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Full Length Research Paper

Partial Characterization of Protease activity from *Rhynchophorus palmarum* (Palm Weevil)

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Palm weevil (*Rhychophorus palmarum)* poses treat to cultivated palm trees in central and southern Africa. It is also a natural source of protein in Asia and Africa. It is expected therefore, that protease from this source could have interesting properties and possible industrial applications. Protease produced by *R. palmarum* was purified by differential centrifugation and partially characterized by their caseinolytic activities. The enzyme optimal in the ranged of pH 8.4 to 9.2 and temperature of 23^{o}C. The Michaelis-Menten's constant (K_M) of the enzyme for its substrate, casein and the maximum attainable velocity (V_{Max}) were 5.51 x 10^{-2} mM and 45.0µg/min respectively. Metalions such as Sl^{+}, Al^{3+}, Ca^{2+}, Hg^{2+}, Na^{+}, Ba^{2+} inhibited the activity of the enzyme, with Na^{+} producing the highest inhibition (32.5%) and Al^{3+} produced the least inhibition (2.9%). The paper discusses the various possible industrial applications of this protease and concludes that palm weevil can be a source of protease for some industrial applications.

Key Words: Protease activity, edible insect, economic value, industrial application, kinetics parameter

INTRODUCTION

Proteases are degrading enzymes that catalyze the hydrolysis of protein (Stryer, 1995), by cleaving peptide bonds in proteins. They may be classified as serine, aspartic, cysteine and metallo proteases depending on the nature of the functional groups at the active site (Al-sherhri and Mostafa, 2004). They differ in their ability to hydrolyse various peptide bonds as each type of protease has a specific kind of peptide bond it breaks.

Protease can be obtained from animals, plants and microbial sources. Some examples include: trypsin, chymotrypsin (animal pancrease), pepsin (gastric stomach), rennin (stomach of all nursing mammal), papain (papaya latex), ficin (ficus latex), bormelain (pineapple), substilisin (Bacillus substilis), collagenase (clostridia), fungal protease, viral proteases and other bacterial proteases (Rao et al., 1998).

Proteolytic enzymes execute a large variety of complex physiological functions; their importance in conducting essential metabolic and regulatory functions is evident from their occurrence in all forms of living organisms (Egwim et al., 2006). They also have important biotechnological applications and represent one of the three largest groups of industrial enzymes. They find applications in detergent, leather, food, pharmaceutical industries and bioremediation processes (Anwar and Saleemuddin, 1998; Gupta et al., 2002). Probably the largest application of protease is in laundry detergents where they help removing protein based stains from clothing (Banerjee et al., 1999). Ndafi and Deobagkar (2005), reported that protease from Pseudomonas aeruginosa PD100 enabled the removal of bloodstain very easily without addition of any detergent and could be used as an alkaline protease in detergent powder and solution owing to the ability to act in the presence of solvents and detergents. In the food industry, proteases have been routinely used for various purposes including: use of papain for tenderizing fresh meats (Soper, 1998). Use of deferent types of proteases in food protein modification (Impoolsup et al., 1981), such as in the modification of cereal protein to produce miso and tofu, in the manufacture of condiments such as soy sauce and tamar sauce, specific diets and processed meats (Ihekoronye, and Ndoggy, 1985). In diary processing, chymosin, a protease with high specificity for casein is used for cheese making (Godfrey and West, 1996). In

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view of the recent trends of developing environmental friendly technologies, proteases have been shown to have extensive applications in leather industry (Kamini, et al., 2004); and in pharmaceutical industry for debridement of wounds (Rao et al., 1998) in which case they offer a gentle and selective debridement, supporting the natural healing process in the successful local management of skin ulcerations by the efficient removal of necrotic material (Sjodahl et al., 2002). Ishikawa, et al., (1993) developed an interesting application of alkaline protease in wish case an alkaline protease was used to decompose the gelatinous coating of x-ray films from which silver was recovered.

The inability of plant and animal proteases to meet the increasing demand of proteases has led to an increased interest in microbial proteases both for basic understanding of enzyme mechanisms and industrial applications (Rao et al., 1998), moreover, microganisms require limited space for their cultivation, exhibit rapid growth and are easily susceptible to genetic manipulation (Nadafi and Deobagkar, 2005).

