RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

Fish waste causes a lot of pollution. One way of partially overcoming this pollution is utilizing these wastes for the isolation of industrially useful enzymes like protease.

The present study entitled **"Comparison of Various Precipitation techniques and Partial Purification of Protease isolated from Visceral Organ and Head and Tail Wastes of Indian Oil Sardine (*Sardinella longiceps*) Fish"** was aimed at isolating and purifying protease from the visceral organ and head and tail wastes of a selected type of fish. The selected fish, the Indian Oil Sardine (*Sardinella longiceps)*, commonly called "Matthi" in Tamil is one of the most commonly consumed fish in Coimbatore. The wastes of this fish were collected from a local fish stall in Coimbatore. They were cleaned, homogenized, precipitated with ammonium sulphate, acetone and ethanol in varying concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70% 80%, 90% and 100%), the fractions from each types of precipitation which gave the highest protease activity were selected for further purification by dialysis and Sephadex G-100 column.

The findings of the study are discussed under the following headings:

4.1 Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by ammonium sulphate

4.2 Purification scheme of protease from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by ammonium sulphate

4.3 Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps)* fish precipitated by acetone

4.4 Purification scheme of protease from head and tail wastes of Indian oil sardine *(Sardinella longiceps)* fish precipitated by acetone

4.5 Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by ethanol

4.6 Purification scheme of protease from head and tail wastes of Indian oil sardine (Sardinella longiceps) fish precipitated by ethanol

4.7 Purification scheme of protease from visceral organ wastes of Indian oil sardine *(Sardinella longiceps)* fish precipitated by ethanol Gel filteration pattern of protease on Sephadex G-100

4.1 Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by ammonium sulphate

Table-I depicts the purification scheme namely, protein content, protease activity, specific activity, purification fold and recovery % for the protease enzyme isolated from the visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish. Figures 1, 2 and 3 depict the protein content, protease activity and specific activity respectively of the protease enzyme isolated from the visceral organ wastes of Indian oil sardine *(Sardinella longiceps*) fish.

Protein content

From Table-I and Figure-1, it is understood that the protein contents of ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate precipitated + Sephadex G-100 run samples from the

TABLE- I

Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps)* fish precipitated by ammonium sulphate

S.No	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	8.9±0.0015	26.8±0.846	3.0±0.091	1±SD	100±SD
II	Ammonium sulphate precipitated					
1	10%	0.54±0.09	8.54±0.239	15.37±0.357	4.95±00.02	36±0.002
2	20%	0.47±0.00	8.12±0.084	17.1±0.180	5.62±0.03	34±0.002
3	30%	0.48±0.003	9.23±0.199	19.12±0.330	6.2±0.072	39±0.004
4	40%	0.56±0.015	19.66±0.432	22.15±0.332	7.4±0.505	72±0.021
5	50%	0.51±0.01	10.06±0.491	20.51±0.151	6.72±0.06	55±0.004
6	60%	0.27±0.03	5.54±1.93	20.05±0.80	6.63±0.06	54±0.002
7	70%	0.24±0.005	5.04±0.415	20.57±0.240	6.12±0.02	53±0.002
8	80%	0.21±0.015	3.27±1.04	14.84±0.295	4.9±0.0017	46±0.001
9	90%	0.21±0.027	3.75±0.520	8.25±0.188	3.4±0.03	34±0.002
10	100%	0.2±0.0152	2.06±0.88	9.68±0.790	2.7±0.0152	32±0.0007
III	40% Ammonium sulphate precipitated +dialysed	0.27±0.040	11.13±3.74	37.99±1.592	12.47±0.440	64±0.0177
IV	40% Ammonium sulphate precipitated + Sephadex G-100	0.87±0.020	10.13±0.30	32.77±0.250	11.69±0.01	50±0.0036
CD (0.05)		**0.470**	**0.545**	**0.263**	**4.717**	**6.705**

All values are mean of triplicates

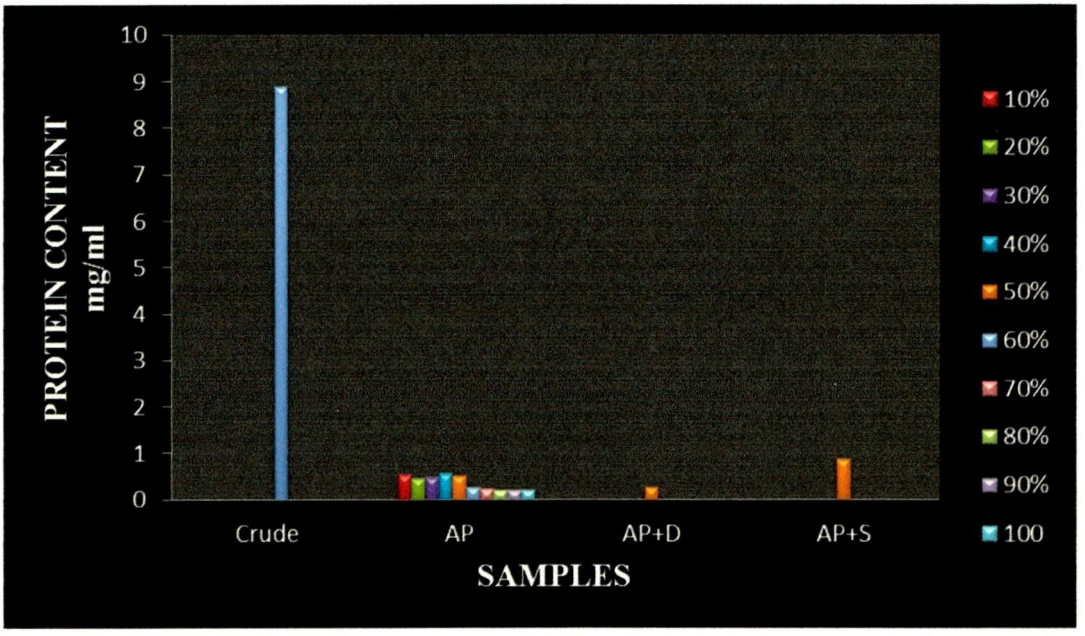
visceral organ wastes of the fish decreased significantly ($P<0.05$) on comparison with the protein content of the crude enzyme (8.90 mg/ml).

Among the ammonium sulphate precipitated samples, the 40% precipitated sample registered the highest protein content (0.56 mg/ml), which was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by Sephadex G-100 of the ammonium sulphate precipitated sample significantly ($P<0.05$) gave a higher protein content (0.87 mg/ml), which was significant ($P<0.05$) than purification of the ammonium sulphate precipitated sample by dialysis (0.27 mg/ml).

