Digestive alkaline proteases from the Tunisian barbell (*Barbus callensis*): Characterization and application as a detergent additive, in chicken feather-degradation and as a dehairing agent

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Abstract

Alkaline crude enzymes from the viscera of the Tunisian barbel (*Barbus callensis*) were extracted and characterized. Proteolytic crude extract from barbel viscera was active and stable in alkaline solution. The optimum pH and temperature were 11.0 and 55 °C, respectively, using casein as a substrate. The crude alkaline protease was extremely stable in the pH range of 5.0-12.0. Zymography activity staining using casein as a substrate showed the presence of at least five distinct proteases. The crude alkaline proteases showed stability towards various surfactants, bleach agents and compatibility with some commercial detergents. Alkaline proteases from the viscera of the barbel were tested in chicken feather-degradation and showed important feather degrading activity. Complete solubilisation of whole feathers was observed after 24h of incubation at 50°C. Additionally, crude alkaline protease demonstrated powerful capabilities of hair removal from skin and the collagen, the major leather-forming protein, was not significantly degraded. Considering its promising properties, alkaline crude enzyme from the viscera of the Tunisian barbel may be considered as a potential candidate for future use in several biotechnological processes.

Keywords: Digestive protease, *Barbus callensis*, Detergent, Keratine-degradation, Dehairing function.

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Introduction

In several major fish-producing countries by-products of seafood harvesting comprise about 50% of the entire harvest. These materials, which cause an environmental problem to the fisheries industries, constitute an important source of proteins and enzymes, especially digestive proteases. In recent years, recovery and characterisation of proteolytic enzymes, from the internal organs of fish, have been reported and this has led to the emergence of some interesting new applications of these enzymes (Shahidi and Kamil, 2001). Proteases account for nearly 60% of the total world-wide enzyme sales and then represent one of the most important enzymes from an industrial point of view (Joo and Chang, 2006). Proteases have diverse applications in a wide range of industries, such as the detergent, food, pharmaceutical, leather and silk industries (Gupta *et al*., 2002). This is mainly due to high tissue concentration of enzymes in these animals (Simpson *et al*., 1991) and partly due to a better understanding of enzymes and their commercial availability from marine sources (Stefansson and Steingrimsdottir, 1990; Han, 1993).

The detergent industry has now emerged as a major consumer of several hydrolytic enzymes acting at alkaline pH. Detergent proteases account for at least a quarter of all protease sales throughout the world (Anwar and Saleemuddin, 1998; Gupta *et al*., 2002). They are primarily used as detergent additives since they are biodegradable and increase performance/cost ratios (Gupta *et al*., 2002). Moreover, although the enzymes selected for detergent composition have been subtilisins, they are not the ideal enzymes for detergents due to their low thermal stability, the presence of detergents and also because of their short shelf life (Samal *et al*., 1990). Thus, it is relevant to search for proteases from new sources presenting high thermal stability, alkaline activity and more compatibility with washing systems (Banerjee *et al*., 1999). These properties have already been observed in trypsin-like enzymes.

Leather processing is an important economic activity in many developing countries. The leather-making industry has a negative image due to its production of pollution. Pre-tanning leads to inefficiency and ecological imbalance. There is a need to revamp leather processing by removing this method. This approach can successfully use enzymes instead of chemicals (Swarna *et al*., 2009; Valeika *et al*., 2009). Enzyme-assisted dehairing reduces the pollution load to some extent and is currently being employed in some parts of the world (Valeika *et al*., 2009; Jian *et al*., 2011). A wide range of proteases are used in leather processing, such as neutral proteases in soaking, alkaline proteases in dehairing and acid proteases in bating (Aravindhan *et al*., 2007; Jian *et al*., 2011). However, most proteinases from marine organisms are extracellular digestive enzymes with characteristics differing from homologous proteases from warm-blooded animals (De Vecchi and Coppes, 1996). They are more active catalysts at relatively low temperature, compared with similar enzymes from mammals, thermophylic

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organisms and plants (Simpson and Haard, 1987).

Tunisian barbel, *B. callensis*, is a group of small carp-like fish that has a wide distribution in northern and central Tunisia. It is relatively important in the fish catches of Tunisia. In Tunisia, barbel (*B. callensis)* catches were about 80 tonnes in 2010 (FAO, 2010). So far, no information regarding digestive enzymes from the barbel has been documented.

The present paper describes the extraction and characterization of alkaline proteases from *B. callensis* viscera. Their compatibility with commercial laundry detergents, oxidants and surfactants agents, their dehairing capacity, as well as the ability of alkaline proteases, from the viscera of the barbel, to accomplish the whole keratin-degradation of various keratinacious wastes is also investigated.

