Trypsin from the Processing Waste of the Lane Snapper (*Lutjanus synagris*) and Its Compatibility with Oxidants, Surfactants and Commercial Detergents

Talita S. Espósito, Marina Marcuschi, Ian P. G. Amaral,
Luiz B. Carvalho, Jr., and Ranilson S. Bezerra*

Laboratório de Enzimologia, Departamento de Bioquímica and Laboratório de
Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Brazil

A trypsin from the viscera of the lane snapper (*Lutjanus synagris*) was purified by heat treatment, fractionation with ammonium sulfate and affinity chromatography. The molecular weight of the enzyme was estimated to be 28.4 kDa (SDS−PAGE). The purified enzyme was capable of hydrolyzing the specific substrate for trypsin benzoyl-arginine-*p*-nitroanilide (BApNA) and was inhibited by benzamidine and tosyl lysine chloromethyl ketone (TLCK), synthetic trypsin inhibitors and phenylmethyl-sulfonyl fluoride (PMSF), which is a serine-protease inhibitor. The enzyme exhibited maximal activity at pH 9.0 and 45 °C and retained 100% of the activity after incubation at the optimal temperature for 30 min. At a concentration of 10 mM, activity was slightly activated by Ca^{2+} and inhibited by the following ions in decreasing order: $Cd^{2+} > Hg^{2+} > Cu^{2+} > Zn^{2+} > Al^{3+}$. The effects of Ba^{2+}, K^{1+} and Li^{1+} proved to be less intensive. Using 1% (w/v) azocasein as substrate, the enzyme revealed high resistance (60% residual activity) when incubated with 10% H_2O_2 for 75 min. The enzyme retained more than 80% activity after 60 min in the presence of different surfactants (Tween 20, Tween 80 and sodium choleate). The alkaline protease demonstrated compatibility with commercial detergents (7 mg/mL), such as Bem-te-vi, Surf and Ala, retaining more than 50% of initial activity after 60 min at 25 °C and 30 min at 40 °C. The thermostability and compatibility of this enzyme with commercial detergents suggest a good potentiality for application in the detergent industry.

KEYWORDS: Lane snapper (*Lutjanus synagris*); protease; trypsin; purification; affinity chromatography; detergent compatibility

1. INTRODUCTION

The lane snapper (*Lutjanus synagris*) is a reef-associated lutjanid distributed in the western Atlantic from the southeast of the USA to southern Brazil (*1*). This species is an important component of commercial fishery in northeastern Brazil and is captured by artisanal and commercial fishing fleets (*2*). Brazil exports the lane snapper either whole or without viscera, mainly to the USA (*3*). According to the Brazilian environmental agency IBAMA (*2*), the Brazilian production of *L. synagris* in 2006 was of 1863 tons, the majority of which was captured on the northeastern coast.

Fish processing generates large amounts of solid and liquid waste, such as heads, tails, skin, bones and viscera. This processing waste is a huge problem for the fishery industry, and its disposal has a major economic and environmental impact (*4*−*7*). The use of fish viscera as a source of biomolecules for biotechnological application is a viable alternative. This waste is regarded as one of the richest sources of proteolytic enzymes, and it is possible to

recover about 1 g of the enzyme per 1 kg of viscera (*8*−*10*). Proteases have been purified from the processing waste of various fish species, such as tambaqui, Nile tilapia, Monterey sardine, Japanese anchovy, spotted goatfish, true sardine, arabesque greenling, jacopever, elkhorn sculpin and sardine (*9*, *11*−*17*). The studies cited describe the isolation, purification and characterization of trypsin (EC 3.4.21.4), which is one of the main digestive proteases detected in the pyloric cecum and intestine of fishes.

Alkaline proteases, mainly trypsin and subtilisin, are the most important group of industrial enzymes, with applications in the leather, food and pharmaceutical industries as well as bioremediation processes (*18*, *19*). However, their major application (about 60% of all protease sold) is in the detergent industry. Biological detergents are commonly used in domestic laundry soaps because the enzymes provide the additional benefit of low temperature washes with improved cleaning performance. The addition of proteases to detergents considerably increases the cleaning effect by removing protein stains and the consumption of surface-active substances, thereby decreasing the pollution load (*20*, *21*).

Currently, subtilisins are chosen as the enzyme for detergent formulations (US patent nos. 1,240,058, 374,971, 370,482, and 4,266,031 and UK patent no. 13155937), despite not being the ideal detergent enzymes due to low thermal stability in the presence of

*Corresponding author. Mailing address: Laboratório de Enzimologia—LABENZ, Departamento de Bioquímica, Universidade Federal de Pernambuco, Cidade Universitária, Recife-PE, Brazil, CEP 50670-420. Tel: + 55-81-21268540. Fax: + 55-81-21268576. E-mail: ransoube@uol.com.br.