FIGURE -1

Protein content of ammonium sulphate precipitated and purified samples from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish



ASP – Ammonium Sulphate Precipitated sample

ASP + D- Ammonium Sulphate Precipitated + Dialysed sample

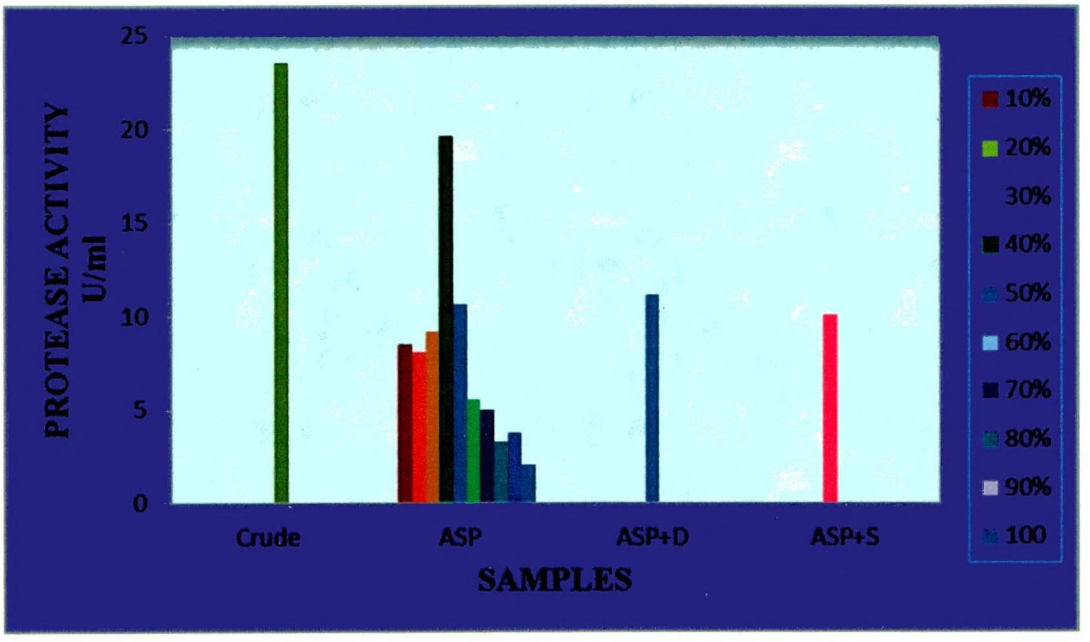
ASP + S-Ammonium Sulphate Precipitated + Sephadex G-100 run sample

Protease activity

A similar trend as that of the protein content was followed for protease activity (Table I and Figure-2) also where the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate precipitated + Sephadex G-100 run samples of the visceral organ wastes of fish showed significantly ($P<0.05$) lower values than that of crude enzyme sample (26.8 U/ml).

FIGURE- 2

Protease activity of ammonium sulphate precipitated and purified samples from visceral organ wastes of Indian oil sardine *(Sardinella longiceps)* fish



ASP- Ammonium sulphate Precipitated

ASP + D - Ammonium Sulphate Precipitated + Dialysed

ASP + S- Ammonium Sulphate Precipitated + Sephadex G-100

Here also, in the ammonium sulphate precipitated group, the 40% ammonium sulphate precipitated sample exhibited the highest protease activity (19.66 U/ml), which was very significant ($P<0.05$).

Purification by dialysis of the ammonium sulphate precipitated sample gave a higher protease activity (11.13 U/ml), which was significant ($P<0.05$) than purification by Sephadex G-100 (10.13 U/ml).

Thus, it can be stated that the highest protease activity was exhibited by the 40% ammonium sulphate precipitated + dialysed sample (11.13 U/ml).

The finding that the protease activity of all the samples were significantly ($P<0.05$) lower than that of the crude enzyme is supported by the findings of Klomklao *et al.*, (2007) who stated that the protease activity of the 50-60% ammonium sulphate fish waste samples was lesser than the activity of the crude.

Thus it can be deduced from the study on protease activity that precipitation with ammonium sulphate and purification by dialysis resulted in a significantly ($P<0.05$) highest value (11.13 U/ml).

Specific activity

From Table -1 & Figure 3,it can be stated that the specific activity of, the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate precipitated + Sephadex G-100 run samples of the enzyme isolated from the visceral organ waste of fish increased significantly ($P<0.05$) on comparison with the specific activity of the crude enzyme (3.08 U/mg).

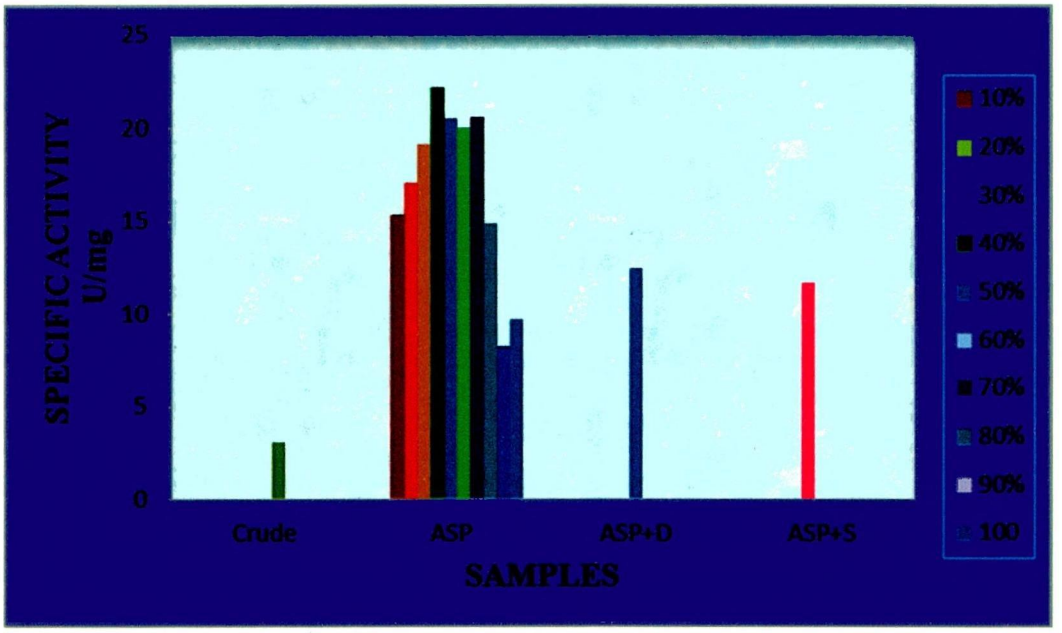
Among the ammonium sulphate precipitated samples, the 40% ammonium sulphate precipitated sample registered the highest specific activity (22.15 U/mg) which was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by dialysis of the ammonium sulphate precipitated sample gave a higher specific activity (37. 99 U/mg), which was significant ($P<0.05$) than purification by Sephadex G-100 (32.77 U/mg).

Thus it can be concluded from Table1 and Figure 3 that precipitation with ammonium sulphate followed by purification by dialysis increased the specific activity of the protease enzyme to the maximum.

