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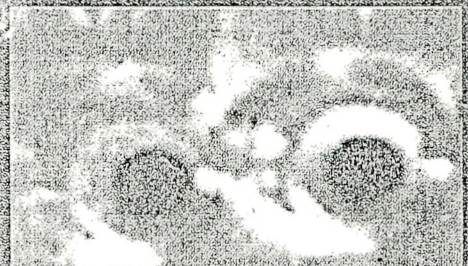

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Utilization Of Visceral Organ Waste Of Catla (*Catla Catla*) For The Isolation, Purification And Partial Characterization Of Protease

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Abstract

Protease is an industrially important enzyme isolated from various sources. Its isolation from the visceral organ waste of Catla, showed the highest specific activity in 50-60 % ammonium sulphate precipitation. The change in increase of specific activity from 2.2 to 3.5 U/mg was noted with the dialyzed fraction. Further purification on Sephadex G-100 column revealed an increase of specific activity from 3.5 to 3.7 U/mg. The molecular weight of the enzyme was found to be 28.0 KDa. On Native PAGE it was found to be 48.0 KDa, which indicated the multimeric nature of the isolated protein. The optimum pH and temperature of the isolated enzyme was found to be 8 and 60°C respectively.

Keywords: *Catla*, alkaline protease, fish waste, enzyme.

Introduction

Fish processing generates large amounts of solid and liquid wastes. Normally, more than half of the raw material weight is unused, e.g., only about 15% of the round shrimp becomes a canned shrimp product. However, fishery by-products are typically used as feeds and fertilizers that have a low dollar value. There is a growing interest in obtaining higher-value biochemicals and pharmaceuticals from fishery wastes, notably enzymes (El-Beltagy, *et al.*, 2004).

Enzymes are biochemical catalysts vital for living beings, because they accelerate chemical reactions between organic constituents within the cells that otherwise would take an extremely long time to complete. Proteolytic enzyme preparations are economically the most important group of enzymes, and their use is well established in the food industry (Kristinsson and Rasco, 2000).

Fish processing creates a large amount of wastes of high nutrient content which, if not properly processed for use in human or animal nutrition, is likely to be deposited in the environment creating pollution problems (Kotzamanis, *et al.*, 2001).

The viscera are one of the most important wastes of the fish industry, for example the sardine industry and this product is recognized with high potential as a source of digestive enzyme. Proteases from fish viscera could be used in industrial applications, so the recovery of this kind of waste might be an alternative to the pollution problem that the fish industry produces (Castillo *et al.*, 2004).

Catla catla, is commonly known as Carp, Minnows, Ray-finned fish, Rohu and Catla (Tamil). They occur in rivers, lakes and culture ponds and breeds in rivers. They are surface and mid-water feeders, mainly omnivorous with juveniles feeding on aquatic and terrestrial insects, detritus and phytoplankton.

Materials and Methods:

Materials:

The visceral organ waste of the fish was collected soon after cutting. The waste was washed well with distilled water, weighed, cut into small pieces and homogenized in 0.02M Tris HCl buffer, pH 7.8. The homogenate was then filtered through cheesecloth to obtain a clear solution.

Enzyme purification:

Ammonium sulphate precipitation is often used as the first purification and concentration procedure. Protease was precipitated from the crude homogenate at varying concentrations of ammonium sulphate ranging from 0-80%. The protease activity and the total protein content in each of the ammonium sulphate precipitated fractions were estimated. The fraction showing the highest specific activity was dialyzed to facilitate

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desalting of the proteins. SDS-PAGE of the dialyzed fraction was carried out on a 10% gel (Sambrook and Russell, 2001). Native PAGE of the dialyzed fraction was also done on a 10% gel. The dialyzed fraction was further purified on a Sephadex G-100 column equilibrated with 0.02M Tris HCl buffer (pH 7.8). The fraction showing high protease activity was taken for the estimation of protein.

Characterization:

Optimization of pH

The optimum pH of the purified protease was determined by preparing the substrate casein in buffers of varying pH ranging from 6-12.

Optimization of temperature

The optimum temperature for the enzyme was determined by incubating the enzyme -substrate mixture at temperatures ranging from 20°C- 80°C followed by checking its activity

Results:

Commonly Consumed Fishes In Coimbatore

A survey relating to the fishes consumed was done in the local fish markets of Coimbatore. Among the fishes sold, Catla was found to be the most commonly consumed fishes with over 120 kg sold respectively. This generated large amounts of waste.

Protease Activity, Protein Content And Specific Activity Of Ammonium Sulphate Precipitated Samples

The protease activity, protein content and specific activity of crude and ammonium sulphate precipitated fish sample were recorded in Table I.

Protease activity, protein content and specific activity of crude and ammonium sulphate precipitated samples of catla

S.No	Fish waste samples	Protease activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)
1	Crude	18.84	10.4	1.811
	Ammonium sulphate precipitated samples			
2	0-30%	1.52	1.8	0.83
3	30-40%	3.9	3.0	1.32
4	40-50%	6.1	5.6	1.10
5	50-60%	17.62	8.0	2.20
6	60-70%	8.8	6.2	1.42
7	70-80%	5.5	4.2	1.40

Table 1

From Table 1, it can be inferred that the maximum protease activity was exhibited by the crude sample and followed by the 50-60% ammonium sulphate precipitated fraction when compared to the other fractions.

