

UTILIZATION OF VISCERAL WASTE OF RIBBON FISH (*TRACHIPTERUS TRACHYPTERUS*) FOR THE EXTRACTION, PURIFICATION AND CHARACTERIZATION OF PROTEASE

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Abstract: Ribbon fish (*Trachipterus trachipterus*) is one of the most commonly consumed fish in Coimbatore. Ribbon fish possesses all the characteristics of fish living in very great depth. Their fins especially and the membrane connecting them are of a very delicate and brittle structure. There is some potential for gaining more value from fish waste. It is rich in valuable minerals, enzymes, pigments and flavours that are required by many industries including food, agriculture, aquaculture and pharmaceuticals industries. Fish waste can also be utilised in the production of organic fertilisers and composts, which have significant benefits over chemical-based products. The aim of the present study was to utilize the visceral waste of ribbon fish (*Trachipterus trachipterus*) for the extraction, purification and characterization of protease. The wastes of the fish *Trachipterus trachipterus* were collected, cleaned, homogenized, precipitated with Ammonium sulphate of varying concentrations (0-80%), purified by dialysis, Sephadex-G100 chromatography, molecular weight determined by SDS-PAGE, the presence of protease confirmed by Zymography and its activity assayed and analyzed. The activity was found to be highest at the alkaline pH 7.8 and maximum protease activity remained at 50°C.

Key words: Fish waste, *Trachipterus trachipterus*, Protease.

INTRODUCTION

Fish Industry waste are an important environmental contamination source. Research has been carried out in order to develop methods to convert these wastes into useful products [1]. A way of minimizing the environmental problems generated by the high amount of fish waste is its transformation to a product to be incorporated as ingredient in animal rations [2].

Fish waste proves to be a great source of minerals, proteins (58% dry matter (d.m)) and fat (19% d.m). Fatty acids (monosaturated acids, palmitic acid and oleic acids) are abundant in fish waste, while the high ash content (22% d.m) indicates

high percentage of minerals in fish meal [3].

Proteases and peptidases are enzymes that are essential for all life. Their actions are exquisitely selective, each protease being responsible for splitting very specific sequences of amino acids under a preferred set of environmental conditions [4]. Alkaline protease in the intestine of marine animals can help in the digestion of protein in the compound diet. Therefore, alkaline protease in the gut of marine animals has received much attention in recent years [5].

Alkaline proteases are one of the most important groups of industrial enzymes widely used in the detergent, food and leather tanning industries.

Alkaline proteases can also be used in the hydrolysis of fibrous proteases such as horn, feather and hair, converting them in to useful biomass [6].

Fish waste can be used as a source for the isolation of enzymes, which are of commercial value. Whole viscera can also be used because these are raw by-products of the processing line. Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms and are essential for cell growth differentiation. The extra cellular proteases are of commercial value and find multiple applications in various industrial sectors. [7].

MATERIALS AND METHODS

The present study was aimed at utilizing the visceral waste of Ribbon fish (*Trachipterus trachipterus*) to isolate the beneficial enzyme protease. The viscera of fish were removed as soon as possible, kept in ice and transported from the fish market to the work place and deep frozen until use. The stored fish visceral organ wastes were washed thoroughly with distilled water, the required amount weighed, cut into small pieces and homogenized in 0.02M Tris HCl buffer. The homogenate obtained was filtered to obtain a clear solution. This is the crude enzyme extract. Protease was precipitated from the crude fish homogenate at varying concentrations of ammonium sulphate ranging from 0-80%. The precipitated enzyme was dialyzed against the Tris-HCl buffer.

The total protein content, enzyme activity, specific activity and the purification fold of protease of the crude sample, ammonium sulphate fractionated sample, dialyzed sample and gel filtered sample were estimated. SDS PAGE of the dialyzed enzyme fraction was carried out on a 12% gel. Analysis of protein was carried out by native PAGE in 12% polyacrylamide gel. The proteolytic activity of the enzyme was evaluated by zymography. The action of protease on various substrates were carried out by studying its ability to remove blood stain and degum silk.