However, the potentials of edible insects such as caterpillar, grubs and palm weevil that serve as natural food protein sources in Asia and Africa have not been explored as source of protease. This work is therefore designed to study some physio-chemical properties of R. palmarum (palm weevil) protease. This insect poses a great threat to cultivated palm trees in South and Central Africa as they feed on the trunk of the tree. Both the young and adult plants can be infected by this weevil, once infected, it is difficult to detect their presence by simply visual inspection (Bain and Fedon, 1951; Sanchez-sotos and Nakano, 2002). A female may lay an average of 245±155 eggs during a period of 30 -50 days. However, investigators have reported up to 718 eggs (Hagley, 1965). The prepupal stage lasts 4-17 days, during which the larvae makes a cocoon using vegetative fibres. The pupal metamorphosis lasts for about 30 days and the adult remains in the cocoon for another 7-10days before emerging. The life span is approximately 50days (Sanchez et al., 1993)

To our knowledge, there is no literature showing any study of protease from palm weevil. The present study was therefore initiated to characterize this protease for possible industrial applications and upgrade the economic value of this weevil, since proteases are of increasing importance in industrial applications.

MATERIALS AND METHODS

MATERIAL

The palm weevil (R. palmarum) used for this work was obtained from infected palm tree in Bida area of Niger State, Nigeria in November, 2005. Most of the chemicals used were of analytical grade.

Preparation of Crude Enzyme Extract

Palm weevil (10g) was weighed and ground in 50ml of chilled 0.02M phosphate buffer (pH 7.0) and centrifuged at 4000rpm for 10min, the resulting supernatant was decanted and frozen for further analysis.

Protease Assay

Protease activity was assayed spectrophotometrically using a modified burette method according to (Henry, and Marmion, 1974). To 3ml crude enzyme in 0.2M phosphate buffer (pH 7), was added 3ml of 0.03% casein. The mixture was incubated at 27oC for 30min; the reaction was stopped by boiling the mixture in water for 10min. Then, 1ml of the mixture was added to 4ml of burette reagent and the absorbance was measured at 540nm. The value of protein degraded was read off a protein standard curve (0.1-1µg/ml). One unit of enzyme activity was defined as the amount of enzyme required to degrade a unit of casein per minute under assay condition.

Determination of Kinetic Parameters

Optimum temperature and pH were determined by assaying for enzyme activity at different temperature and pH ranges.

The Michaelis-Menten's constant (KM) and the maximum attainable velocity (VMax) were determined at different substrate concentrations [s]. KM and VMax values were obtained from a plot Lineweaver and Burk.

RESULTS AND DISCUSSION

Optimal Temperature

The enzyme in the present study demonstrated maximum proteolytic activity on 0.03% casein at 23oC (Figure 1). This is an indication that the enzyme may function well in some industrial processes involving mild temperature conditions such as in the cold food industries. Kaimini et al (2004), has reported that protease activity within this range is considered a cold protease. Aoki et al (2004), has shown that the enzymes from northern shrimp (Pandalus borealis) (adapted to cold) with optimum temperature less than 20^{o}C can tenderize beef in cold condition and has a potential for application in the food industry, where working at lower temperature to prevent undesirable chemical reactions is necessary. The