Materials and methods

Reagents

N-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-p-nitroanilide (SAAPNA), casein sodium salt from bovine milk, glycine, ammonium sulphate and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis MO, USA). Soybean trypsin inhibitor (SBTI) and Na-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) were obtained from Fluka Biochemica (USA). Sodium dodecyl sulphate (SDS), N,N,N,N'-tetramethyl ethylenediamine (TEMED) and Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories (Mexico). Tris (hydroxymethyl) amino-methane was obtained from Panreac Quimica SA (Spain). All other reagents were of analytical grade.

Barbel viscera

The barbel samples used in the present work were obtained from Barrage SIDI SAAD, Tunisia. The samples were packed in polyethylene bags, placed on ice (sample/ice ratio of about 1:3 (w/w)), and transported to the laboratory. The internal organs were separated and then stored in sealed plastic bags at -20°C.

Preparation of crude alkaline protease extract

Viscera from barbel (150 g) were rinsed in distilled water and homogenised for 5 min with 150 mL of extraction buffer A (10 mM Tris-HCl, pH 8.0) using a Moulinex R123 homogenizer. The homogenate was centrifuged at 8,500g for 30 min at 4°C using a refrigerated centrifuge. The pellet was discarded and the supernatant was collected and used as the crude protease extract.

Protease activity assay

Protease activity in the alkaline crude extract was measured by the method of Kembhavi *et al*. (1993) using casein as a substrate. A 0.5 mL aliquot of the crude enzyme extract, suitably diluted, was mixed with 0.5 mL of 100 mM Glycine-NaOH (pH 11.0) containing 1% (w/v) casein, and incubated for 15 min at 55 °C. The reaction was stopped by addition of 0.5 mL 20% (w/v) trichloroacetic acid. The mixture was allowed to stand at room temperature for 15min and then centrifuged at 10,000g for 15min to remove the precipitate. The acid soluble material was estimated spectrophotometrically at 280nm. A standard curve was generated using

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solutions of 0-50 mg L^{-1} tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate 1 µg tyrosine per min under the experimental conditions used.

Detection of protease activity by zymography

Casein-zymography was performed on native-PAGE according to the method of Garcia-Carreno *et al*. (1993).

Effect of pH on activity and stability of barbel proteases

The effect of pH was determined with casein 1 % (w/v) as a substrate. Protease activity was studied over a pH range of 5.0-12.0 at 50°C. For the measurement of pH stability, barbel crude extract was pre-incubated in buffers at different pH in the range of 5.0-12.0 for 1 h at 30°C. Aliquots were withdrawn and residual proteolytic activities were determined under standard assay conditions. The following buffer systems were used: 100 mM sodium acetate buffer for pH 5.0-6.0; 100 mM potassium phosphate buffer for pH 7.0; 100 mM Tris-HCl buffer for pH 8.0; 100 mM glycine-NaOH buffer for pH 9.0-11.0 and 100 mM KCl-NaOH buffer for pH 12.0-13.0.

Optimum temperature and thermal stability of goby proteases

The effects of temperature on barbel protease activities were studied from 30 to70°C using casein as a substrate for 15 min in 100 mM glycine-NaOH buffer, pH 11.0. Thermal inactivation was examined by incubating the crude protease extract for 60 min at different temperatures. Aliquots were withdrawn at desired time intervals to test the remaining activity at standard assay conditions. The non-heated enzymes were considered as control (100 %).

Effects of enzyme inhibitors on protease activity

The effects of various enzyme inhibitors on protease activity were studied using PMSF, SBTI, benzamidine, pepstatin A, □-mercaptoethanol, EDTA, DNTP, TPCK, and TLCK. The crude protease extract was preincubated with inhibitors for 30 min at 30 °C, and then the remaining enzyme activities were estimated using casein (1 %) as a substrate at pH 11.0 and 55°C. The activity of the crude enzyme assayed in the absence of inhibitors was taken as control.

Effects of metal ions

The effects of various metal ions (5 mM) on enzyme activity were investigated by adding monovalent (Na^+ or K^+) or divalent metal ions (Ca^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Ba^{2+}, Mg^{2+}) to the reaction mixture. The effect of $CaCl_2$ concentration on trypsin activity was also studied. The activity of the crude enzyme in the absence of metal ions was chosen as 100 %.

Effect of NaCl concentration on barbel proteases

Enzyme activity was assayed in the presence of NaCl at various concentrations (0-30 % (w/v)). The relative enzyme activity was determined at 55°C for 15 min, using casein as a substrate.

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Effect of oxidizing agents, surfactants and detergents on proteases activities

The effects of some surfactants (Triton X-100, Tween 20, Tween 80 and SDS) and oxidizing agents (sodium perborate) on barbel proteases stability were studied by pre-incubating enzymes for 1 h at 30°C. The residual activities were measured at pH 11.0 and 55°C. The activity of the enzyme without any additive was chosen as 100 %.