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detergents and short shelf life (*22*). Thus, the search for new proteases with novel properties from as many different sources as possible is desirable (*23*).

The lane snapper has a typical carnivorous digestive tract (*24*) composed of the stomach followed by the pyloric cecum, which precedes a very short intestine. The developed pyloric cecum is likely responsible for a higher amount of alkaline proteases. No information regarding the characteristics of trypsin from the intestine and pyloric cecum of the lane snapper (*Lutjanus synagris*) has been reported, despite the importance of this species for the Brazilian market (mainly the northeastern region) and exportation as an appreciated marine fish.

The aim of the present study was to purify this enzyme and test the viability of its biotechnological use in detergent formulations.

2. MATERIALS AND METHODS

2.1. Preparation of Crude Extract. Crude extracts from the intestine and pyloric cecum of *Lutjanus synagris* were prepared using the method described by Bezerra et al. (*11*). Fresh fish were collected in both the dry and rainy seasons. The intestines and pyloric ceca of these fish were collected and homogenized (Bodine Electric Company, Chicago, IL) at a proportion of 1 g of tissue for each 5 mL of 0.9% NaCl (w/v) prepared in 0.1 M Tris-HCl buffer (pH 8.0). The homogenate was centrifuged (Sorvall RC-6 Superspeed Centrifuge, NC) at 10000*g* for 10 min at 4 °C and the supernatant (crude extract) was used for the purification steps.

2.2. Precipitation of Enzymes. For the partial purification of the enzymes, the crude extracts were first submitted to a heat treatment at 45 °C for 30 min and centrifuged at 10000*g* for 10 min at 4 °C (*11*). The supernatant was used in a two-step fractionation with ammonium sulfate (40 and 80% saturation). The precipitate formed at 0–40%, and 40–80% saturation of ammonium sulfate was collected by centrifugation at 10000*g* at 4 °C for 15 min, resuspended in 0.1 M Tris-HCl buffer (pH 8.0) and dialyzed twice against 4 L of 0.01 M Tris-HCl buffer (pH 8.0) for 12 h. All steps in the enzyme precipitation process were carried out at 4 °C.

2.3. Purification of Trypsin. Aliquots of the fraction with 40–80% ammonium sulfate saturation (5 mg·mL^{-1} protein) were applied to a column of *p*-aminobenzamidine Sepharose 6B (1.5 × 0.2 cm^2). The matrix was balanced with 0.1 M Tris-HCl buffer (pH 8.0). For the elution of trypsin from the column, 0.2 M of KCl buffer (pH 2.0) was used. Fractions of 0.5 mL were collected at a flow rate of 30 mL·h^{-1}, and 30 μL of 1 M Tris-HCl, pH 9.0, was added to each tube. Fractions containing detectable protein using the Warburg and Christian method (*25*) were pooled and dialyzed twice against 2 L of 0.01 M Tris-HCl buffer, pH 8.0, for 12 h. This procedure was repeated five times to obtain 13.6 mg of the purified enzyme.

2.4. Assay for Alkaline Protease and Trypsin Activity. Protease activity was assayed using 1% azocasein (w/v) as substrate, as described by Bezerra et al. (*11*), using a microplate reader. In triplicate, using microcentrifuge tubes, 1% azocasein (w/v) was incubated with the sample for 60 min at 25 °C. Trichloroacetic acid (TCA) was added to stop the reaction, and after 15 min the tubes were centrifuged for 5 min at 8000*g*. The supernatant was then added to 1 M NaOH on a microtiter plate, and the absorbance of the mixture was measured in a microtiter plate reader at 450 nm against a blank in which distilled water was used instead of the tryspsin-like enzyme. One unit (U) of enzyme activity was defined as the amount of enzyme able to hydrolyze azocasein to produce a change of 0.001 unit of absorbance per minute.

Trypsin activity was determined using the method described by Souza et al. (*17*) with adaptations, using 8 mM benzoyl-arginine-*p*-nitroanilide (BApNA) as a substrate. *p*-Nitroaniline release was followed at 405 nm using a microtiter plate reader (Bio-Rad 680). One unit of enzyme activity was defined as the amount of enzyme required to hydrolyze 1 μmol of BApNA per minute.

2.5. Protein Determination. The protein content was determined based on the method described by Warburg and Christian (*25*), measuring the absorbance of the samples at 280 and 260 nm and using the following equation: (protein) mg/mL = Abs$_{280nm}$ × 1.5 − Abs$_{260nm}$ × 0.75. Porcine trypsin (Sigma) was used as a standard protein.