FIGURE- 3

Specific activity of ammonium sulphate precipitated and purified samples from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish



ASP – Ammonium Sulphate Precipitated sample

ASP + D - Ammonium Sulphate Precipitated + Dialysed sample

ASP + S - Ammonium Sulphate Precipitated + Sephadex G-100 run sample

Purification fold

From Table I it is understood that the purification fold, of the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed and ammonium sulphate precipitated + Sephadex G-100 run samples of the visceral

organ wastes of fish recorded significantly ($P<0.05$) higher values than the crude sample (1.00)

Among the ammonium sulphate precipitated samples, the 40% precipitated sample registered the highest purification fold of 7.4, which was significant ($P<0.05$).

Purification by dialysis of the ammonium sulphate precipitated sample gave a significantly ($P<0.05$) higher value (12.47), than purification by Sephadex G-100 (11.69).

Thus it is clear from the data on purification fold that precipitation with 40% ammonium sulphate and purification by dialysis recorded the highest value (12.47) for purification fold.

Recovery %

The recovery % of all the samples from the visceral organ wastes of fish as shown in Table 1 were significantly ($P<0.05$) lower than those of the crude sample (100%).

Among the ammonium sulphate precipitated samples, the highest recovery was recorded by the 40% precipitated sample (72%).

Purification by dialysis of the 40% ammonium sulphate precipitated sample increased the recovery % (64%), significantly ($P<0.05$) than purification by Sephadex G-100 (50%).

Thus it can be inferred from the above study that the maximum recovery % was with the ammonium sulphate precipitated sample (72%) and then purified by dialysis (64%).

4.2 Purification scheme of protease from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by ammonium sulphate

TABLE- II

Purification scheme of protease from head and tail wastes of Indian oil sardine (*Sardinella longiceps)* fish precipitated by ammonium sulphate

S. No	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	7.7±0.03	23.56±0.03	3.08±0.03	1±SD	100±SD
II	Ammonium Sulphate precipitated					
1	10%	0.05±0.009	11.55±0.002	25.7±0.23	8.48±0.357	45±0.002
2	20%	0.04±0.001	12.36±0.03	30.46±0.8	9.91±0.180	48±0.002
3	30%	0.3±0.003	14.32±0.0072	47.56±0.1	15.69±0.330	55±0.004
4	40%	0.31±0.15	16.23±0.050	51.64±0.4	16.39±0.332	63±0.021
5	50%	0.33±0.01	19.42±0.06	58.33±0.4	19.24±0.151	75±0.004
6	60%	0.28±0.03	15.66±0.061	55.36±1.9	18.21±0.805	54±0.002
7	70%	0.26±0.005	12.54±0.020	47.98±0.4	15.84±0.240	48±0.002
8	80%	0.21±0.015	9.82±0.01	46.12±1.0	15.21±0.295	37±0.001
9	90%	0.23±0.027	9.09±0.03	44.94±0.5	14.82±0.188	35±0.002
10	100%	0.25±0.01	9.1±0.015	36.46±0.8	12.02±0.790	35±0.007
III	50% Ammonium Sulphate precipitated +Dialysed	0.61±0.04	15.26±0.44	23.31±3.7	27.69±0.1592	60±0.0177
IV	50% Ammonium Sulphate Precipitated + Sephadex G-100	0.66±0.02	14.28±0.01	23.73±0.3	27.83±0.250	55±0.0033
CD (0.05)		**0.643**	**0.45**	**0.325**	**7.213**	**7.1**

All values are mean of triplicates

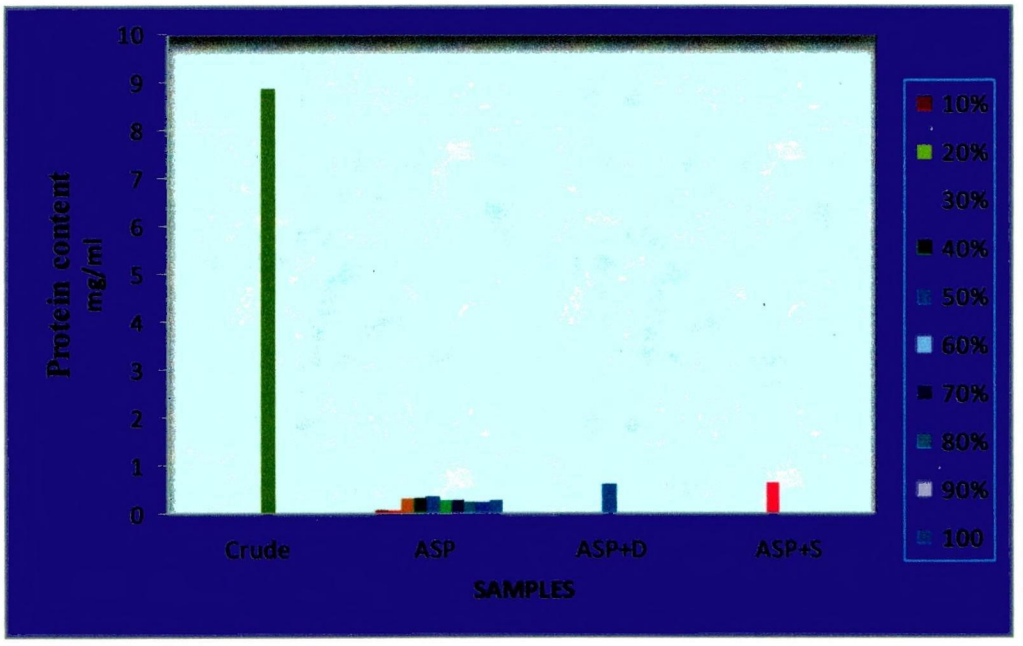
protease enzyme isolated from the visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish. Figures 4, 5 and 6 depict the protein content, protease activity and specific activity respectively of the protease enzyme isolated from the visceral organ wastes of Indian oil sardine *(Sardinella longiceps*) fish.

Protein content

From Table-2 and Figure-4, it is understood that the protein contents of ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate precipitated + Sephadex G-100 run samples from the enzyme isolated from the visceral organ wastes of fish decreased significantly ($P<0.05$) on comparison with protein content of the crude extract (7.7 mg/ml)

FIGURE- 4

Protein content of ammonium sulphate precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