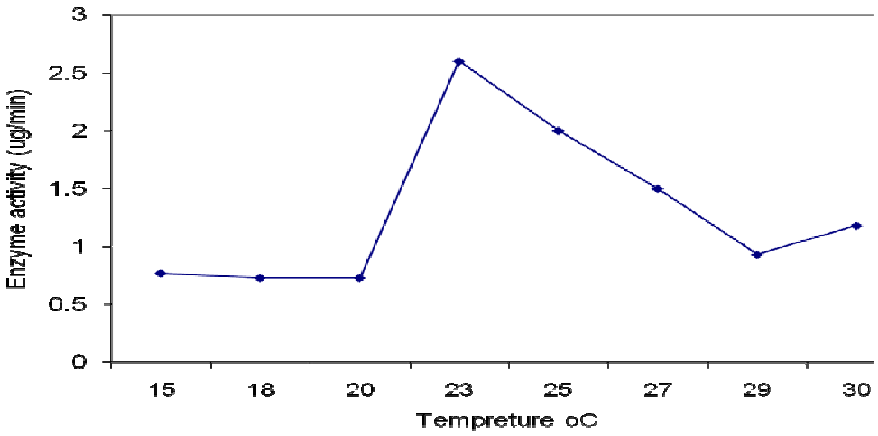

Figure 1. Effect of temperature on Enzyme activity

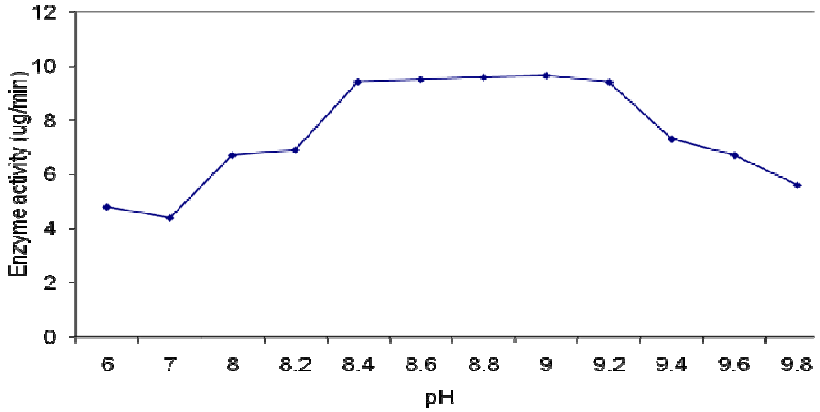

Figure 2. Effect of Ph on Enzyme activity

protease from edible palm weevil in the present study may also find application in food processing requiring mild temperature and the detergent industry where emphasis is now placed on using enzymes at low temperatures at which traditional chemicals often is no longer effective, in order to save energy in the home and reduce environment emissions like CO_2 .

PH Optimum

The enzyme showed maximum activity at pH 8.4 and 9.2 (Figure 2) when the crude protease was allowed to act on 0.03% casein. This reflects that an alkaline protease was involved. This enzyme may thus be useful in various processes requiring alkaline protease, particularly in the industrial food, detergents and leather industries where alkaline proteases have been shown to have extensive applications (Banerjee et al., 1999; Kamini et al., 2004). For instance, Impodsup et al (1981) partially purified an alkaline protease, which was optimally active on casein at pH 8 and 11, from Koji mold used in the production of soy sauce, this compares well with protease (optimum pH 7 - 9) obtained in this study. Young and Wood (1974) also reported that alkaline proteases play a significant role in the digestion of soybean protein during soy sauce processing. Also, Njafi and Deobagkar (2005), have reported the potential of alkaline protease from Pseudomonas aeruginosa PD100 as ingredient in detergent powder or solution, while Kaimini et al (2004), have described the applications of alkaline proteases at different stages of leather processing. It therefore implies that protease from palm weevil may find several industrial applications.

Enzyme Kinetics

The result of varying substrate concentration on proteolytic activity revealed that enzyme activity follows normal Michelis-Menten curve (Figure 3). This implies that the characteristics of the enzyme under study may

