the methodology described by Khaled et al. (2011), with modifications to substrate concentration, incubation time, temperature and pH of the buffer. A mixture (100mL) containing 2% hemoglobin (w/v) pH 2.5, 50 µL of crude stomach extract, and 350 µL of glycine – HCl 0.1 M buffer pH 2.5 was incubated for 30 min at 37 °C. 500 µL of 10% trichloroacetic acid (w/v) was added after 30 minutes and the mixture was incubated for a further 15 min at 25 °C. The mixture was centrifuged at 10,000 × *g* for 10 min and the absorbance of the supernatant was analyzed at 280 nm. A control reaction was performed by replacing the crude stomach extract with 0.15 M NaCl. In addition, another control was performed by replacing the 2% hemoglobin with glycine-HCl 0.1 M pH 2.5 buffer.

One unit of total protease activity (U) was expressed as the amount of enzyme required to hydrolyse azocasein or hemoglobin and produce a 0.001 change in absorbance per millilitre per minute. Specific protease activity was expressed in units per milligram of protein. All values were presented as means ± standard deviations.

2.4. Specific enzyme assays

Activites of trypsin, chymotrypsin, and leucine aminopeptidase were determined in microplates according to Buarque et al. (2010) using their specific substrates: BapNA (Nα-benzoyl-DL-arginine-p-nitroanilide), 8 mM Sapna (succinyl - alanine phenylalanine proline alanine - p - nitroanilide), and 4 mM p-Nan-Leu (leucine-p nitroanilide) 8 mM. DMSO (dimethyl sulfoxide) was used as the solvent for all substrates. All assays were performed in triplicate. Crude intestine extract (30 µL) was incubated with 140 µL of Tris-HCl 0.1 M, pH 8.0, and 30 µL of the substrate for a period of 15 min at 25 °C. Absorbance readings were measured and recorded using a microplate reader (Bio-Rad Model X-Mark ™, USA) at 405 nm wavelength. One unit of activity (U) was defined as the amount of enzyme required to produce 1 µmol of p-nitroanilide per minute. The specific activity was expressed as units per milligram of protein. All values were presented as means ± standard deviations.

2.5. Amylolytic activity

Total amylase activity was based on the method of Bernfeld (1955) using starch solution at 2% (w/v) as substrate. The reaction consisted of incubating 20 µL of the crude liver extract and intestine (crude liver or intestine extract) with 125 µL of 0.1 M Tris-HCl, pH 8, and 125 µL of substrate at 37 °C for 10 minutes. Then, 30 µL of solution was incubated with DNSA (acid 3.5 - dinitrosalicylic) at 100 °C for 10 minutes. Immediately after cooling, 200 µL of the solution was transferred to a microplate and the absorbance was measured at 570 nm using a microplate reader (Bio-Rad Model X-Mark™, USA). One unit of enzyme activity was expressed in µg of maltose liberated at 37 °C per minute per mg protein. A calibration curve using commercial maltose was used to determine the concentration of liberated maltose. All values were presented as means ± standard deviations.

2.6. Effects of pH and temperature on enzyme activity

The effects of pH and temperature on amylolytic and proteolytic activity from crude extract of intestine, stomach and liver of Brazilian flounder were evaluated using Tris-HCl, citrate-phosphate and glycine-NaOH buffers (at a final concentration of 0.1 M, pH ranging from 4.5 to 11.5 for alkaline activity and 1.5 to 6.5 for acid activity and temperature of 25-70 °C for both pH ranges). Thermal stability was evaluated by analyses of enzymatic activity at 25 °C after pre-incubation for 30 minutes at temperatures ranging from 25-70 °C (Bezerra et al., 2005). All values were presented as means ± standard deviations.

2.7. Inhibitiors assays

Inhibitors were prepared in DMSO at concentrations of 8 mM: phenylmethylsulfonyl fluoride (PMSF - serine peptidase inhibitor), TLCK (tosyl lysine chloromethyl ketone) and benzamidine (classic trypsin inhibitors), TPCK (tosyl phenylalanine chloromethyl ketone), classic chymotrypsins inhibitor (Bezerra et al., 2005).