ASP- Ammonium sulphate

ASP + Dialysed - Ammonium Sulphate Precipitated + Dialysed

ASP + S - Ammonium Sulphate Precipitated + Sephadex G-100 run sample

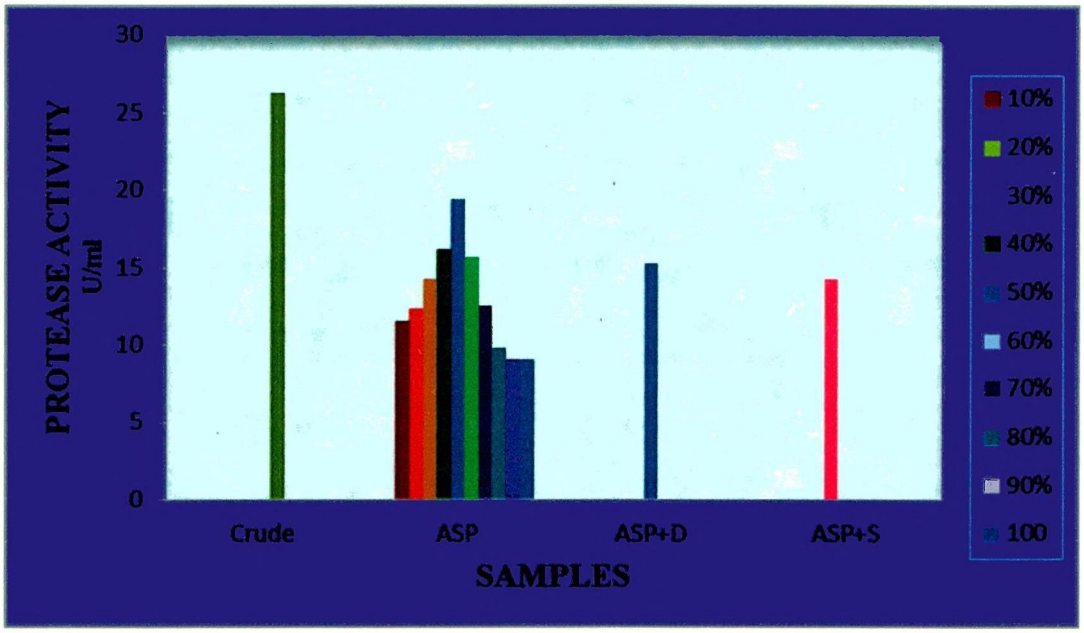
Among the ammonium sulphate precipitated samples, the 50% ammonium sulphate precipitated samples registered the highest protein content (0.33 mg/ml), which was significant ($P<0.05$) when compared to all the other samples of the same group.

Protease activity

A similar pattern as that for protein content was also followed for protease activity (Table II & Figure-5) where the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate

FIGURE- 5

Protease activity of ammonium sulphate precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps)* fish



ASP - Ammonium sulphate

ASP + Dialysed - Ammonium Sulphate Precipitated + Dialysed

ASP + S - Ammonium Sulphate Precipitated + Sephadex G-100 run sample precipitated + Sephadex G-100 run samples from head and tail wastes of fish showed values lower than that of the crude extract sample (23.56 U/ml).

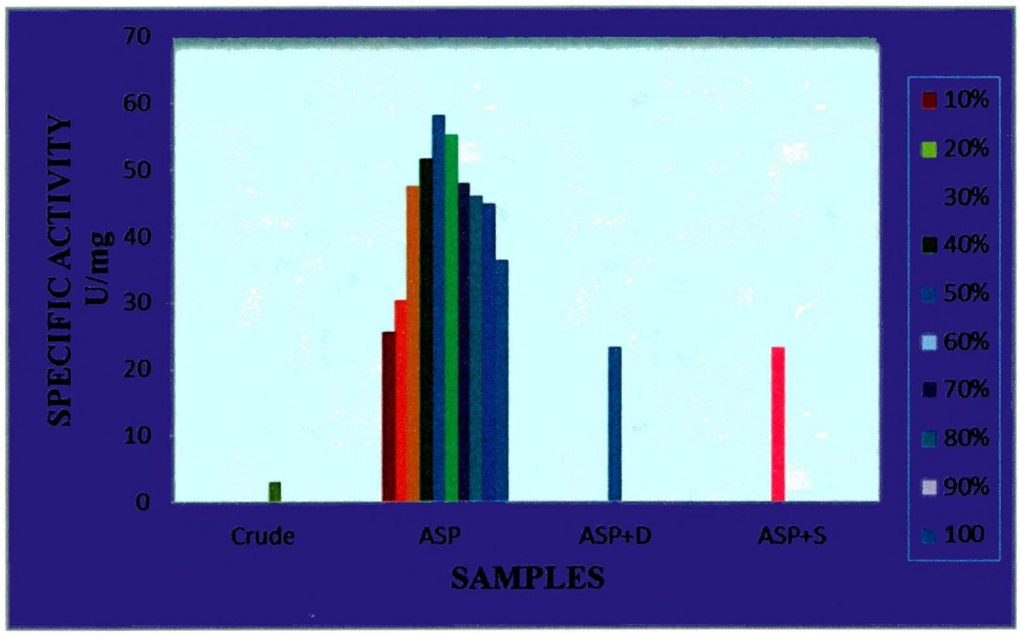
Here also, in the ammonium sulphate precipitated group the 50% precipitated sample exhibited the highest protease activity (19.42 U/ml), which was significant ($P<0.05$).

Specific activity

From Table II and Figure-6 it can be stated that the specific activity of head and tail wastes of Indian oil sardine increased significantly ($P<0.05$) in the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed, ammonium sulphate precipitated + Sephadex G-100 run samples on comparison with the specific activity of crude enzyme sample (3.08 mg/ml).

FIGURE- 6

Specific activity of ammonium sulphate precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps)* fish



ASP- Ammonium sulphate

ASP + Dialysed -Ammonium Sulphate Precipitated + Dialysed

ASP + S - Ammonium Sulphate Precipitated + Sephadex G-100 run sample

Among the ammonium sulphate precipitated samples, the 50% ammonium sulphate precipitated sample registered the highest specific activity (58.33 U/mg) when compared to all the other samples of the same group.

Purification by dialysis and Sephadex G-100 column of the 50% ammonium sulphate precipitated sample gave similar specific activities of 23.31 U/mg and 27.73 U/mg which were significant ($P<0.05$). These values are on parr with those reported by Qi *et al.*, (2007) who stated that the specific activity of protease enzyme from Sea cucumber fish purified by ion exchange chromatography was 21.94 U/mg.

Thus it can be inferred from the studies on specific activity that precipitation with 50% ammonium sulphate followed by purification on Sephadex G-100 column recorded the highest value of 23.73 U/mg.

Purification fold

As regards the purification fold, Table II shows that the ammonium sulphate precipitated, ammonium sulphate precipitated + dialyzed sample and ammonium sulphate precipitated + Sephadex G-100 run samples from head and tail wastes of fish recorded an increase in the purification fold as compared to the crude enzyme (1.00).

Among the ammonium sulphate precipitated samples, the 50% ammonium sulphate precipitated sample registered a significantly ($P<0.05$) highest purification fold (19.24) as compared to the other samples of the same group.

Purification by dialysis of the 50% ammonium sulphate precipitated samples gave a similar purification fold (27.69) to that of purification by Sephadex G-100 run sample (27.83).

Thus it can be inferred from the data on purification fold that precipitation with ammonium sulphate followed by purification with Sephadex G-100 registered the highest purification fold of 27.83 which was significant ($P<0.05$).

Recovery %

The Recovery % of all the samples from the head and tail wastes of fish were decreased on comparison with the crude (100).

Among the ammonium sulphate precipitated samples, the highest recovery was exhibited by the 50% ammonium sulphate precipitated sample (75%), and this value was significant ($P<0.05$). These results are similar to the one reported by Arulmani *et al.*, 2007 who revealed that the recovery of protease from thermostable alkalophilic *Bacillus laterospores* strain by acetone precipitation was 74.7%.