2.6. Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out using a 4% stacking gel (w/v) and 15% separating gel (w/v) (Vertical Electrophoresis System Bio-Rad Laboratories, Inc.) based on the method described by Laemmli (*26*). The gel was stained with 0.1% Coomassie Brilliant Blue (w/v) and destained with 10% acetic acid (v/v) and 25% methanol (v/v). The dialyzed trypsin (50 μg of protein) was concentrated by lyophilization.

2.7. Effect of Protease Inhibitors. The effect of inhibitors was determined based on the methods described by Alencar et al. (*27*) and Bezerra et al. (*11*), incubating trypsin from the lane snapper with different specific protease inhibitors (phenylmethylsulfonylfluoride, PMSF; tosyl lysine chloromethyl ketone, TLCK; and benzamidine) at 8 mM. After incubating the mixture for 30 min, 8 mM of BApNA was added, the residual activity was measured and the percentage of inhibition was calculated.

2.8. Kinetic Studies. The kinetic parameters V_{max} and K_m were calculated by fitting the reaction rates to a Michaelis–Menten graph using the Origin Version 6.0 software program (Microcal Software, Inc.). Activity was assayed with different final concentrations of BApNA prepared in DMSO ranging from 0.01875 to 1.8 mM. The reactions were prepared in triplicate in a 96-well microtiter plate: 30 μL of enzyme, 140 μL of 0.1 M Tris-HCl buffer (pH 9.0) and started by the addition of 30 μL of BApNA. Blanks were prepared without the enzyme.

2.9. Effect of Metal Ions. The effect of metal ions was assayed using the methods described by Souza et al. (*17*). Samples of the purified enzyme (30 μL) were added to a 96-well microtiter plate with a 10 mM solution (70 μL) of AlCl$_3$, BaCl$_2$, CdSO$_4$, CuSO$_4$, HgCl$_2$, KCl, LiCl, Pb and ZnSO$_4$. After 30 min of incubation, Tris-HCl buffer (70 μL), pH 8.0, and 8 mM of BAPNA (30 μL) were added. The *p*-nitroaniline produced was recorded in a microplate reader (Bio-Rad 680) at 405 nm after 30 min of reaction.

2.10. Effect of pH and Temperature. To evaluate the effects of pH and temperature on trypsin, activity was determined in different 0.1 M buffer solutions (phosphate, pH 6.5–7.5; Tris-HCl, pH 7.2–9.0; and NaOH–glycine, pH 8.6–11.0) at 25 °C. Temperature dependencies of enzyme activity were determined at pH 9.0 and various temperatures (25–75 °C). Thermal stability was recorded at 25 °C after preincubating the enzyme for temperatures ranging from 25 to 60 °C at intervals of 5 °C at pH 9.0 for 30 min.

2.11. Effect of Oxidizing Agents. Hydrogen peroxide stability of the proteases from the lane snapper was investigated by incubating samples (600 μL) with H$_2$O$_2$ (600 μL) at concentrations of 5%, 10% and 15% at 40 °C. Samples (150 μL) were withdrawn at 15, 30, and 75 min intervals to establish their activities (duplicates) on azocasein and to compare them to the nontreated sample (*21*).

2.12. Effect of Surfactants. Stability with regard to ionic (SDS and sodium choleate) and nonionic surfactants (Tween 20 and Tween 80) was investigated by incubating the purified enzyme in a 1% concentration of surfactant solution (w/v) for 30 and 60 min at 40 °C, after which enzyme activity was assayed (*21*).

2.13. Compatibility with Commercial Detergents. The trypsin from the lane snapper at a concentration of 0.20 mg mL^{-1} was incubated at 40 °C with commercial detergents: Ala (Procter & Gamble); Bem-te-vi (Alimonda); Omo Multi Ação (UniLever) and Surf (UniLever) in a final concentration of 7 mg mL^{-1}. Samples were collected at 10 min intervals for 60 min. The residual proteolytic activity in each sample was determined at 25 °C and compared with the control sample incubated in Tris-HCl, pH 9.0, at 40 °C. Protease activity was assayed using 1% azocasein (w/v) as substrate, as described by Bezerra et al. (*11*), using a microplate reader. In triplicate, using microcentrifuge tubes, 1% azocasein (w/v) was incubated with the sample for 60 min at 25 °C. Trichloroacetic acid (TCA) was added to stop the reaction and, after 15 min, the tubes were centrifuged for 5 min at 8000*g*. The supernatant was then added to 1 M of NaOH on a microtiter plate and the absorbance of the mixture was measured in a microtiter plate reader at 450 nm against a blank in which distilled water was used instead of the tryspsin-like enzyme. One unit (U) of enzyme activity was defined as the amount of enzyme able to hydrolyze azocasein to produce a change of 0.001 unit of absorbance per minute.