The characterization of the isolated enzyme was carried out by determining its pH and temperature. The optimum pH of the isolated protease was determined using casein as a

substrate at varying pH. The optimum temperature of the extracted protease was determined by incubating the enzyme substrate mixture at temperatures ranging from 20 °C - 80°C followed by checking its activity. The effect of Azocasein,

Table 1: Protein content, protease activity, specific activity and purification folds of ammonium sulphate precipitated fish waste samples

S.No	Samples	Protein content (mg)	Protease activity (u/ml)	Specific activity (u/mg)	Purification folds
1	Crude	0.81	1.7	2.09	1
2	Ammonium sulphate ppt 0-40%	0.73	1.33	1.82	0.8
3	40-80%	0.43	1.23	3.08	1.5

Table 2: Protein content, protease activity, specific activity and purification folds of dialyzed fish waste samples

S.No	Samples	Protein content (mg)	Protease activity (u/ml)	Specific Activity (u/mg)	Purification folds
1	Crude	0.81	1.7	2.09	1
2	Dialyzed 0-40%	0.59	2.15	3.6	1.74
3	40-80%	0.29	2.37	8.17	3.91

Table 3: Comparison of protein content, protease activity, specific activity and purification folds crude, dialyzed and sephadex G-100 purified protease

S. No.	Waste	Protein Content (mg)	Protease activity (u/ml)	Specific Activity (u/mg)	Purification folds
1	Crude	0.81	1.7	2.09	1
2	Ammonium sulphate ppt 40-80%	0.43	1.23	3.08	1.5
3	Dialysed 40-80%	0.29	2.37	8.17	3.91
4	Sephadex G-100	0.23	2.95	12.83	6.12

Table 4: Effect of pH

pH	Protease Activity (U/min)
3.6	1.15
4.0	1.02
4.4	0.67
5.2	0.75
6.0	4.50
6.8	2.27
7.0	3.94
7.2	1.93
7.8	6.07
8.0	3.77
8.2	1.56
8.8	1.45
9.0	1.13

Table 5: Effect of temperature

Temperature °C	Protease Activity (U/min)
20	3.70
30	5.80
40	9.50
50	12.70
60	10.50
70	5.90
80	2.40

Table 6: Substrate specificity

Substrate	Protease activity (U/ml)
Azocasein	7.78
Casein	10.57
BSA	3.28

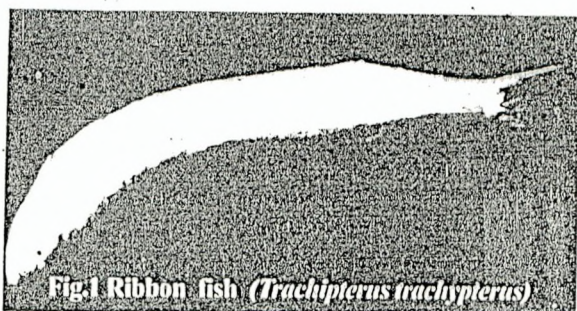


Fig.1 Ribbon fish (*Trachipterus trachipterus*)

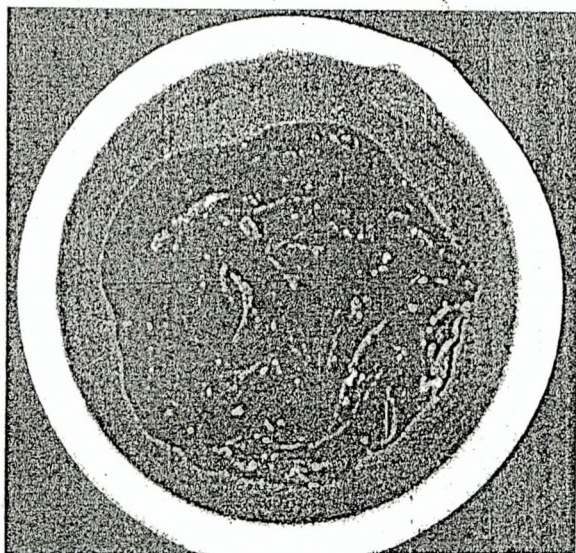


Fig. 2: visceral waste of ribbon fish,

BSA and Caesin on the purified protease was determined by incubating the enzyme substrate mixture in Tris Hcl buffer pH 7.8 followed by checking its activity.

RESULTS

From table 1 it is clear that the protein content and proteolytic activity was found to be higher in the crude fish waste sample and ammonium sulphate precipitated samples. The 40-80 % Ammonium sulphate precipitated sample exhibited the maximum specific activity and highest purification fold as compared to that of other samples. This may be due to the impurities present in the crude sample.