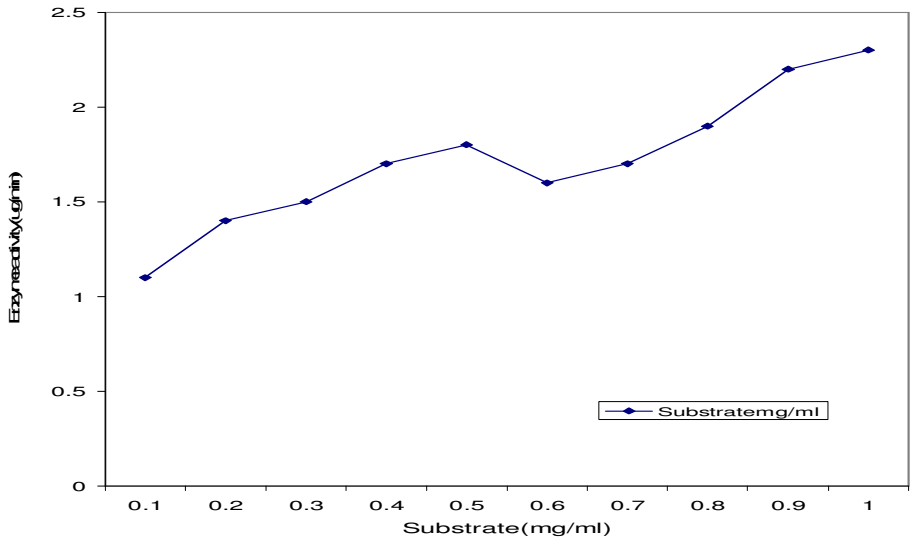

Figure 3. Variation of substrateconcentration on enzyme activity

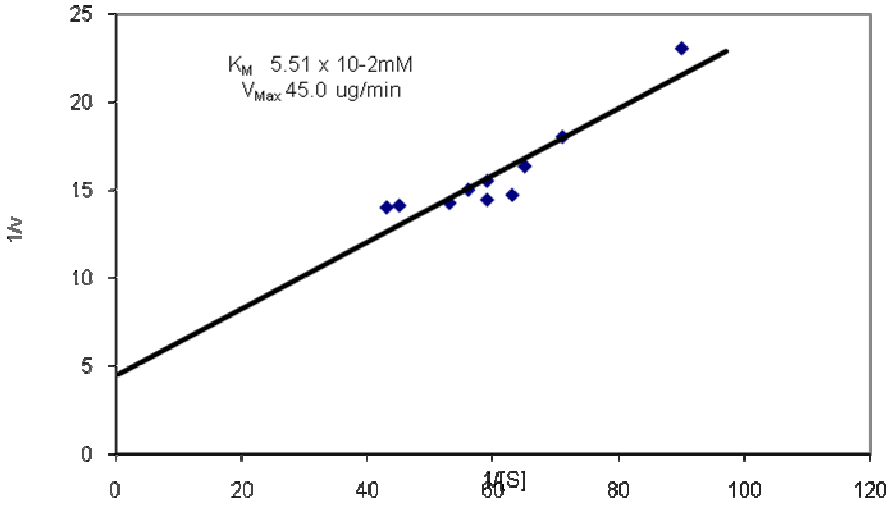

Figure 4. Lineweaver-Burk plot

not be uniquely different from known protease properties.

From the Lineweaver-Burk reciprocal plot (Figure 4), the KM and VMax of the crude protease from edible palm weevil (R. palmarum) were established as 5.51 x 10-2 mM and 45.0□g/min respectively. Protease from palm weevil (R. palmarum) may be a better enzyme source for industrial processes when compared with values for protease obtained from Mucor racemous (Km = 0.9 x 10-4M, 5.93□g/min) isolated from Korean traditional Meju (Yoo et al ,1999) and fermenting locust bean and melon seed (5.8 x 10-2 M; 5.1 x 10-2 S-1; 4.8 x 10-2 M; 3.43 x 10-2 S-1 respectively) (Egwim et al., 2009). This it is because of the lower Km (5.51 x 10-5M) which suggest higher affinity for the substrate, and a higher Vmax (45.0□g/min) which suggest a greater efficiency. However, Yoo et al (1999) elucidated that Mucor racemosus was very strong protease producer acting on Meju fermentation, which further underscores that protease from palm weevil may be having a great potential for application in food processing operations particularly those requiring food protein modification and in animal culture media (Chiphonikar et al., 1985). Noteworthy is the fact that palm weevil (R. palmarum) itself is an edible insect which serves as natural food

Table 1. Effect of heavy metals on protease activity

Metal Ions	Enzyme Activity(µg/min)	% Deactivation
Control	1.33 x 10_{-2}	-
Ca^{2+}	1.05 x 10^{-2}	21.2
Na^{+}	0.90 x 10^{-2}	32.5
Cu^{2+}	0.95 x 10^{-2}	28.3
Sr^{+}	0.99 x 10^{-2}	25.5
Al^{3+}	1.29 x10^{-2}	2.9
Hg^{2+}	0.94 x 10^{-2}	28.8
Ba^{2+}	0.95 x 10^{-2}	28.3
Pb^{2+}	1.58 x 10^{-2}	18.9

protein source in central Africa.

Effect of Some Metal Ions

Heavy metals including Cu2+, Al3+, Ba2+ and Sr+ were all found to inhibit the protease activity (Table 1). Na.+ induced the highest inhibition while Al3+ induced the least inhibition. The present observation is in agreement with earlier findings which have shown that different heavy metals reduce proteolytic activity to different degrees (Chen and Zall, 1986; Pena, et al., 2006)

With respect to the physio-chemical properties established for the protease in this study, it can be postulated that palm weevil (R. palmarum), hitherto seen as a destructive agent in cultivated palm trees in South and Central Africa, could after all be exploited for some industrial application ranging from food, detergent to even leather production.

The present work has clearly demonstrated an alkaline protease from palm weevil. The kinetic parameters show that this enzyme can be exploited for industrial processes. Further studies at the molecular level; isolating, cloning and expressing this protease gene in microorganisms may be a good idea in making this enzyme in commercial and industrial quantities.

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