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ISOLATION PURIFICATION AND CHARACTERIZATION OF PROTEASE ENZYME FROM PARROT FISH (*SCARUS GHOBBAN*) WASTES

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Introduction

Enzymes are widely distributed in biological systems. The main sources of enzymes used in food processing are from plants, animals and microorganisms. Proteases are one of the most important groups of industrial enzymes that account for nearly 60 percent of the total enzymes (Safey *et al*, 2004). Proteases are involved in digesting long protein chains into short fragments splitting the peptide bonds that link amino acid residues. About 100 million tons of fish are harvested yearly. Of the total catch, 30 percent is transformed into fishmeal. More than 50 percent of the remaining fish tissue is considered to be a processing waste. This includes fins, skin, internal organs, head, bone and so on that are not used as food (Park *et al*, 2002). Fish processing creates large amounts of waste 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).

Even though fish plays beneficial roles, fish wastes or material processing operations cause foul odour if not managed properly. Most fish wastes degrade rapidly in warm weather and cause aesthetic problems and strong odour as a result of bacterial decomposition if not stored properly or disposed off quickly (Haard, 2000). Fish viscera is a potential source for recovering enzymes such as proteinases that may have some unique properties for industrial application eg: in the detergent, food, pharmaceutical, leather and silk industries.

Parrot fish are nearly all herbivorous that occur on coral reefs. They feed by grazing algae off coral. Parrot fish are a family of marine tropical fish distributed in Indian Pacific and Atlantic oceans. They are characterized by fused teeth and bright colors, which make them known as parrotfish. Parrot fish (*Scarus ghobban*) is generally valued as a fish for human consumption (Bariche *et al*, 2005). The present study aimed at isolating protease from the visceral organ waste of fish.

Methodology

Selection and collection of fish wastes

The fish species, namely, *Scarrus ghobban* is a commonly consumed fish in Coimbatore. The fish visceral organs, head

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and tails were collected soon after cutting into clean plastic bags, maintained in ice and brought to the place of study.

Preparation of crude homogenate

The fish visceral organs, head and tails were washed well with distilled water and stored at 4°C. The wastes were then weighed and cut into small pieces with a knife. These pieces were homogenized in Tris-HClbuffer and the homogenatecentrifuged at 3000g for 30 minutes at 4°C to obtain a clear solution.

Precipitation of protease by ammonium sulphate fractionation

Protease was collected from the crude supernatant by precipitation with saturated ammonium sulphate in varying concentrations (0-40%, 40-50%, 50-60%, 60-70% and 70-80%). They were mixed well for 30 minutes and then allowed to settle for 24 hours at 4°C. The mixture was then centrifuged and the precipitate collected.

Purification of protease fractions by dialysis

Desalting of protein fractions by dialysis were carried out with all the ammonium sulphate precipitated samples (0-40%, 40-50%, 50-60%, 60-70% and 70-80%) to prevent interference of salts with the protein fraction.

Assay of protease

Protease activity in the crude extract sample in ammonium sulphate precipitated samples and in the ammonium sulphate precipitated + dialyzed samples were calorimetrically assayed using casein as the substrate.

Estimation of protein content

The protein contents of the crude, ammonium sulphate precipitated and ammonium sulphate precipitated + dialyzed samples were determined by Lowry's method

Molecular weight determination of protease by SDS-PAGE

SDS-PAGE was carried out with both the ammonium sulphate precipitated samples and the ammonium sulphate precipitated + dialyzed samples using 12 percent gradient gel on a discontinuous buffer system according to the procedure of Laemmli. The protein bands were stained with Coomassie Brilliant blue.

Native-PAGE

Like SDS-PAGE, Native-PAGE was carried out with the ammonium sulphate precipitated and ammonium sulphate precipitated + dialyzed samples using 12 percent gradient gel.

Confirmation of protease by zymography

Casein zymography was done for the ammonium sulphate fractionated samples and the ammonium sulphated precipitated+dialysed samples using a 12percent polyacrylamide gel containing casein.

Characterization of protease

Effect of pH and temperature

The optimum pH for the protease activity was determined by preparing the casein substrate in buffers of varying pH (6 to 10). The optimum temperature for the protease activity was also determined by incubating the enzyme substrate mixture at various temperature intervals (30-80°C).

Testing for presence of protease

With coagulated egg white

Protease has the ability to dissolve coagulated egg white. The crude enzyme was incubated with coagulated egg white in Tris-HCl buffer at 30°C and observed.

With blood stain

The destaining ability of protease was examined with a piece of cloth soaked in blood and incubated with the purified protease enzyme at 45°C-50°C over night.

With animal skin

Protease has the ability to remove the hair of cow skin. The enzyme was incubated with cow skin at 37°C for 12 hours and observed.

Results and discussion

Purification scheme for protease from visceral organ wastes of parrot fish

Table 1 depicts the purification scheme for the protease enzyme isolated from visceral organ waste of Parrot fish (*Scarus ghobban*).