Purification by dialysis of the ammonium sulphate precipitated sample gave a significantly ($P<0.05$) increased recovery % (60%) than purification by Sephadex G-100 run sample (55%). A study by Arulmani *et al.*, 2007 also reported a similar finding that purification of protease from thermostable alkalophilic *Bacillus laterospores* strain by Sephadex G-100 recorded a 55% recovery.

Thus, it can be concluded from the study on the Recovery % that precipitation with 50% ammonium sulphate gave the maximum recovery % (75%) of the enzyme protease isolated from the fish wastes.

4.3 Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by acetone

Table-III depicts the purification scheme, namely, protein content, protease activity, specific activity, purification fold and recovery % for the protease enzyme isolated from the visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish. Figure 7, 8 and 9 depict the protein content, protease activity and specific activity respectively of the protease enzyme

TABLE – III

Purification scheme of protease from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by acetone

S. No	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	8.85±0.001	26.82±0.84	3.0803±0.01	1±SD	100±SD
II	Acetone precipitated					
1	10%	0.34±0.015	5.94±0.020	27.05±1.1	3.91±0.11	22±0.72
2	20%	0.38±0.004	10.88±0.06	31.42±1.4	3.94±0.71	41±1.10
3	30%	0.42±0.005	11.3±0.05	35.76±0.5	4.18±0.49	42±1.47
4	40%	0.49±0.01	12.46±0.15	37.03±1.0	6.15±0.54	47±1.72
5	50%	0.54±0.011	16.84±0.05	52.85±1.8	7.07±1.14	62±2.04
6	60%	0.45±0.005	15.36±0.07	41.68±1.1	6.07±0.17	58±1.98
7	70%	0.42±0.01	15.28±0.03	33.72±0.4	5.42±0.34	54±1.62
8	80%	0.39±0.09	14.08±0.03	27.08±0.5	5.32±0.07	53±1.48
9	90%	0.38±0.01	10.49±0.05	17.55±2.5	5.28±0.78	39±1.05
10	100%	0.32±0.07	9.38±0.05	12.19±0.1	4.02±0.12	35±1.23
III	50% Acetone precipitated +Dialysed	0.49±0.07	15.34±0.07	31.58±5.8	8.27±1.35	57±1.93
IV	50% Acetone precipitated + Sephadex G-100	0.45+0.01	12.58±0.34	23.30±0.6	7.87±0.34	47±2.08
CD (0.05)		**0.263**	**0.470**	**0.545**	**3.977**	**5.620**

All the values are mean of triplicates

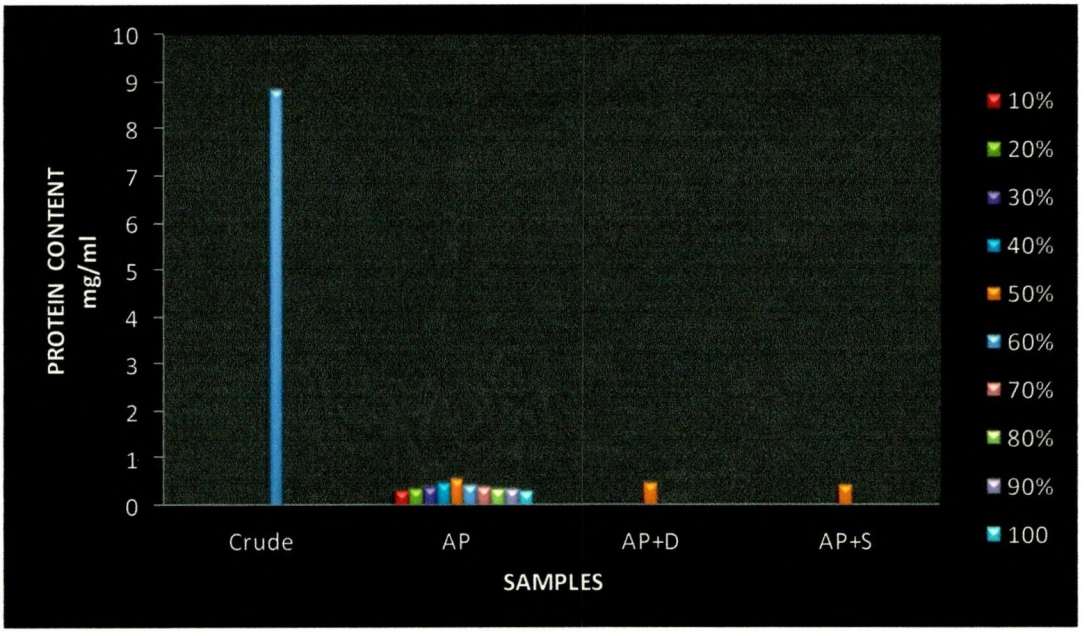
Isolated from the visceral organ wastes of Indian oil sardine *(Sardinella longiceps)* fish.

Protein content

From Table-III and Figure-7, it is understood that the protein contents of acetone precipitated, acetone precipitated+ dialyzed, acetone precipitated + Sephadex G-100 run samples from the visceral organ wastes of fish decreased significantly ($P<0.05$) on comparison with the protein content of the crude (8.85 mg/ml) enzyme extract.

FIGURE- 7

Protein content of acetone precipitated and purified samples from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone Precipitated sample

AP + D – Acetone Precipitated + Dialysed

AP + S – Acetone Precipitated + Sephadex G-100 run sample

Among the acetone precipitated samples, the 50% acetone precipitated sample registered significantly ($P<0.05$) the highest protein content (0.54 mg/ml), when compared to all the other samples of the same group.

Purification by dialysis of the acetone precipitated gave significantly higher protein content (0.49 mg/ml) than purification by Sephadex G-100 (0.45 mg/ml).

Thus it can be inferred from the values of protein content that precipitation with 50% acetone followed by dialysis gave significantly ($P<0.05$) the highest value (0.54 mg/ml).

The above results indicating the protein content of the acetone precipitated fish waste samples to be significantly ($p<0.05$) lesser than the protein content of the crude sample agrees with the study of Kurtovic *et al.*, (2006) who stated that the protein content of the crude extract of Chinook salmon fish was found to be higher than the protein content of the acetone precipitated samples.