The stability of alkaline proteases in the presence of solid and liquid laundry detergents was examined by incubating the crude protease extract for 1 h at 30 and 40 °C with various common detergent preparations, and then the residual activities were determined. The enzyme activity of a control sample (without detergent), incubated under the similar conditions, was chosen as 100 %. The solid detergents used were Dixan (Henkel-Spain), Nadhif (Henkel-Alki, Tunisia), Ariel (Procter and Gamble, Suisse), New Det (Sodet, Tunisia) and Axion (Colgate-Palmolive, France). The detergents were diluted in tap water to give a final concentration of 7 mg/ml to simulate washing conditions. The liquid detergents used were Dixan (Henkel-Spain), Persil (Unilever, France) and Ariel (Procter and Gamble, Suisse) that were diluted 100-fold in tap water to simulate washing conditions. The endogenous proteases contained in these detergents were inactivated by heating the diluted detergents for 1h at 65°C prior to the addition of the crude protease extract.

Keratin-degradation determination

The keratin-degradation ability of alkaline proteases from the viscera of the barbel was investigated using whole chicken feather as keratinacious materials. Chicken feathers were collected from a local slaughter-house, rinsed to remove excess blood, and autoclaved to be sterilized. Disintegration of whole chicken feathers was assessed by incubation with the crude enzyme (5,000 U casein activities) at different temperatures ranging from 30 to 60 °C.

Dehairing test

Pieces of bovine skin with hair (5cm×5cm) were incubated with 5000U mL^{-1} of alkaline proteases from the viscera of the barbel at 25, 30 and 37°C and shaken at 150 rpm in a shaking incubator. After 24 h of incubation, skins were taken out and the hair was gently pulled with hand. The dehairing efficacy was assessed according to the depilated area of the skin at the end of the process and the quality of the dehaired skin was estimated according to the appearance observed by the naked eye after treatment.

Statistical analysis

All data were submitted to Analysis of Variance (ANOVA) and differences between means were evaluated by Duncan's Multiple Range Test. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 10.0 (Chicago, Illinois, USA). Differences were considered significant at $p<0.05$.

Results

Characterization of viscera enzyme extract

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SDS-PAGE and zymography of crude alkaline proteases

A preliminary study on the characterization of the crude enzyme extract was carried out. In order to estimate the number of proteases in the alkaline crude enzyme extract, the sample was separated by SDS-PAGE, and then the activity was revealed by casein zymogram activity staining. Fig. 1 shows the separation of various protease bands.

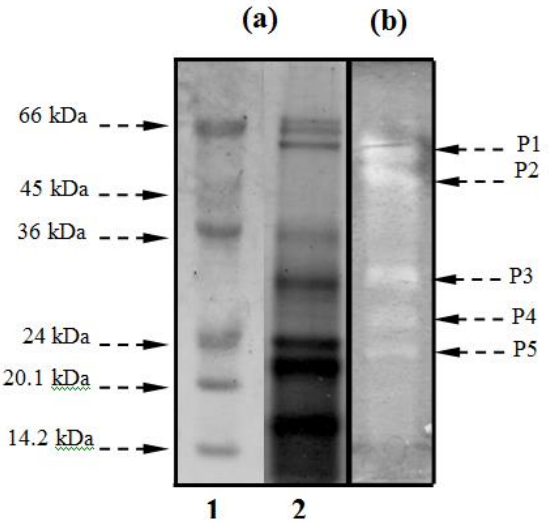


Figure 1: (a) SDS-PAGE of alkaline proteases from the viscera of *Barbus callensis*. Lane 1: standard proteins marker of different molecular weights; lane 2: crude enzyme extract; (b) zymogram of the crude extract.

The crude enzyme extract showed at least five clear zones of protease activity with different molecular weights. This result suggests that at least five major proteinases were present in Tunisian barbel viscera.

Effect of pH on activity and stability of barbel crude protease extract

The pH activity profile of the crude protease extract is shown in Fig. 2a. The proteases of the barbel viscera displayed maximum activities at a pH range of 10.0 to 11.0, with an optimum around pH 11.0. The relative activities at pH 8.0, 9.0, 10.0 and 12.0 were about 79.8, 90, 98.5 and 78%, respectively, of that at pH 11.0.