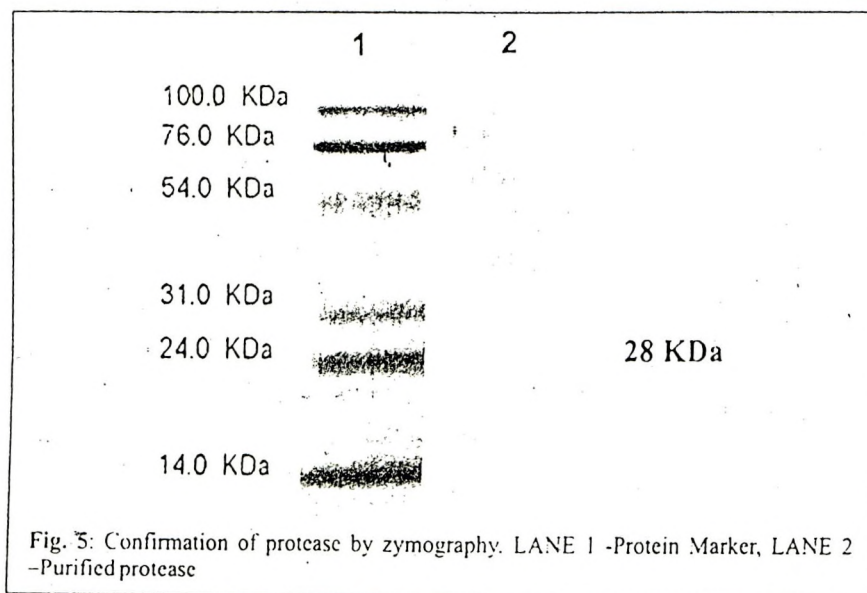
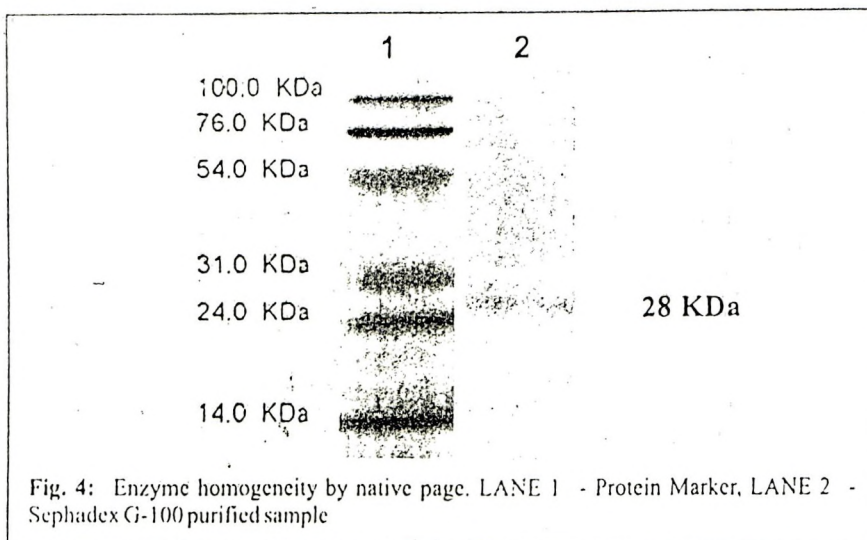
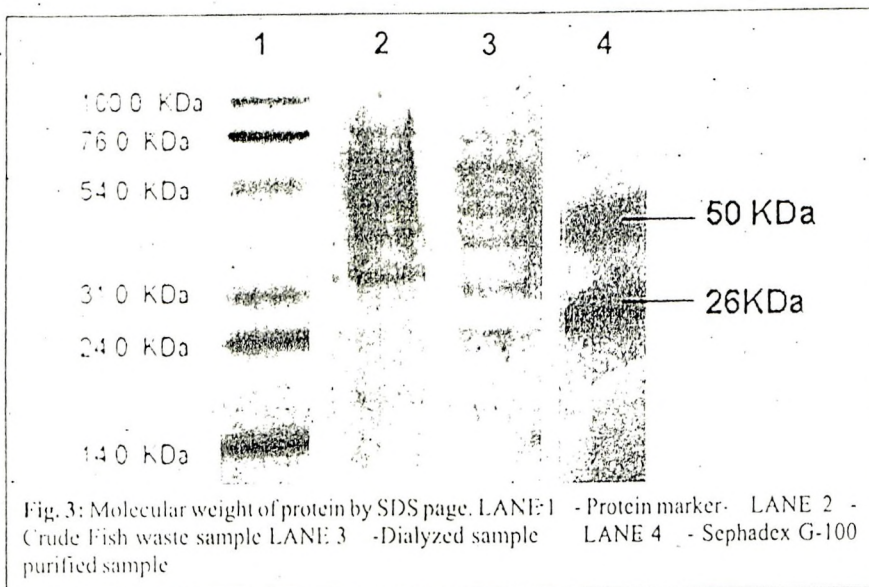
Table 2 shows that the protein content was found to be highest in the crude sample when compared to the dialyzed fish waste samples. The protease activity and specific activity of the 40-80% dialyzed sample was found to be higher when compared to crude and 0-40% dialyzed sample. The purification fold was increased after dialysis from 1.5 to 3.91.