Protease activity

From Table III & Figure 8,it can be observed that the protease activity of the acetone precipitated, acetone precipitated + dialyzed, acetone precipitated + Sephadex G-100 run samples from viscersal organ waste of fish showed values lower than that of the crude sample (26.82 U/ml)

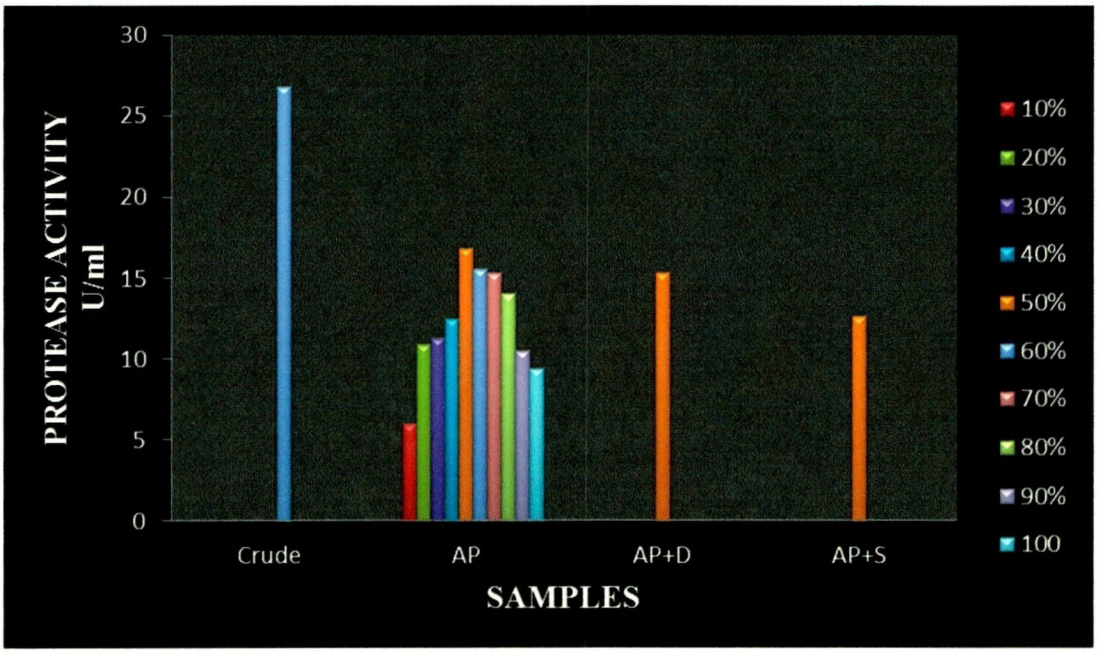
Here also, in the acetone precipitated group, the 50% precipitated sample exhibited the highest protease activity (16.84 U/ml), which was significant ($P<0.05$) when compared to all the other acetone precipitated samples of the same group.

Purification by dialysis of the 50% acetone precipitated samples gave a significantly ($P<0.05$) higher protease activity (15.34 U/ml) than purification by Sephadex G-100 (12.58 U/ml).

Thus it can be deduced from the studies on protease activity that precipitation 50% with acetone registered the highest activity for protease (16.84 U/ml).

FIGURE- 8

Protease activity of acetone precipitated and purified samples from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone Precipitated

AP + D – Acetone Precipitated + Dialysed

AP + S – Acetone Precipitated + Sephadex G-100 run sample

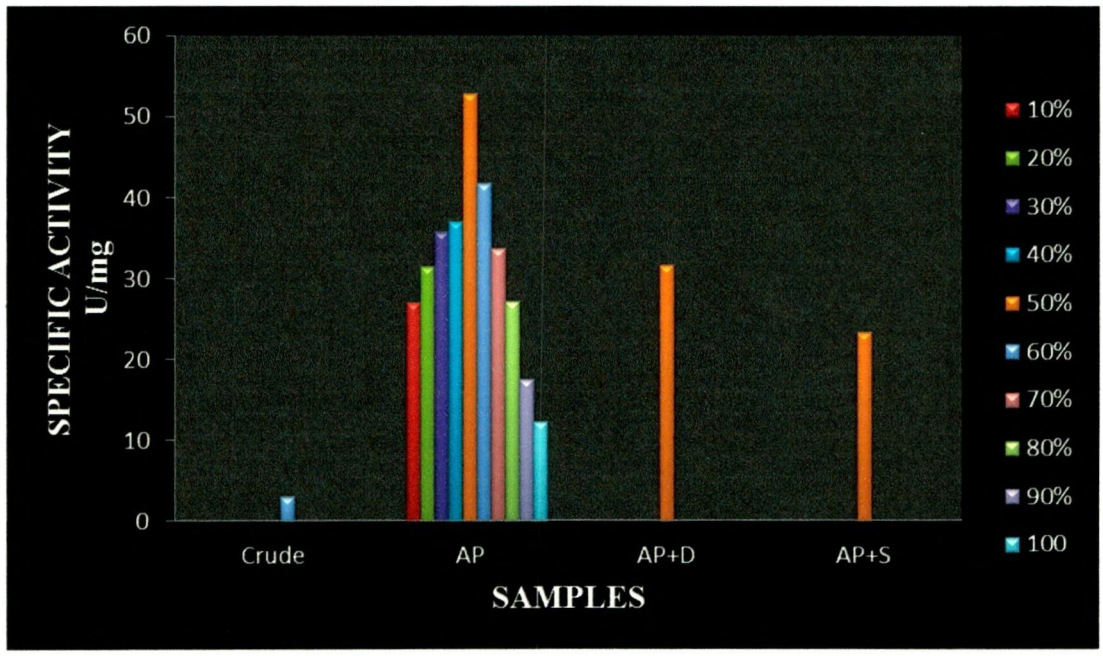
Specific activity

It can be seen from Table II & Figure-9 that the specific activity of the enzyme from the visceral organ waste of Indian oil sardine fish exhibits values significantly ($P<0.05$) higher than the crude (3.08 U/mg), for acetone precipitated + dialysed, acetone precipitated + Sephadex G-100 run samples.

Among the acetone precipitated samples, the 50% precipitated sample registered the highest specific activity (52.85 U/mg) which was significant ($P<0.05$) when compared to all the other samples of the same group.

FIGURE- 9

Specific activity of acetone precipitated and purified samples from visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone Precipitated

AP + D – Acetone Precipitated + Dialysed

AP + S – Acetone Precipitated + Sephadex G-100 run sample

Purification by dialysis of the acetone precipitated sample gave a specific activity (31.58 U/mg), which was significantly ($P<0.05$) higher than the value for Sephadex G-100 run sample (23.30 U/mg).

Thus it can be inferred from the studies on specific activity that precipitation 50% with acetone recorded the highest specific activity of the protease enzyme (52.85 UU/mg).

The finding that the specific activity increased on dialysis of the 50% acetone precipitated sample is in agreement with the study of Hinsui *et al.*, (2006) who stated that the enzyme activity and protein decreased at each stage of purification while the specific activity increased.

Purification fold

As regards the purification fold, (Table II) the acetone precipitated, acetone precipitated + dialyzed and acetone precipitated + Sephadex G-100 run samples from visceral organ waste of fish recorded significantly higher purification fold on comparison with the crude sample (1.00)

Among the acetone precipitated samples, the 50% acetone precipitated sample registered the highest purification fold (7.07), which was significant ($P<0.05$) when compared to the other samples of the same group.

Purification by dialysis of the 50% acetone precipitated samples gave a better purification fold (8.27) which was significant ($P<0.05$) than purification by Sephadex G-100 run sample (7.87).

Thus it can be inferred from the data on purification fold that precipitation with acetone and purification by dialysis increased the purification fold significantly ($P<0.05$) to the maximum extent (8.27).

Recovery %

The Recovery % of all the samples (Table III) from the visceral organ wastes of fish were increased on comparison with the crude (100%).