The protein content and protease activity of both ammonium sulphate precipitated as well as ammonium sulphate

Table 1. Purification scheme of protease from visceral organ wastes of parrot fish (*scarus ghobban*)

	Samples	Total Protein (mg/ml)	Total protease activity (U/ml)	Specific activity of protease (U/mg)	Purification fold	Recovery %
I	**Crude extract**	8.961	16.9	1.9	1.0	100
II	**Ammonium Sulphate Precipitated**					
1.	0 -40 %	0.706	8.5	11.9	6.3	50
2.	40-50 %	0.744	10.2	13.8	7.3	60
3.	50-60 %	0.775	14.2	18.2	9.6	84
4.	60-70 %	0.715	9.5	13.2	6.9	56
5.	70-80%	0.693	8.2	11.9	6.3	48
III	**Ammonium Sulphate Precipitated +Dialyzed**					
6.	0 -40 %	0.567	4.9	8.6	4.5	28
7.	40-50 %	0.618	5.9	9.5	5.0	34
8.	50-60 %	0.643	8.9	13.9	7.3	52
9.	60-70 %	0.605	5.4	8.9	4.7	31
10.	70-80%	0.558	4.4	7.9	4.2	26

precipitated + dialyzed samples decreased on comparison with the protein content of the crude extract. The 50-60 percent ammonium sulphate precipitated and ammonium sulphate precipitated + dialyzed samples also exhibited the highest protein content (0.744 mg/ml & 0.643 mg/ml), highest protease activity (14.2 U/ml & 8.9 U/ml), highest specific activity (18.2 U/mg & 13.9 U/mg), maximum purification fold (9.6 & 7.3) and maximum recovery percentage (84% & 52%) respectively.

Purification scheme for protease from head and tail wastes of parrot fish

Table 2 presents the purification scheme for the protease enzyme isolated from the head and tail wastes of Parrot fish (*Scarus ghobban*).

Table 2. Purification scheme of protease from head and tail wastes of parrot fish (*scarus ghobban*)

	Samples	Total Protein (mg/ml)	Total protease activity (U/ml)	Specific activity of protease (U/mg)	Purification fold	Recovery %
I	Crude extract	1.320	9.2	1.9	1.0	100
II	Ammonium Sulphate Precipitated					
1.	0 -40 %	0.164	2.3	14.4	7.3	25
2.	40-50 %	0.293	6.1	20.9	10.7	65
3.	50-60 %	0.400	7.4	21.2	10.9	81
4.	60-70 %	0.211	3.5	16.8	8.6	38
5.	70-80%	0.118	1.5	12.3	6.3	16
III	Ammonium Sulphate Precipitated +Dialyzed					
6.	0 -40 %	0.125	1.3	10.3	5.3	14
7.	40-50 %	0.243	4.4	18.3	9.4	47
8.	50-60 %	0.354	6.1	18.6	9.5	70
9.	60-70 %	0.157	2.7	16.9	8.7	29
10.	70-80%	0.068	0.6	8.9	4.4	6

The protein content and protease activity of both the ammonium sulphate precipitated and ammonium sulphate precipated+ dialyzed sample decreased on comparison with the protein content of the crude extract. The 50-60% ammonium sulphate precipitated samples and 50-60% ammonium sulphate precipitated + dialyzedrecorded the highest protein content (0.400 mg/ml & 0.354 mg/ml) respectively, protease activity (7.4U/ml & 6.1 U/ml), specific activity (21.2U/mg & 18.6 U/mg), maximum purification fold

(10.9 & 9.5) and maximum recovery % (81% & 70%).

Molecular weight determination of protease by SDS-PAGE and Native PAGE

The ammonium sulphate precipitated and ammonium sulphate precipitated + dialyzed samples of fish wastes (visceral organ and head & tail wastes) were analyzed by SDS-PAGE and Native PAGE for determining the molecular weight of the protease. The molecular weight of the protease was compared with that of the marker, which is a mixture of proteins that resolve into sharp bands in the range of 14.4 kDa to 116.0 kDa. It is clear from the electrophoratogram that a distinct band at 24 kDa present for the ammonium sulphate precipitated + dialyzed samples. Native PAGE for 50-60percent of ammonium sulphate precipitated and 50-60percent of ammonium sulphate precipitated + dialyzed protease from the visceral organ waste and head and tail also showed a single band.□

Confirmation of presence of protease by Zymography

The proteolytic activity of the SDS-PAGE bands was confirmed by zymography using casein incorporated SDS-PAGE. Plate 4 shows the zymogram of 50-60percent of ammonium sulphte precipitated and 50-6 percent of ammonium sulphate precipitated + dialyzed protease isolated from fish wastes (visceral organ, head and tail respectively). The zymogram revealed the presence of a clear band, which represents the caseinolytic activity of the enzyme.

Characterization of protease

Effect of pH

The effect of pH on the activity of purified protease from visceral organ waste and head and tail wastes were examined from pH 6 to pH 10. The activity of the protease from both the wastes was maximum at pH 9. Hence the isolated protease may be an alkaline protease.

Effect of temperature

The optimum temperature for the activity of protease from both the visceral organ waste and head and tail wastes of the selected fish was 60°C. The enzyme seemed to have almost lost its activity at 30°C and 80 °C in the case of both the wastes. The optimum temperature was found to be 60 °C since it gave the maximum activity.

Action of protease on coagulated egg white

The protease enzyme of visceral organ and head and tail waste of fish had the ability to digest albumin. Protease could hydrolyse egg protein thereby making them soluble in appearance.

Bloodstain

Protease could easily remove stains from a blood stained cloth without even using a detergent.

Animal skin

Protease could remove hairs from the animal skin to a large extent.

Conclusion

Isolation and purification of protease from visceral organ, head and tail waste of the fish *Scarus ghobban* was an aim to exploit a source for the enzyme protease. Protease has the ability to digest proteins with fibrin and albumin base. Hence it can be concluded that visceral organ, head and tail of fish waste which are simply disposed away causing pollution problems can be effectively and usefully managed by using it as a good source of enzyme.

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