3. RESULTS AND DISCUSSION

In the present study, a trypsin was purified from the intestine and pyloric cecum of the lane snapper in three steps: heat treatment, ammonium sulfate precipitation and affinity chromatography. The enzyme was purified 63.85-fold from the crude extract

Table 1. Purification of Trypsin from the Intestine and Pyloric Cecum of the Lane Snapper

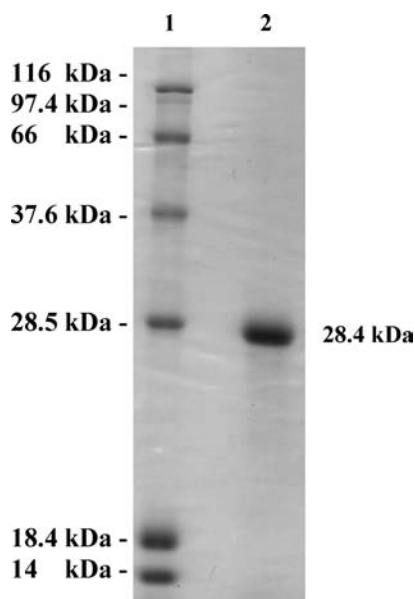| purifn steps	total act. (U)	total protein (mg)	sp act. (U/mg protein)	recovery (%)	purifn (fold)
crude extract	121,410.99	142.71	850.76	100.0	1.0
step 1: heat treatment	124,747.25	61.29	2035.36	102.7	2.4
step 2: ammonium sulfate precipitation					0.3
F1 $(NH_4)_2SO_4$ (0−40%)	664.84	2.85	233.58	0.5	0.3
F2 $(NH_4)_2SO_4$ (40−80%)	102.626.37	49.50	2073.26	84.5	2.4
step 3: affinity chromatography	109,076.92	2.01	54,317.8	89.84	63.85

Figure 1. SDS−PAGE of intestine and pyloric cecum purified trypsin from the lane snapper; lane 1, standard proteins; lane 2, pool collected by *p*-aminobenzamidine Sepharose 6B; molecular weight was estimated using the protein standards galctosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (37.6 kDa), carbonic anhydrase (28.5 kDa), myoglobin (18.4 kDa) and lysozyme (14 kDa).

Figure 2. Michaelis−Menten plot for trypsin kinetics; BApNA concentrations (1.8−0.01875 mM); R^2 = 0.99.

(**Table 1**). Protein purification strategies are generally of high cost and time-consuming. Indeed, this is an important limiting factor for the commercial use of fish processing waste as a source of proteases. However, the procedures employed in the present study are of relatively low cost and the raw material (fish viscera) has little or no cost at all, often being discarded. The present study also confirms the previously observed efficiency of heat treatment and ammonium sulfate precipitation as steps in purifying trypsins from tropical fish (*9, 11, 12, 17*). *p*-Aminobenzamidine is a highly effective and specific ligand for purification of trypsin (*13, 28, 29*) and was used effectively in the present study as the final purification step. This technique was able to purify a protease (only one band in SDS−PAGE) with an estimated molecular weight of 28.4 kDa (**Figure 1**). Similar results have been found for trypsin from other fish: *Oreochromis niloticus* (23.5 kDa) (*11*), *Sardina pilchardus* (25 kDa) (*12*), *Engraulis japonica* (24 kDa) (*14*), *Sardinops melanostictus, Pleuroprammus azonus* (24 kDa) (*15*), *Sebastes schlegelii, Alcichthys alcicornis* (24 kDa) (*16*) and *Pseudupeneus maculatus* (24.5 kDa) (*17*).

As this protease was obtained from a biological source that lives in an open system (marine fish), it is subject to seasonal changes that could be reflected in many aspects of its physiology, including protease synthesis in the digestive tract. In tropical northeastern Brazil, the seasonal effect is minimized; the water temperature generally ranges from 24 to 28 °C throughout the year. Moreover, fish diet may vary seasonally in tropical coastal environments, changing in both quality and quantity, mainly with
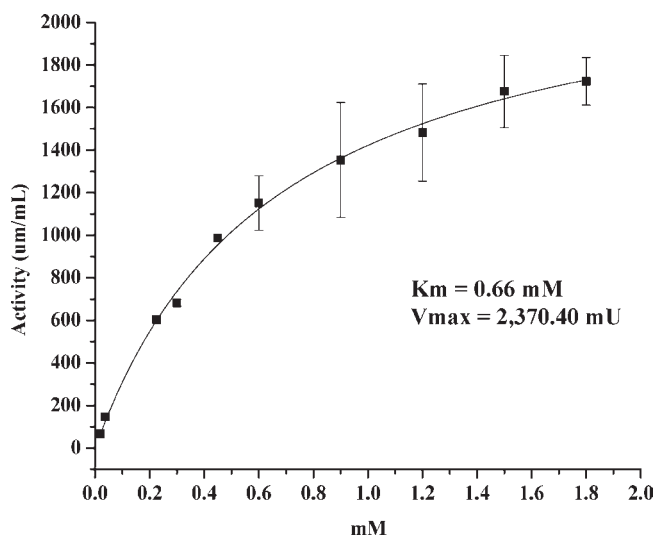
oscillation of salinity generated during the rainy (pluviometric index above 200 mm per month) and dry (pluviometric index < 50 mm per month) seasons. The lane snapper is known to be a generalist carnivore and trophic opportunist, preying on a wide range of food sources (*30, 31*). Despite the different environmental conditions (e.g., salinity, temperature and food availability) between the dry and rainy seasons, no differences were observed in trypsin activity in the lane snapper (54.3 and 54.2 U/mg, respectively).