Inhibitor type I of α-amylase was obtained from the plant *Triticum aestivum* and was prepared in distilled water at a concentration of 1 mg/mL. Triplicate samples containing mixtures of crude extracts of intestine (trypsin, chymotrypsin and aminopeptidase), liver (amylase), and inhibitor solution at a ratio of 1:1 were incubated for 30 min at 25 °C. After incubation, enzyme activity assays were carried out as described previously. Negative controls of the enzyme extract and reaction were performed by replacing the substrate buffer and crude extract by 0.15 M NaCl, respectively. A positive control (100%) was conducted using DMSO without inhibitors.

2.8. Statistics

All values were presented as means ± standard deviations. The inhibition results were analyzed by ANOVA, followed by a post hoc (Tukey) test, when indicated. Differences between groups were accepted as significant at the 95% confidence level ($p < 0.05$).

3. Results

Table 1 shows the activity of digestive peptidases and amylolytic activity present in the digestive tract of juvenile Brazilian flounder. Trypsin (BapNA) and leucine-aminopeptidase (Leu-p-Nan) activities were observed in crude extract of intestine. Enzymatic activity using nonspecific substrates was also observed in both alkaline activity (azocasein) as well as the acid activity (hemoglobin) in crude intestine and stomach extract, respectively.

Table 1. Enzymatic activity present in the crude extract of juvenile Brazilian flounder *Paralichthys orbignyanus* using different substrates.

	Substrates					
	***BA*p*NA 8mM**	***Sa*p*Na 4mM**	***Leu-*p*-Nan 8mM**	***Azocasein (1%)**	***Hemoglobin**	****Starch (2%)**
Intestine	0.51 ± 0.2	2.62 ± 1.8	0.97 ± 0.83	3.58 ± 2.36	-	0
Liver	0	0	0	0.002 ± 0.001	-	0.03 ± 0.00
Stomach	-	-	-	-	0.36 ± 0.25	-

BA*p*NA - benzoyl arginine ρ-nitroanilide, specific for trypsin; Sa*p*Na - N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, specific for chymotrypsin; Leu-*p*-Nan – aminoacil de β – naftilamide, specific for leucine - aminopeptidase; Azocasein, nonspecific for alkaline peptidases; Hemoglobin, nospecific for pepsin; Starch, nonspecific for amylases. – denotes no enzyme mearsurements. (n=14); *mU mg^{-1} protein ± SD; **µg maltose/mg protein.

Amylolytic activity was detected only in crude extract of the liver, using the nonspecific substrate starch (Table 1). The enzymatic activity of chymotrypsin was also detected in this crude extract, using starch as substrate, while unspecific proteolytic activity was observed using azocasein.

Table 2 indicates the effect of some inhibitors on the enzymatic activity of trypsin, chymotrypsin and leucine aminopeptidase in crude intestine extracts. The inhibitor of serine peptidases (PMSF), benzamidine, and TLCK demonstrated inhibition in all three enzymes (trypsin, chymotrypsin and leucine aminopeptidase). The TPCK specific inhibitor of chymotrypsin did not inhibit trypsin, however did inhibit 60% of leucine aminopeptidase activity and 79% of the activity of chymotrypsin. In Table 3 it is possible to observe inhibition effects with the two concentrations of type I inhibitor of α-amylase obtained from *Triticum aestivum* (500 and 1,000 mg/mL = 26% and 36%).

The effects of temperature on the activity of trypsin, chymotrypsin and leucine aminopeptidase present in the crude extract of intestine are shown in Figure 1A, 1D and 1G, respectively. Maximum activity of the three enzymes was

Table 2. Effect of the specific inhibitors on peptidases activity present in the intestine crude extract of juvenile Brazilian flounder *Paralichthys orbignyanus*.