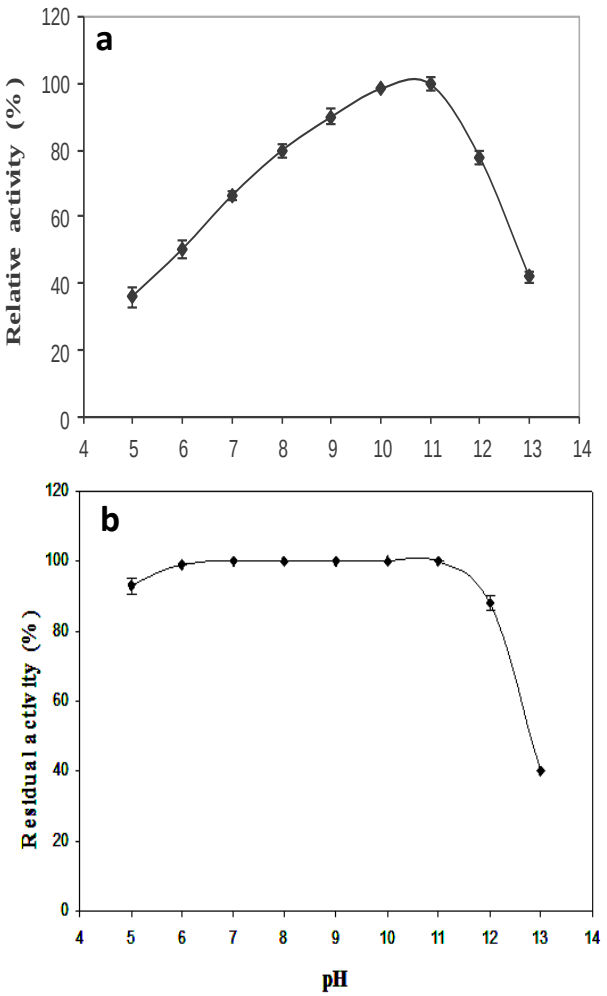


Figure 2: Effect of ph on activity (a) and stability (b) of barbel crude extract. The protease activity was assayed in the pH range 5.0-12.0 using buffers of different pH values at 50 °C.

As displayed in Fig. 2b, the crude enzyme extract is highly stable over a wide pH range, maintaining more than 90% of its original activity between pH 5.0 and 11.0 and 88 % at pH 12 after 1 h incubation at 30 °C.

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Effect of temperature on the activity and stability of the visceral crude enzyme extract

The effect of temperature on protease activity was determined by assaying enzyme activity at different temperatures (Fig. 3a). The crude extract from barbel viscera was active at temperatures from 40 to 60 °C with an optimum around 55 °C. The relative activities at 50 and 60°C were about 95 and 90 %, respectively.

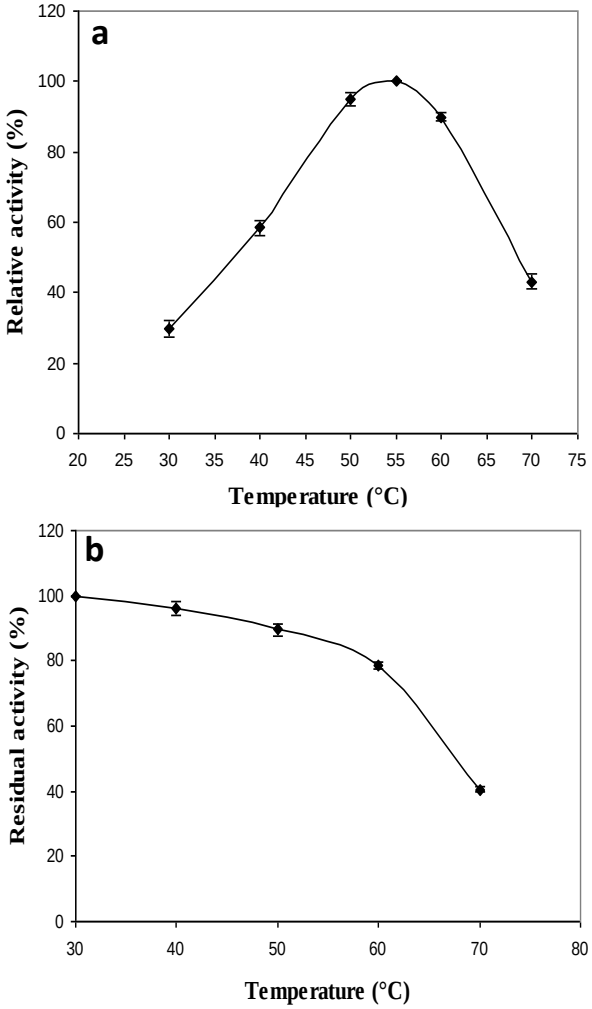


Figure 3: Effect of temperature on activity (a) and stability (b) of barbel crude extract. The temperature profile was determined by assaying protease activity at temperatures between 30 and 70°C.