The trypsin from the lane snapper was inhibited by the serine proteinase inhibitor PMSF (45%) and exhibited strong inhibition in the presence of TLCK (81.22%) and benzamidine (77.75%), which are synthetic trypsin inhibitors. These results indicate that this enzyme is probably a trypsin. Similar results are reported in a study by Bezerra et al. (*11*), in which an alkaline protease from Nile tilapia intestine was also inhibited by PMSF, TLCK and benzamidine (approximately 55, 100 and 87.5%, respectively).

The BApNA hydrolysis rates obeyed the Michaelis−Menten kinetics model regarding the concentration of substrate examined in the present study (**Figure 2**). K_m and V_{max} values for the trypsin from the lane snapper acting on BApNA were 0.66 ± 0.044 mM and 2370.40 ± 65.93 mU, respectively. This K_m value is similar to that reported by Martinez et al. (*32*) for trypsin from anchovy (*Engraulis encrasicolus*), which is a pelagic marine fish that feeds on plankton, unlike the lane snapper, which is a reef-associated marine fish that feeds on small fish, bottom-living crabs, shrimp, worms, gastropods and cephalopods. In comparison to other marine fish, this value is lower than trypsin from the spotted goatfish (*Pseudupeneus maculates*, K_m = 1.94 and 1.82) and higher than trypsin from Monterey sardine (*Sardinops sagax caerulea*, K_m = 0.051), grey triggerfish (*Balistes capriscus*, K_m = 0.068 mM), other species of anchovy (*E. japonica*, K_m = 0.049) and red snapper (*Lutjanus vitta*, K_m = 0.507 mM) (*17, 13, 33−35*). The K_m value for the lane snapper was also different from trypsin found

Table 2. Ion Effect on the Trypsin from the Intestine and Pyloric Cecum of the Lane Snapper

ion (10 mM)	residual activity $\pm$ SD (%)
Al^{3+}	57.69 ± 8.81
Ba^{2+}	72.11 ± 1.14
Ca^{2+}	115.25 ± 1.24
Cd^{2+}	6.07 ± 0.8
Cu^{2+}	13.09 ± 0.76
Hg^{2+}	11.39 ± 1.97
K^{+}	90.52 ± 4.10
Li^{+}	96.02 ± 5.10
Zn^{2+}	56.36 ± 3.11

in freshwater fish: lower than Nile tilapia (*Oreochromis niloticus*, $K_m = 0.772$) and higher than carp (*Cyprinus carpio*, $K_m = 0.039$) (*11, 36*).

Table 2 displays the effects of the ions on trypsin activity from the intestine of the lane snapper. This enzyme proved sensitive to ions, mainly Al^{3+}, Cd^{2+}, Cu^{2+}, Hg^{2+} and Zn^{2+}. It is known that Cd^{2+}, Co^{2+} and Hg^{2+} act on sulfhydryl residues in proteins (*37*). The inhibition caused by these metal ions suggests the relevance of sulfhydryl residues for the catalytic action of this protease (*11*). In other studies on tropical fish proteases, these ions also inhibited trypsin activity in samples from the intestine of the Nile tilapia and the pyloric cecum of the spotted goatfish (*11, 17*). Despite the effects of Cd^{2+}, Zn^{2+} and Al^{3+} (respectively, 6.07%, 56.39% and 57.69%) on enzyme activity from the intestine of the lane snapper, its influence is lesser than that recorded for the Nile tilapia enzyme (respectively, 3.0%, 30.5% and 0%). However, the effects of Cu^{2+} and Hg^{2+} (respectively, 13.09% and 11.39%) proved to be stronger than those recorded for the Nile tilapia enzyme (respectively, 43.9% and 38.0%). The inhibition effects of Li^{+}, K^{+} and Ba^{2+} on lane snapper enzyme activity were less intensive than those displayed by the ions mentioned above. Only Ca^{2+} increased enzyme activity. This result suggests that this enzyme possesses the primary calcium-binding site found in mammalian pancreatic trypsin and trypsin from other fish (*38, 39*).