Inhibitors (8mM)	Residual activity (% ± SD)		
	Ba*p*Na**	**Succ-phe-*p*-NAN****	**Leu-*p*-NAN****
Control*	100.0 ± 0.7a	100.0 ± 3.2a	100.0 ± 5.9a
PMSF	52.0 ± 2.3b	42.0 ± 1.2b	43.0 ± 1.4b
TLCK	33.0 ± 1.8c	45.0 ± 0.3c	44.0 ± 4.1b
TPCK	100.0 ± 1.2a	79.0 ± 4.5d	60.0 ± 1.8c
Benzamidine	41.0 ± 2.3b	58.0 ± 0.5e	40.0 ± 1.2b

*Activity without addition of inhibitors; PMSF: phenylmethylsulphonyl fluoride, serine protease inhibitor; TLCK: tosyl lysine chloromethyl ketone, trypsin inhibitor; TPCK: tosyl phenylalanine chloromethylketone, chymotrypsin inhibitor; Benzamidine: trypsin inhibitor. (n=14); **mU mg^{-1} protein ± SD; Different superscript letters in column represent statistical differences ($p < 0.05$).

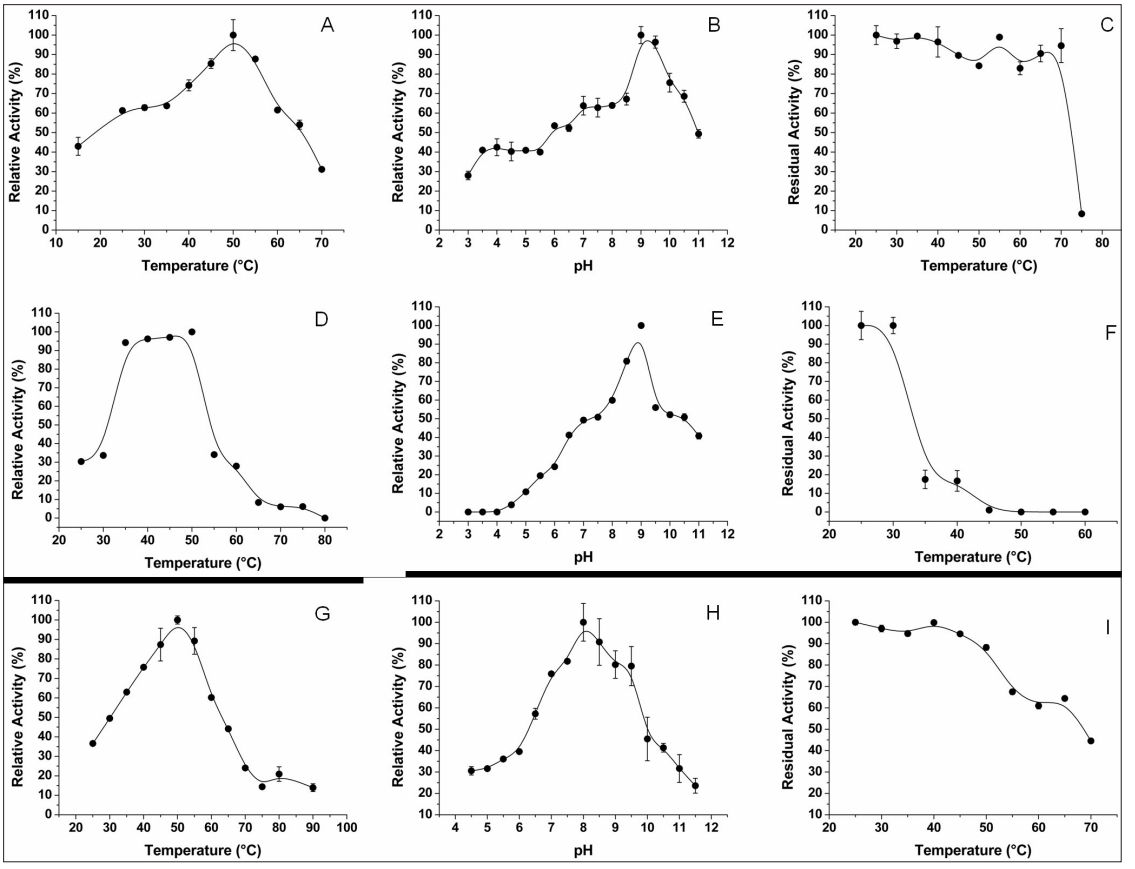

Figure 1. Effects of temperature (A, D, G), pH (B, E, H) and thermal stability (C, F, I) in the trypsin, chymotrypsin, leucine-aminopeptidase, respectively, activity present in the intestine crude extract of flounder. (n=14); Values are presented as mean ± SD.