The thermal stability profile showed that alkaline enzyme extract is fully active for at least 60 min at 30 and 40 °C. The alkaline crude extract retained more than 78 and 40 % of its initial activity after 60 min incubation at 50 and 60 °C, respectively (Fig. 3b).

Effects of enzyme inhibitors on barbel crude protease extract activity

In order to determine the nature of barbel protease, the effect of a variety of enzyme inhibitors, such as chelating agent and a specific group reagent on barbel proteases activity was investigated (Table 1). The relative inhibition of PMSF, soybean trypsin inhibitor and benzamidine towards the proteinases from barbel viscera indicated the presence of serine proteinases, especially trypsin.

Table 1: Effect of various enzyme inhibitors on the activity of barbel crude protease extract.

Inhibitors	Concentration (mM)	Remaining activity (%)
None	-	100
SBTI	1 mg mL^{-1}	77.41 ± 1.2
PMSF	1	80.15 ± 1.13
Benzamidine	1	79.60 ± 0.9
TLCK	2	63.98 ± 1.8
TPCK	2	83.33 ± 1
EDTA	2	100
β-mercaptoethanol	2	100
DNTP	1	100
Pepstatine A	0.1	100

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Effects of ions

The effects of some ions, at a concentration of 5 mM, on the activity of *B. callensis* visceral crude enzyme extract were studied at pH 11.0 and 55 °C by the addition of ions to the reaction mixture (Table 2). As shown in Table 2, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} did not affect protease activity. However, Cu^{2+}, Ag^{2+} and Mn^{2+} affected the enzyme activity, with 53.7; 40.1 and 31 % inhibition, respectively.

Table 2: Effects of various ions on the activity of the alkaline crude enzyme extract from *Barbus callensis*.

Chemicals	Concentration	Relative Activity (%)
None	-	100
Ca^{2+}	5 mM	100
Ba^{2+}	5 mM	96.4±2
Zn^{2+}	5 mM	88 ±1.1
Cu^{2+}	5 mM	53.7 ± 0.9
Hg^{2+}	5 mM	34.5 ± 1
Mg^{2+}	5 mM	100
Mn^{2+}	5 mM	97 ± 2.2
Ag^{2+}	5 mM	40.1 ± 0.4
K^{+}	5 mM	100
Na^{+}	5 mM	100

Effect of NaCl on barbel proteases activity

The effect of NaCl on the activity of barbel crude extract was studied at pH 11.0 and 55 °C by the addition of NaCl to the reaction mixture (Fig. 4). Proteolytic activity of crude extract decreased gradually with increasing NaCl. The decrease in activity might be due to the denaturation of enzymes. The 'salting out' effect was postulated to cause the enzyme denaturation. The activity at 15 % NaCl was about 58% that of the control (no NaCl).

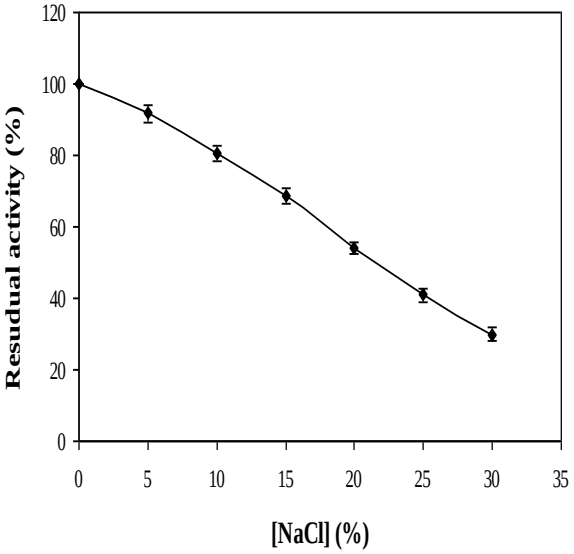


Figure 4: Effect of NaCl concentration on the activity of barbel crude extract.

Stability of the alkaline crude extract from goby in the presence of oxidizing agents, surfactants, solid and liquid detergents

The suitability of the *B. callensis* protease as a detergent additive was determined by testing its stability in oxidants and surfactants. As shown in Table 3, the alkaline crude enzyme extract is highly stable in the presence of the non-ionic surfactants like Tween 20, Tween 80 and Triton X-100, retaining 100 % of its activity. However, the *B. callensis* proteases were less stable against the strong anionic surfactant (SDS). Interestingly, *B. callensis* protease activity was little influenced by oxidizing agent, and retained about 100 %, 78.6 % and 66.9 % of its activity after incubation 1 h at 30 °C in the presence of 0.1%, 1% and 2% sodium perborate, respectively.