The maximum specific activity was also seen in the 50-60% precipitated fraction and hence this fraction was used for further purification.

Protease Activity Of Dialysate Of Ammonium Sulphate Fractionated Fish Waste Sample

The 50 - 60 % precipitated fraction, which showed the maximum specific activity was dialyzed and the protein content and specific activity is recorded in the Table 2.

Protease activity of dialysate of ammonium sulphate fractionated fish waste sample of Catla

Protease activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)
Before dialysis 17.62	8.0	2.2
After dialysis 20.1	5.8	3.5

Table 2

It is clear from the table, that the protein content of the 50 - 60 % precipitated fraction had decreased (5.8 mg/ ml) after dialysis, but the specific activity had increased from 2.2 to 3.5 U/mg, which indicates the purification level.

Elution Profile Of Protease On Sephadex G-100 Column

The dialysate of the fractioned sample was further purified on Sephadex G-100 column.

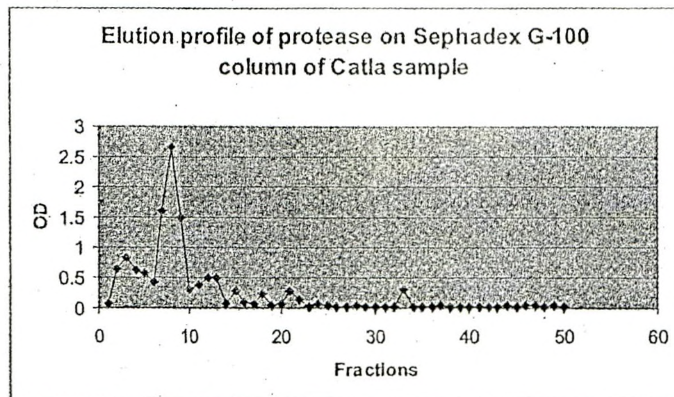

Figure 1: Elution profile of protease on Sephadex G-100 column of Catla sample

The fraction 7- 9, which showed the highest peak, was pooled and taken for the estimation of protein.

From the Figure, the protein was found that protein have been eluted in the earliest fraction (7-9) in Catla sample. Those fractions are taken for the estimation of protein.

Protease Activity In Crude, Ammonium Sulphate Precipitated, Dialyzed And Sephadex G-100 Column Purified Samples

The enzyme activity, protein content and specific activity at each purification step was done. Table 3 and 4 explains the results.

From the table, it is clear that there is an increase in the specific activity of the enzyme after each successive purification step and there is an increase in the purity level of the protein. The purification fold has also increased (0- 2.1) successively, showing the purity of the enzyme.

Molecular Weight Determination Of Protease

The molecular weight of the enzyme protease was determined by SDS-PAGE and Native-PAGE.

Determination of optimum temperature	
Temperature(°C)	Protease Activity (U/mg)
20	1.52
30	1.23
40	1.80
50	2.43
60	3.50
70	2.80
80	1.80

Table 5

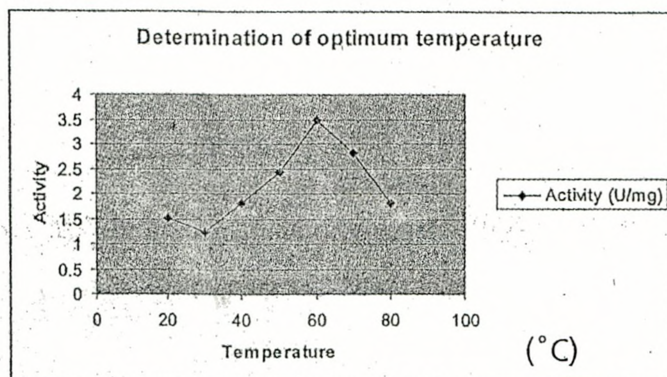

Figure 5:Determination of optimum temperature

Discussion

From the above study, it was seen that the protease isolated from the visceral organ waste of Catla, showed the highest specific activity with 50 - 60 % ammonium sulphate precipitation and was taken for further purification process.

The dialyzed fraction was found to have an increase in the specific activity from 2.2 to 3.5 U/mg. Further purification of the dialyzed fraction on Sephadex G-100 column also revealed increase in specific activity from 3.5 to 3.7 U/mg.

The molecular weight of the isolated enzyme was found to be 28.0 KDa on SDS PAGE. On Native PAGE it was found to be 48.0 KDa Catla, which showed that the protein isolated was multimeric.

The optimum pH of the isolated enzyme was shown to be 8 where the protease activity was highest and the optimum temperature was found to be 60°C. From this can be concluded that the enzyme isolated is an alkaline protease.

Thus from the present study it was shown that the huge amounts of fish waste generated which causes pollution can be used effectively for isolating industrially important enzymes like protease.