Protein content, protease activity, specific activity and purification folds of crude, dialyzed and sephadex G-100 purified protease are depicted in table 3. The protein content of the crude sample was found to be high when compared to the sephadex G-100 purified samples. The protease activity of the column sample showed higher activity than the other samples. It is also evident from the table that there was a gradual increase in the specific activity of the enzyme when subjected to purification. When compared to crude sample, sephadex G-100 purified protease showed 6.4 folds purification. These results were supported by the work for characterizing the trypsin isozymes from the viscera of Japanese Anchovy, stated that gel filtered sample yielded 38 folds purification when compared to the crude sample [8].


Figure 1 shows the Ribbon fish (*Trachipterus trachipterus*) and figure 2 shows its visceral organ waste. The banding pattern of the crude, Ammonium sulphate precipitated, dialysed and Sephadex G-100 purified samples are shown in Fig. 3. When compared to the other samples, column sample showed 2 clear bands at the range of 26 and 50 KDa. Figure 4 shows the single bands that correspond very closely to the single 28 Kda band produced in Lane 1 by the marker. The zymogram of a single band indicating the proteolytic activity of protease is depicted in Figure 5. Hence it can be confirmed that the enzyme isolated from fish visceral organ waste was a protease.

Figure 6 shows the action of protease on the blood stain. Protease treated blood stained cloth was found to be destained when compared with untreated cloth. Figure 7 shows that the 35 g of yarn was reduced to 26 g after the treatment of purified protease. Therefore, the 25.7 % of the sample lost weight due to the treatment of purified protease. This confirmed that the purified protease had the ability to degum silk.

The protease activity of the sample was determined over the pH range 3.6-9 using acidic and alkaline buffers are depicted in table 4. The activity was found to be highest at the alkaline pH 7.8 and acidic pH shows very low activity. The protease activity at different temperature varying from 20 °C - 80 °C in Tris buffer pH 7.8 are shown in table 5. The maximum protease activity remained at 50 °C and low activity was



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detected when assayed at 80°C. Table 6 indicates that the substrate casein had more protease activity when compared to the substrate Azocasein and BSA.

DISCUSSION

The protein content and protease activity was found to be higher in the crude fish waste sample than the ammonium sulphate precipitated samples and dialyzed samples. These results are similar to that reported by the work in which trypsin extracted from the pyloric caeca of a fish named Tambaqui, showed the highest specific activity in the 40-50% Ammonium sulphate precipitated samples. [9].

There was a gradual increase in the purification folds when it was dialyzed. [10] also reported that the dialysis of alkaline protease from the viscera of Boltifish increases from the purified fold of 7.60 to 8 after precipitation with Ammonium sulphate fraction. The Sephadex G-100 purified protease had high specific activity and purification fold. These results were supported by the work characterizing the trypsin isozymes from the viscera of Japanese Anchovy, which stated that gel filtrated sample yielded 38 folds purification when compared to the crude sample[8].

The molecular weight of the samples was found to be between 26-50 KDa by SDS-PAGE. The proteolytic activity was confirmed by its ability to destain blood stained cloth and its property to degum silk.

The enzyme had an optimum pH of 7.8. This was in accordance with the trypsin purified from the pyloric caeca of the fish Tambaqui which also exhibited high activity at alkaline pH. It had an optimum temperature of 50°C.

CONCLUSION

It is concluded from the present that the visceral organs of fish can be effectively and usefully managed by using it as a good source of enzymes protease which is in great demand in the commercial market today

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