Enzymes used in detergent formulations should have high optimal pH and thermal stability (*21*), which are characteristics of the protease purified from the lane snapper (**Figure 3**) and other reef-associated marine fish, such as the cunner (*Tautogolabrus adspersus*) (*39*). Compared to bacterial enzymes used as additives in detergent (Alcalase, Savinase, Esperase, Novozymes, Denmark; Maxatase, Gist-brocades, The Netherlands), the enzyme from the lane snapper has a lower optimal temperature activity (**Figure 3A**), but retained 100% of its activity after 30 min of incubation at 45 °C, while most of the bacterial enzymes used have low thermal stability above this temperature (**Figure 3B**).

The effect of pH on trypsin activity is illustrated in **Figure 3C**. The enzyme hydrolyzed BApNA effectively at alkaline pH with optimal activity at pH 8.0−10.5, which is similar to that of other fish trypsins (*9, 11, 17, 27, 33, 40−44*). This characteristic likely contributes to its physiological role in intestinal tissue, where pH is high (*37*), and is a relevant aspect that enables its use in detergent formulations, as the pH of laundry detergents is commonly alkaline (*23*).

The performance of proteases in detergent is influenced by several factors (e.g., pH, ionic strength, washing temperature, detergent composition, bleach systems and mechanical handling). To test the compatibility of this enzyme, its proteolytic activity was assayed under different conditions resembling these factors. Oxidizing agents are commonly found in bleach based detergent. Oxidants act on proteinaceous stain by oxidizing the methionine residues to sulfoxides, thus denaturing it and facilitating the wash (*23*). However, oxidizing agents can also affect protein structure and
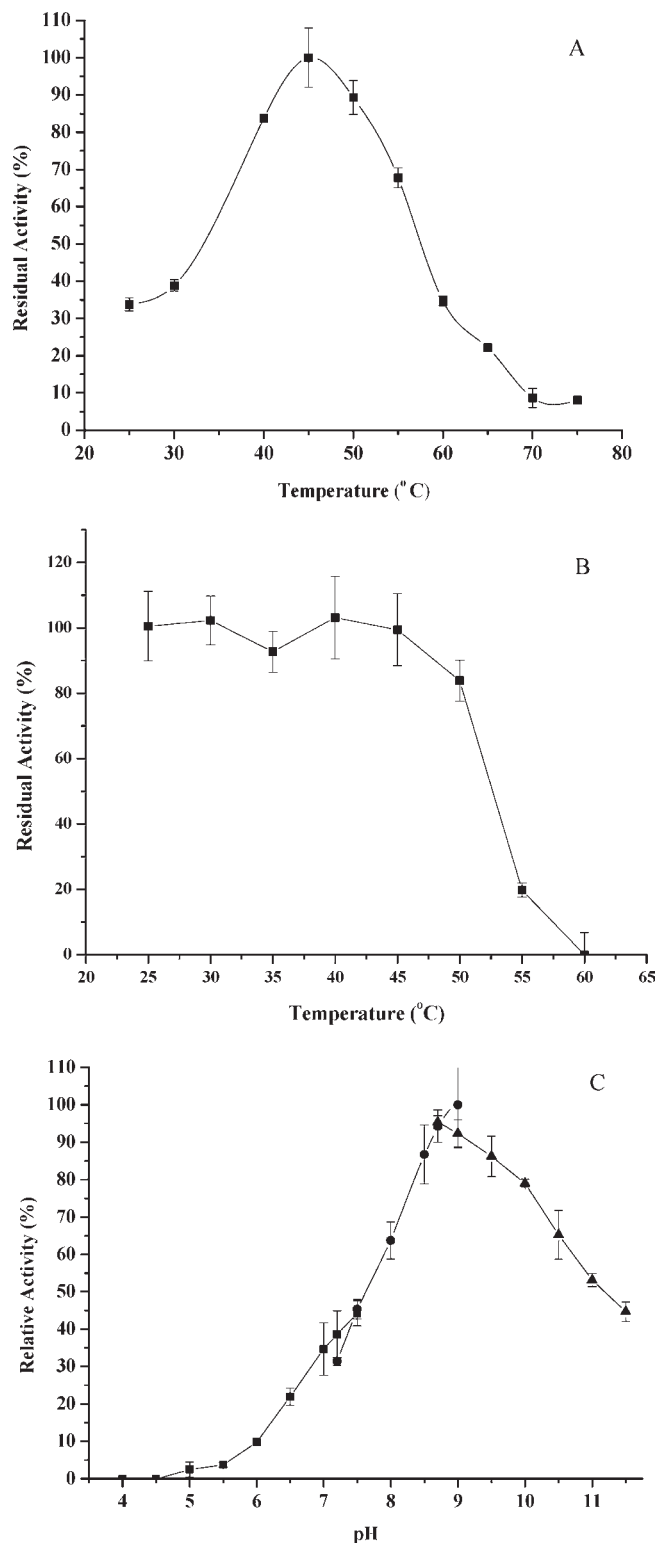