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Table 3: Stability of barbel alkaline crud protease extract in the presence of various surfactants and oxidizing agents.

Tensioactifs/Oxidizing agents	Concentrations (%)	Residual activity (%)
Control	0	100
SDS	0.1 (w/v)	56.6
	0.5	11.72
	0.1 (w/v)	100
Sodium perborate	1	78.6
	2	66.9
Triton X-100	1 (v/v)	100
	5	100
Tween 20	1 (v/v)	100
	5	100
Tween 80	1 (v/v)	100
	5	83.2

To check the compatibility of the alkaline crude extract with solid detergents, the crude enzyme was preincubated in the presence of various solid commercial detergents for 1 h at 30 and 40 °C. The data presented in Fig. 5a show that the alkaline proteases are highly stable at 30 °C and relatively stable at 40 °C. The alkaline proteases exhibited higher stability with Axion, Ariel and Dixan than, Nadhif and New Det. The crude protease retained 100 % of its activity in the presence of Axion, Ariel and Dixan after 1 h incubation at 30 °C, while 95 %, 87 % and 68 % of activity was retained at 40 °C, respectively. The data presented in Fig. 5b also shows that barbel proteases are extremely stable in the presence of liquid detergents, retaining 100 % of their initial activity with Dixan and Persil and more than 86 % with Ariel even after 1 h incubation at 30 °C.

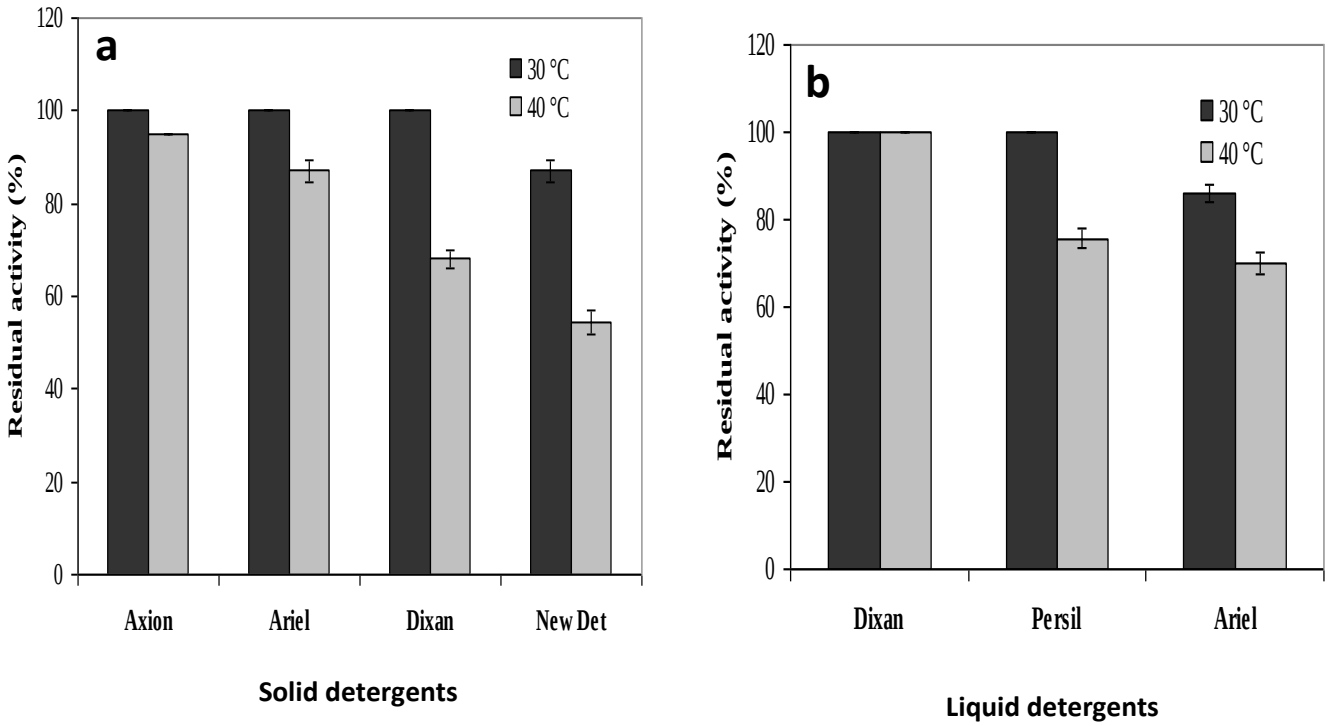


Figure 5: Stability of the barbel alkaline proteases in the presence of various commercial solid (a) and liquid (b) detergents.