observed at 50 °C, although more than 80% of the maximum activity was observed in a range between 40-55 °C for trypsin (Figure 1A), 35-50 °C for chymotrypsin (Figure 1D) and 40-55 °C for leucine aminopeptidase (Figure 1G). Enzymatic pepsin activity present in the crude stomach was higher at 50 °C, with over 80% of its activity being observed in the temperature range of 35 to 50 °C (Figure 2A). Amylolytic activity present in crude extract of the liver showed a peak in activity at 40 °C, while more than 80% of the activity was observed at 30 to 55 °C (Figure 2D).

Results regarding the effects of pH on enzyme activity revealed increased activity of trypsin present in crude extract of the intestine at pH values ranging from 9-10 (Figure 1B), while higher enzymatic activity of both chymotrypsin and leucine aminopeptidase occurred in crude extracts of intestine at pH values ranging from 7.5 to 9.5 (Figure 1E and 1H, respectively). Pepsin activity present in crude extract of the stomach showed higher activity at pH 2.0, but 80% of its activity occurred at pH values ranging from 1.5-3.5 (Figure 2B). Amylolytic activity present in crude extract of the liver presented a range of enzymatic activity at pH 6.5 to 8.0 (Figure 2E).

Trypsin activity present in crude extract of the intestine was stable in the temperature range of 20 to 70 °C, however all activity ceased at 75 °C (Figure 1C). Leucine aminopeptidase present in crude extract of the intestine demonstrated thermal stability from 20 to 50 °C (Figure 1I). The thermal stability of pepsin displayed enzymatic activity above 80% at temperatures between 25-45 °C (Figure 2C). Amylolytic activity present in crude extract of the liver indicated residual activities higher than 80% at temperatures between 25 to 35 °C (Figure 2F).

Table 3. Effect of a specific inhibitor of amylase activity present in the liver crude extract of juvenile Brazilian flounder *Paralichthys orbignyanus*.

Inhibidors (µg/mL)	Residual activity (% ± SD)	Inhibition (%)
Control*	100.0 ±16.1	0.0
500	74.0 ± 9.6	26.0
1000	64.0 ± 9.1	36.0

Activity without addition of inhibitors, inhibitor of α-amylase I obtained from *Triticum aestivum*. (n=14); *mU mg^{-1} protein ± SD.

4. Discussion

Carnivorous fish demonstrate higher pepsin activity than herbivorous fish (Sabapathy and Teo, 1993). The activity of pepsin found in crude extract of the stomach of flounder was higher than that observed for several species of carnivorous fish such as: *Glyptosternun maculatum* (Xiong et al., 2011) and *Thunnus alalunga* (Nalinanon et al., 2010). High activity of proteases is important for digesting shellfish organisms such as crustaceans, the principal food item for *P. orbignyanus* described by Cazorla and Forte (2005).

Amylolytic activity for *P. orbignyanus* was observed only in the liver. However, in other species, amylolytic activity has been detected in the liver, intestine, bile, stomach and pyloric ceca (Deng et al., 2010; Xiong et al., 2011). According to Krogdahl et al. (2005), the detection