Figure 3. Effect of temperature (**A**), thermal stability (**B**) and pH (**C**) on trypsin from lane snapper intestine and pyloric cecum; The purified enzyme collected from *p*-aminobenzamidine sepharose 6B was incubated for 30 min with BApNA (8 mM) at the indicated temperatures and at different 0.1 M buffer solutions: phosphate (■), Tris-HCl (●) and NaOH-glycine (▲). The products were measured at 405 nm. Thermal stability was determined by assaying activity (25−75 °C) after preincubation for 30 min at the temperatures indicated. All the experiments were carried out in triplicate. Values (mean $\pm$ SD) are expressed as percentage of highest activity.

enzymatic activity. Thus it is crucial to assess the enzyme stability in the presence of these chemicals, as a requirement for its use in
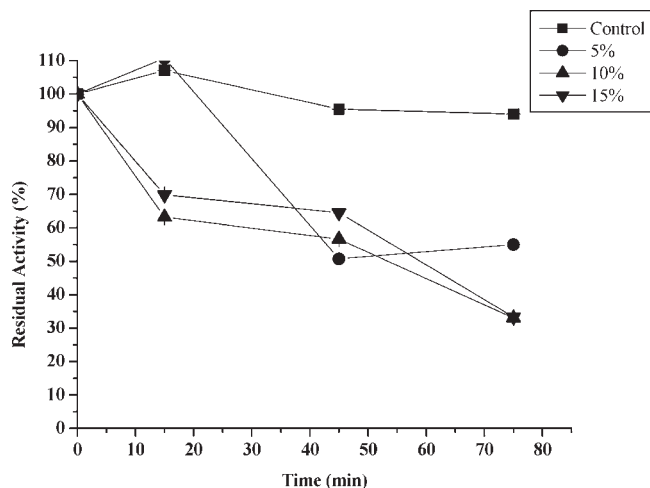
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Figure 4. Inactivation curve of H$_2$O$_2$ on the protease purified from the pyloric cecum and intestine of *L. synagris* precipitated with 40−80% ammonium sulfate. Enzyme preparations were incubated at pH 9.0 and 40 °C, with H$_2$O$_2$ at concentrations of 5% (●), 10% (▲) and 15% (▼). Samples were withdrawn at time intervals; their activities (duplicates) were established using azocasein as substrate and compared to the nontreated sample (■).

Table 3. Effect of Surfactants on the Protease Purified from the Intestine and Pyloric Cecum of the Lane Snapper[a]

surfactants (1% w/v)	residual act. (%)	
	after 30 min	after 60 min
sodium choleate	71.8 ± 10.8	99.1 ± 12.3
SDS	61.1 ± 10.6	3.6 ± 3.1
Tween 20	84.0 ± 5.8	94.5 ± 9.4
Tween 80	133.6 ± 22.9	81.8 ± 6.9

[a] Values are expressed in ± standard deviation; *n* = 3; the specific enzyme activity of the control sample (100%) was 50,000 U/mg using azocasein as substrate.

detergent formulations. **Figure 4** displays the oxidant stability of the lane snapper protease in the presence of hydrogen peroxide. The peroxide inactivation curve indicates that the lane snapper digestive protease is stable even at high concentrations of H$_2$O$_2$ (15% v/v). These results are similar to those found for alkaline proteases from the grey triggerfish, tambaqui and carp (*33,43,44*). As bleach stability has only been attained by site-directed mutagenesis (*45, 46*) or protein engineering (*47*) of bacterial enzymes, this characteristic can be considered relevant from the biotechnological standpoint.

Enzyme activity of the trypsin from the lane snapper was analyzed in the presence of nonionic (Tween 20 and Tween 80) and ionic (sodium choleate) surfactants using azocasein as substrate. **Table 3** shows the high stability of this enzyme when incubated with these surfactants. Tween 80 increased protease activity by 33.6% after 30 min of incubation. Alkaline proteases from other fish, like grey triggerfish, tambaqui and carp, have also been stable to nonionic surfactants (*33, 43, 44*). After 60 min of incubation with Tween 20 and sodium choleate, the protease retained 94.5% and 99.1% of its initial activity. Only sodium dodecyl sulfate (SDS) was capable of strongly inhibiting the enzyme after 60 min. Ionic surfactants, such as SDS, can bind to globular proteins, like trypsin, and form small aggregates around its polypeptide backbone, thus denaturing the protein native structure (*48*). Therefore, most enzymes from other fish are also not very stable in the presence of SDS and other similar surfactants (*33, 43, 44*).