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Degradation of chicken feathers

Alkaline crude enzyme extracted from the viscera of Tunisian barbel showed important feather-degrading activity, so the the crude protease extract was then investigated for the hydrolysis of chiken feathers. Disintegration of whole chicken feathers was assessed by incubation with barbel crude extract (5,000 U using casein as a substrate) for 24h at different temperatures ranging from 30 to 60°C. Complete solubilisation of chicken feathers was observed at 40°C (Fig. 6).

Commercial Subtilisin Carlsberg was used under the same conditions as barbel proteases. The data presented show that the barbel alkaline crude is more efficient than commercial protease after 24h incubation at 40°C.

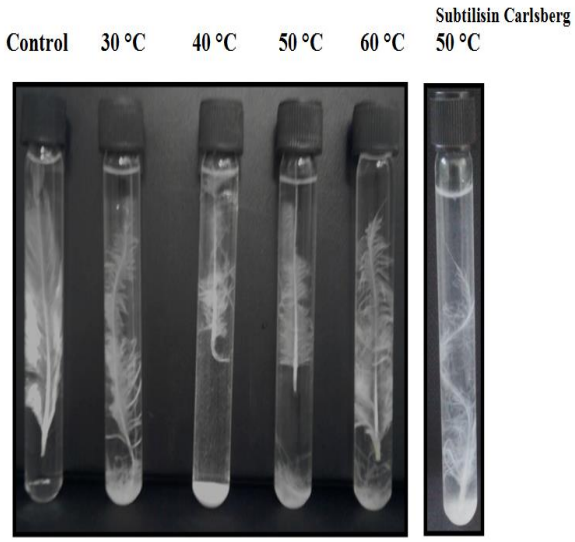


Figure 6: Disintegration of chicken feathers by barbel crude extract at different temperatures for 24 h and by commercial Subtilisin Carlsberg after 24 h incubation at 50°C.

Barbel proteases dehairing function

Incubation of the barbel crude extract with bovine skin for dehairing showed that after incubation for 24 h at pH 10 and at 30 or 37°C, hair was removed very easily from skin (Fig. 7). No dehairing was observed at 25 °C. The dehairing function in leather processing is generally carried out at relatively high pH value of about 8.0-10.0 (Dayanandanet al., 2003). This criterion is satisfied by the barbel proteases which exhibited high activity at pH 8.0-11.0.

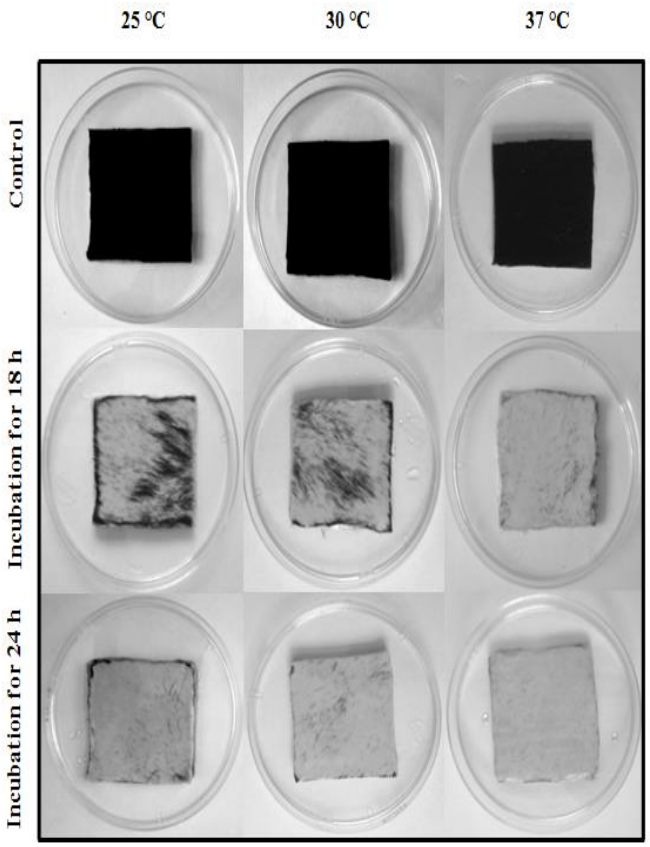


Figure 7: Dehairing function of barbel crude extract. Bovine skins were incubated for 18 h or 24 h at 25 °C, 30 °C and 37 °C with barbel proteases.

Discussion

Fish viscera, one of the most important by-products of the fishing industry, are a rich source of digestive enzymes, whose properties are highly valued in a wide range of industrial applications and processes (Simpson *et al*., 1991). Alkaline proteases from the viscera of the Tunisian barbel were extracted and characterized.