Among the acetone precipitated samples, the highest recovery was recorded by the 50% acetone precipitated sample (62%), and this value was significant ($P<0.05$).

Purification by dialysis of the acetone precipitated gave significantly ($P<0.05$) an increase in the recovery % (57%) than purification by Sephadex G-100 run sample (47%). A study by Arulmani *et al.*, 2007 also reported a similar finding that purification of protease from thermostable alkalophilic *Bacillus laterospores* strain by Sephadex G-100 recorded a 55% recovery of the enzyme.

Thus, it can be concluded from the study on the recovery % that precipitation with acetone and purification by dialysis of the acetone

precipitated samples and Sephadex G-100 decreased the recovery % of the enzyme protease isolated from the fish waste.

4.4 Purification scheme of protease from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by acetone

Table-IV represent the purification scheme, namely, protein content, protease activity, specific activity, purification fold and recovery % for the protease enzyme isolated from the visceral organ wastes of Indian oil sardine (*Sardinella longiceps*) fish. Figures 10, 11 and 12 indicate the protein content, protease activity and specific activity respectively of the protease enzyme isolated from the visceral organ wastes of Indian oil sardine *(Sardinella longiceps*) fish.

Protein content

From Table-IV and Figure-10, it is understood that the protein contents of acetone precipitated, acetone precipitated+ dialyzed, acetone precipitated + Sephadex G-100 run samples from the head and tail wastes of the fish decreased significantly ($P<0.05$) on comparison with the protein content of the crude enzyme (7.65 mg/ml).

Among the acetone precipitated samples, the 60% precipitated sample registered the highest protein content (0.86 mg/ml), and this was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by dialysis of the acetone precipitated samples gave a higher protein content (0.56 mg/ml), which was significant ($P<0.05$) than purification by Sephadex G-100 dialysis (0.25 mg/ml).

Hence it can deduced from the analysis of protein content that the maximum value was given by 60% acetone precipitated sample (0.86 mg/ml)

TABLE – IV

Purification scheme of protease precipitated and purified samples from head and tail

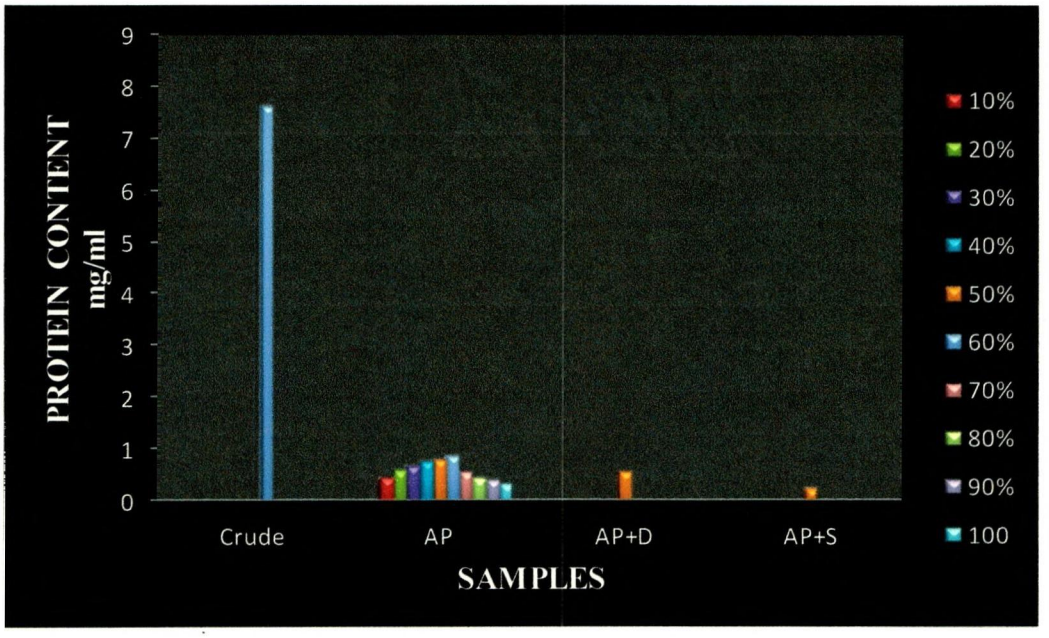
Synod	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	7.65±0.001	23.563±0.8	3.08±0.09	1±SD	100±SD
II	Acetone precipitated					
1	10%	0.44±0.03	8.89±0.04	18.84±1.200	6.22±0.541	33±0.8461
2	20%	0.59±0.025	10.31±0.05	11.94±0.28	3.94±0.031	39±1.370
3	30%	0.67±0.028	11.3±0.04	17.32±0.71	5.71±0.121	42±1.45
4	40%	0.78±0.056	13.65±0.11	15.26±0.93	5.03±0.324	51±1.96
5	50%	0.80±0.035	16.25±0.08	19.03±0.7	6.28±0.390	61±2.15
6	60%	0.86±0.020	18.26±0.03	31.08±1.15	10.51±0.125	67±2.47
7	70%	0.55±0.028	17.33±0.09	28.77±1.64	9.82±0.63	65±2.22
8	80%	0.45±0.080	14.33±0.05	27.72±4.28	9.17±1.69	53±1.81
19	90%	0.42±0.047	13.41±0.155	19.57±1.10	6.46±0.38	50±1.89
10	100%	0.38±0.02	11.27±0.02	14.4±0.51	4.75±0.22	42±3.107
III	60% Acetone precipitated + dialysed	0.56±0.005	16.39±0.01	42.32±1.43	20.38±0.319	57±1.76
IV	60% Acetone precipitated + Sephadex G-100	0.25±0.025	15.24±0.124	23.3±1.41	10.08±0.163	61±1.92
CD (0.05)		**0.533**	**0.673**	**0.865**	**4.960**	**6.280**

All the values are mean of triplicates

Wastes of Indian oil sardine (*Sardinella longiceps*) fish precipitated by acetone

FIGURE- 10

Protein content of acetone precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone precipitated sample

AP + D – Acetone precipitated + Dialysed

AP + S – Acetone precipitated + Sephadex G-100

Protease activity

A similar trend as that of the protein content was followed for protease activity (Table IV and Figure-11) also where the acetone precipitated, acetone precipitated + dialyzed, acetone precipitated + Sephadex G-100 run samples of the head and tail wastes of fish showed significantly lower values than that of crude enzyme sample (23.56 U/ml).