The alkaline protease from *L. synagris* demonstrated stability and compatibility with a wide range of commercial detergents at
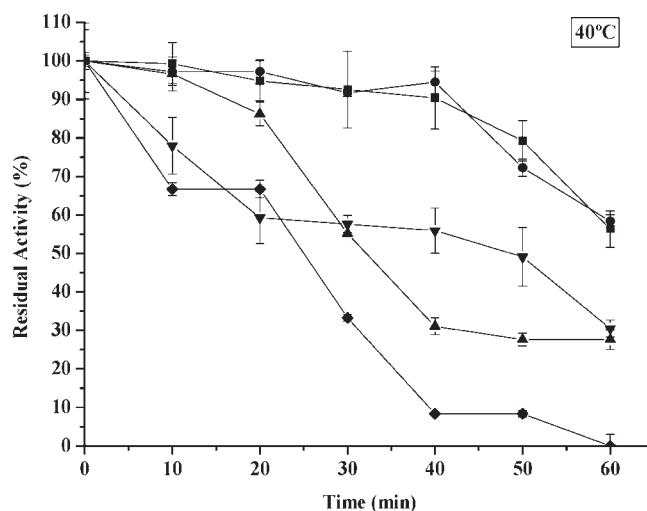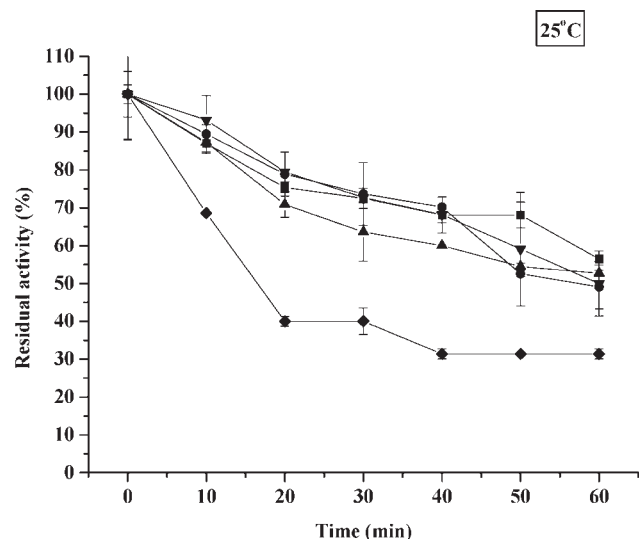

Figure 5. Stability of the protease purified from the pyloric cecum and intestine of *L. synagris* in commercially available detergents. Protease (0.2 mg mL^{-1}) was incubated at 25 and 40 °C in presence of detergents at 7 mg mL^{-1}. Activity of control sample devoid of any detergent incubated under similar conditions (■); Surf (●); Ala (▲); Bem-te-vi (▼); Omo Multi-Ação (♦).

25 and 40 °C (**Figure 5**). The enzyme retained about 50% of its activity after 1 h in the presence of the Surf, Ala and Bem-te-vi detergents at 25 °C. After 1 h at 40 °C, the enzyme retained more than 60% of its activity in the Surf detergent and retained about 50% of its activity in the presence of Ala and Bem-te-vi after 30 min. The Omo detergent inhibited the activity of the enzyme, with about 70% loss of activity after 30 min of incubation at 40 °C. Espósito et al. (*43*) found that tambaqui proteases retained more than 50% of their activity when incubated with the Ala, Bem-te-vi and Omo detergents for 1 h at 40 °C. Maximal stability was observed with the Surf detergent, as the enzyme retained 73.70% of its activity. Studies on the compatibility of proteases from the fungi *Conidiobolus coronatus* and *Nocardiopsis* sp. in the presence of detergents demonstrate activity retention of 64% and 90%, respectively (*46, 21*). Studies on alkaline proteases from species of *Bacillus* describe retention of more than 70% of activity after 1 h at 40 °C (*47*).

Enzymes from fish viscera contribute toward sustainable development by being isolated from waste that is usually discarded. In the search for an alkaline-stable protease for use in the detergent industry and based on the results of the present study, trypsin from

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L. synagris viscera was easily purified through affinity chromatography with high recovery (about 90%). It therefore demonstrates good potential for application in laundry detergents. Moreover, the economy in production would make this enzyme suitable for low-cost operations in the industry.

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