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The optimum pH for barbel proteases was superior to that reported by Sila *et al*. (2012) for proteases extracted from the viscera of *Zosterisessor ophiocephalus*. In addition, alkaline proteases from tambaqui (*Colossoma macropomum*) and common carp (*Cyprinus carpio*) intestine were reported to be active at higher pH values (10.0-12.0) (Espósito *et al*., 2009a). Results suggest that the viscera of *B. callensis* would be a potential source of proteases for certain industrial applications that require high alkaline conditions. The optimum temperature for barbel crude extract was similar to that of crude protease from *Nile tilapia* (Mendes *et al.*, 2009) and lower than that of crude extract from tambaqui (Espósito *et al*., 2009b). The thermal stability profile showed that alkaline enzyme extract is desirable for laundry purposes and from the ecological and economical point of view, mainly, because of saving energy. Sila *et al*. (2012) reported that PMSF, soybean trypsin inhibitor and TLCK effectively inhibited crude proteinases from goby. On the other hand, Pepstatin A, EDTA and DTNB (that are specific for aspartic protease, metalloproteinase and cystein protease, respectively) were practically without influence on the activity of the goby proteases. The effect of NaCl on the activity of barbel crude extract was studied. The barbel crude extract was more active in the presence of NaCl than that from true sardine (Klomklao *et al*., 2008) and gobie (Sila *et al*., 2012) which showed 45 and 55 % initial activities, respectively, of the initial activity under the same conditions. The water molecule is drawn from the protease molecules by salt, leading to the aggregation of those enzymes (Klomklao *et al*., 2004; 2007). Thus, the barbel crude proteases may be used to facilitate the hydrolysis of proteins in high-salt-fermented fish products such as fish sauce.

The suitability of the *B. callensis* proteases as detergent additives was determined. Similar results were reported by Sila *et al*. (2012) for proteases of goby. *B. callensis* protease activity was little influenced by an oxidizing agent. This is a relevant property because bleach stability has been attained only by site directed mutagenesis (Outtrup *et al*., 1993; Outtrup *et al*., 1995) or by protein engineering (Boguslawski and Shultz, 1992) of bacterial enzymes. The high stability of the alkaline proteases towards oxidizing agents is a very important characteristic for their eventual use in detergent formulations. The compatibility with detergents was investigated. The alkaline proteases exhibited hight stability. Sila *et a*l. (2012) and Mendes *et al*. (2009) reported also the stability of goby (*Z. ophiocephalus*) and Nile tilapia (*O. niloticus*) proteases in the presence of several commercial detergents. The goby proteases exhibited higher stability in Axion (100%), Ariel (100%) and Dixan (100%) but were less stable in NewDet (71%) and Nadhif (70%) at 30 °C. Results showed that the proteolytic activity varied with each laundry detergent. The results obtained clearly indicated that the performance of enzymes in detergents depends on number of factors, including the detergents' compounds.

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Complete solubilisation of chicken feathers by the barbel crude extract was observed after 24h incubation compared to Subtilisin Carlsberg. Similar results were reported by Haddar *et al*. (2010) for proteases from *Bacillus mojavensis* A21. Barbel proteases exhibited high dehairing activity at pH 8.0-11.0. Similar results were obtained with the *Aspergillus tamarri* alkaline protease on goat skin after 18-24h at pH 9.0-11.0 and 30-37°C (Dayanandan *et al*., 2003). Alkaline proteases with high keratinolytic activity from *B. pumilus*, were also reported to accomplish the dehairing process on bovine hair (Kumar *et al*., 2008), cowhides (Wang *et al*., 2007), and goat skins (Huang *et al*., 2003). The results obtained indicated that the barbel crude extract could also find application in leather processing.

The present study, reports the extraction, characterization and evaluation of digestive alkaline proteases from Tunisian barbel as detergent additive, in chicken feather-degradation and as a dehairing agent. The crude enzyme extract showed a high activity and stability in high alkaline pH. The alkaline crude enzyme extract showed optimum activity at temperature of 55°C and optimum pH of 11.0. Results showed that the alkaline crude protease extract exhibited a high stability in the presence of various commercial solid and liquid detergents. Furthermore, the barbel crude extract appears suitable for degradation of chicken feather with a potential for biotechnological application. More interestingly, alkaline crude enzyme extracted from the viscera of Tunisian barbel exhibited powerful dehairing function against bovine skin with minimal damage on the collagen. The results obtained indicated that Tunisian barbel proteases offer new and promising opportunities for biotechnological perspective bioprocesses, particularly for leather and poultry processing industries. Moreover, industrial scale recovery of marine enzymes is still under experimental stage. It may be expected that expanding capabilities of this new area will continue to profoundly affect the fish and shellfish industries in the future. However, further research is required to better understand processing lines and to develop new techniques that may be tailored to the specific requirements of production of various food products.

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