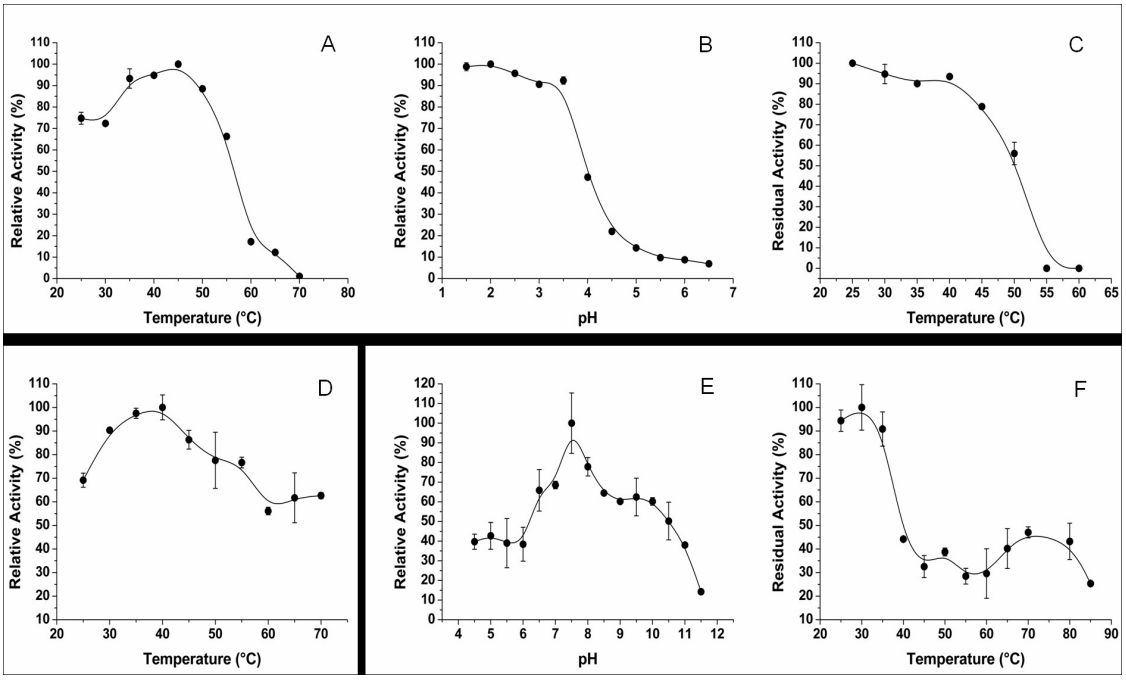

Figure 2. Effects of temperature (A, D), pH (B, E) and thermal stability (C, F) using hemoglobin and using starch as substrate activity present in the stomach crude extract of flounder and liver crude extract, respectively. (n=14) Values are presented as mean ± SD.

of amylase activity in the liver is likely due to the presence of pancreatic tissue, since α-amylase in fish is of pancreatic origin only. Amylolytic activity present in the liver may also be of intracellular origin, in which enzymes are related to glycogenolysis (Mehrani and Storey, 1993). Most carnivorous fish demonstrate low or moderate activity of α-amylase in the intestine and pancreas (Munilla-Morán and Saborido-Rey, 1996), which is related to the low-carbohydrate diet of these fish in the natural environment (Harpaz and Uni, 1999). This could be the reason why no amylolytic activity was detected in the intestine here, as *P. obignyanus* presents a carnivorous feeding habit (Norbis and Galli, 2004).

4.1. Effect of pH

pH exerts an important role on enzyme activity. Márquez et al. (2012) stated that digestion in juvenile/adult fish represents a two-phase process, with an acid phase in the stomach and an alkalinity phase in the intestine. Trypsins of aquatic organisms are active and stable between pH 7.5-10.0, and are able to hydrolyze various substrates (Vecchi and Coppes, 1996). In this study, trypsin activity presented optimal activity at pH 9.5. For *Thunnus orientalist*, the highest trypsin activity was observed at pH 8.0, while the same was observed for *Paralichthys olivaceus* at pH 7.5 (Parra et al., 2007; Kim and Jeong, 2013).

In general, chymotrypsin is more active at pH 7.5 to 8.5, and more stable at pH 9.0 (Simpson, 2000). Here, chymotrypsin in the Brazilian flounder presented the highest activity at pH 9.0. The same result was observed for the freshwater *Pterygoplichthys disjunctivus* (Villalba-Villalba et al., 2013). On the other hand, chymotrypsin from the Japanese sea bass *Lateolabrax japonicas* demonstrated the highest activity at pH 8.0 (Jiang et al., 2010). Leucine aminopeptidase showed maximun activity at pH 8.0. Parra et al. (2007) found the same result for Pacific bluefin tuna.