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Protease activity in crude, ammonium sulphate precipitated, dialyzed and Sephadex G-100 column purified samples of Catla

S.No	Fish waste samples	Protease activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)	Purification Fold
1	Crude	18.84	10.4	1.8	0
2	50-60% ammonium sulphate precipitated fraction	17.62	8.0	2.2	1.2
3	Dialyzed 50-60% sample	20.1	5.8	3.5	1.9
4	Sephadex G-100 purified fraction	17.62	4.8	3.7	2.1

Table 3

SDS-PAGE

The precipitated sample, dialyzed sample and eluted sample were analyzed by SDS-PAGE to determine the molecular weight of the enzyme from the fish sample.

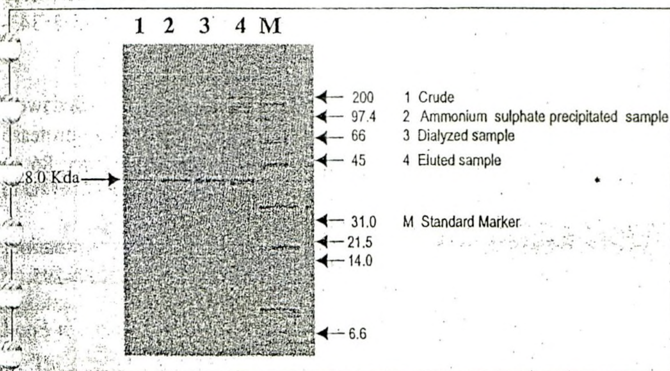

Figure 2

SDS PAGE of protease from Catla

The molecular weight of the isolated protease enzyme was found to be 48 KDa when compared to the standard marker protein of 6.6 -200 KDa. The thickness of the band indicates that there is an increase in the purity level in the dialyzed sample and the column eluted sample.

Native Page

The precipitated sample, dialyzed sample & eluted sample were analyzed by the Native-PAGE to determine the molecular weight of the enzyme.

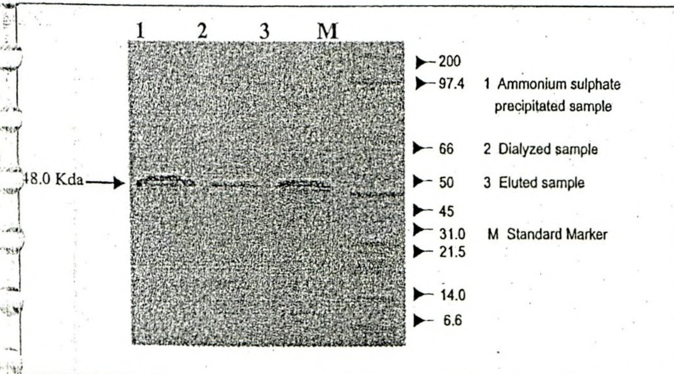

Figure 3

The precipitated sample, dialyzed sample & eluted sample were analyzed by the Native-PAGE to determine the molecular weight of the enzyme.

Native PAGE of protease from Catla

Figure 3 shows the banding pattern of the Catla sample on Native PAGE. From the banding pattern on Native PAGE, it can be deduced that the molecular weight of the native form of enzyme is between 45 to 50 KDa. Hence, it may be concluded that the enzyme isolated from the visceral organ waste of Catla is a multimeric protein having a molecular weight of 48 KDa with the monomeric units having a molecular weight of 28 KDa.

Characterization Of Protease

The purified enzyme was partially characterized based on the pH and temperature.

Determination of optimum pH

The activity of the isolated & partially purified enzyme protease at various pH are recorded in Table 4 and Figure 4.

Determination of optimum pH

pH	Protease Activity (U/mg)
5	1.7
6	1.6
7	2.2
8	3.0
9	2.1
10	2.1
11	1.7

Table 4

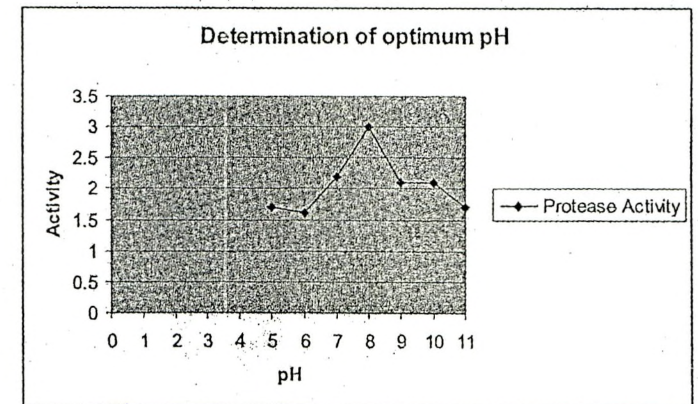

Figure 4: Determination of optimum pH

From Figure 4, it can be inferred that the purified enzyme has highest activity at pH 8.

The enzyme also showed a considerable activity at pH 9 and 10. However the pH started reducing from pH 11. Hence the enzyme can be called as 'Alkaline Protease'.

Determination Of Optimum Temperature

The optimum temperature of the isolated and partially purified enzyme was determined and the results are depicted in the Table 5 and figure 5