The optimal pH for amylase at near neutral pH found in this work has been reported in other studies (Munilla-Morán and Saborido-Rey, 1996). However, Fernandéz et al. (2001) observed amylolitic activity for five fish species in a wide pH range (4-9). Optimum amylase activity varies among fish species, as these enzymes may present more than one isoform (Krogdahl et al., 2005).

Pepsin activity present in crude extract of the stomach showed higher activity at pH 2.0, with 80% of the activity occurring at pH ranging from 1.5-3.5. Optimal pH for pepsin activity has been observed as 2.0 in albacore, wherein its activity was stable in the pH range of 2.0–5.0 (Nalinanon et al., 2010).

4.2. Effect of temperature

Trypsin was active over a wide temperature range, as well as after heat treatment of 30 minutes. Peak trypsin activity occurred at 50 °C, lower than that of other fish species: *Astroctosteus tropicus* (65 °C; Guerrero-Zarate et al., 2014) and tilapia hybrid (60 °C; Wang et al., 2010).

Leucine - aminopeptidase and chymotrypsin present in crude extract of flounder intestine showed similar results to those of trypsin. Sabapathy and Teo (1993) mentioned that leucine - aminopeptidase may compensate for the absence of trypsin activity in bream, digesting oligopeptides into free amino acids for adsorption.

Pepsin activity present in stomach crude extract was active for 30 minutes at temperatures below 45 °C, with a significant loss in activity in temperatures above 50 °C. Similar results were reported by Castillo-Yañes et al. (2004) for *Sardinops sagax caerulea*. These authors suggest that this feature is important for technological applications, especially in the extraction of collagen.

Peak amylase activity found for *P. orbignyanus* was higher (40 °C) than that found by Xiong et al. (2011) for *Glyptosternun maculatum* (30 °C). According to Parra et al. (2007), the enzymatic activity of α-amylase registered for *Thunnus orientalis* demonstrates a large temperature range (35 to 45 °C) when compared with other studies (Munilla-Morán and Saborido-Rey, 1996).

4.3. Effect of inhibitors

The use of specific inhibitors provides important data regarding the classes of enzymes present in tissue extracts, and may indicate the existence of significant structural differences in enzymes from different species. Peptidase inhibition patterns observed here were not similar to those observed in other species of fish, and neither were the degrees of inhibition. Kim and Jeong (2013) observed complete inhibition for purified trypsin using PMSF, benzamidine and TLCK in olive flounder intestine. Klomklao et al. (2007, 2010) also found complete inhibition in both TLCK and for TPCK for *Katsuwonus pelamis* and *Cololabis saira*. Castillo-Yanez et al. (2006) demonstrated 40% inhibition of trypsin using PMSF and 80% using benzamidine for pacific sardine. The commercially used inhibitors were developed based on mammalian enzymes; this may explain the different pattern of inhibition observed for Brazilian flounder and other results reported for peptidases from other species of fish and crustacean (Buarque et al., 2009, 2010; Garcia-Carreño et al., 1994; Fernández-Gimenez et al., 2002).

Amylolytic activity in crude extract of flounder liver was slightly inhibited by the inhibitor of α-amylase obtained from plant *Triticum aestivum*. This result may be associated with a low sensitivity to this inhibitor for this species. Fernandéz et al. (2001) mentions that the efficacy of α-amylase seems to vary between species. The authors further state that the inhibition rate ranged from 0% to 61% in five species of Sparidae.

High pepsin activity observed in stomach of *P. orbignyanus* supports the carnivorous – piscivorous feeding habit of this species, enabling digestion of difficult proteins. Moreover, 80% of pepsin activity was detected between pH values of 1.5 to 3.5, which is indicative of the presence of more than one pepsin isoform. Low amylase activity present in liver, and its absence in the intestine portion, supports the fact that this species may display diffulty in dealing with a high dietary inclusion of carbohydrates. These results provide additional information regarding the biology of

P. orbignyanus and should support further nutritional studies for this species.

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