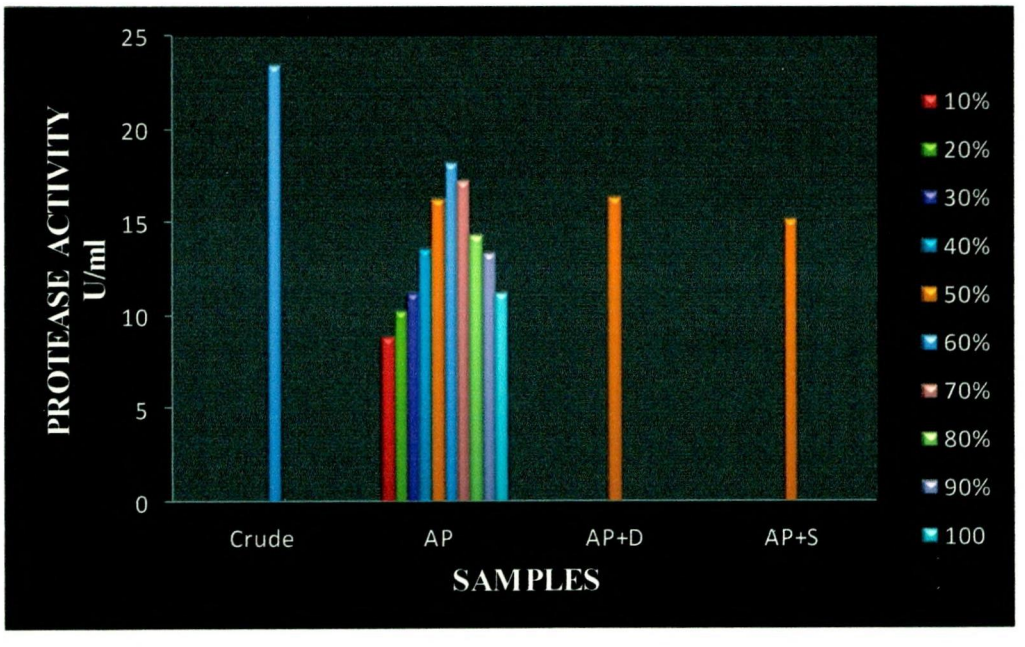
Here also, in the acetone precipitated group, the 60% acetone precipitated sample exhibited the highest protease activity (18.26 U/ml), which was very significant ($P<0.05$).

Purification by dialysis of the acetone precipitated samples gave a higher protease activity (16.39 U/ml), which was significant ($P<0.05$) than purification by Sephadex G-100 (15.24 U/ml).

It can be therefore concluded that the highest protease activity was registered by 60% acetone precipitated sample (18.26 U/ml)

FIGURE- 11

Protease activity of acetone precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone Precipitated

AP + D – Acetone Precipitated + Dialysed

AP + S – Acetone Precipitated + Sephadex G-100

Specific activity

The data in Table IV and Figure 12 reveal that the specific activity of the acetone precipitated, acetone precipitated + dialyzed, acetone precipitated + Sephadex G-100 run samples of the head and tail wastes of fish increased significantly ($P<0.05$) on comparison with the specific activity of the crude enzyme (3.08 U/mg).

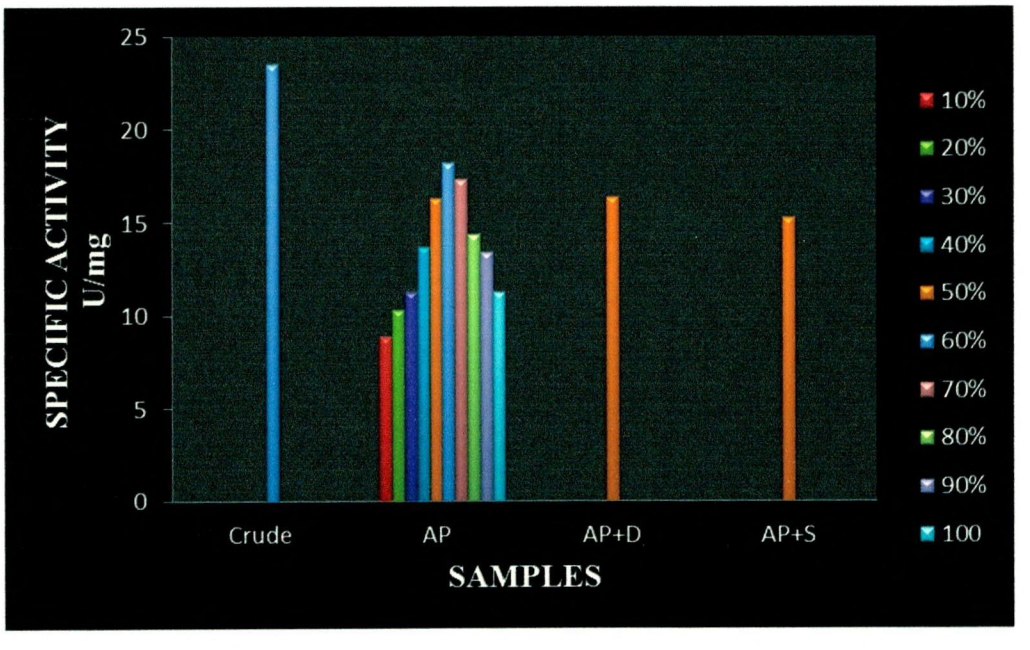
Among the acetone precipitated samples, the 60% acetone precipitated sample registered the highest specific activity (31.08 U/mg) which was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by dialysis of the acetone precipitated samples gave a higher specific activity (42.32 U/mg), which was significant ($P<0.05$) than purification by Sephadex G-100 (23.3 U/mg).

Thus it can be concluded from Table IV and Figure 12 that precipitation with 60% acetone and purification by dialysis of the acetone precipitated samples significantly ($P<0.05$) and increased the specific activity of the protease enzyme to the maximum (42.32 U/mg)

FIGURE- 12

Specific activity of acetone precipitated and purified samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



AP – Acetone Precipitated

AP + D – Acetone Precipitated + Dialysed

AP + S – Acetone Precipitated + Sephadex G-100

Purification fold

From Table IV it is understood that the purification fold of the acetone precipitated, acetone precipitated + dialyzed and acetone precipitated + Sephadex G-100 run samples of the head and tail wastes of fish recorded significantly ($P<0.05$) highest values than the crude (1.00). On comparison with the crude sample (1.00), the purification fold of all the other groups of sample were significantly ($P<0.05$) higher.

Among the acetone precipitated samples, the 60% acetone precipitated sample registered the highest purification fold (10.51), which was significant ($P<0.05$).

Purification by dialysis of the acetone precipitated sample gave a significantly ($P<0.05$) better purification fold (20.38), than purification by Sephadex G-100 (10.38).

Thus it is clear from the data on purification fold that precipitation with acetone and purification by dialysis increased the purification fold of the isolated fish waste enzyme to the maximum (20.38) when compared to that of the crude.

Recovery %

From the observations of Table IV, it is understood that the recovery % of all the samples from the head and tail wastes of fish were significantly ($P<0.05$) on comparison with that of the crude enzyme100%.

Among the acetone precipitated samples, the highest recovery was recorded by the 60% acetone precipitated sample (67%), and this was significant ($P<0.05$).

Purification on Sephadex G-100 column of the acetone precipitated samples gave a significantly ($P<0.05$) higher recovery % (61%), than purification by dialysis (57%).

Thus it is understood from the findings on recovery % that precipitation with 60% acetone gave the maximum recovery % of the enzyme (67%) isolated from fish wastes.

4.5 Purification scheme of protease from visceral organ wastes of fish Indian oil sardine *(Sardinella longiceps)* fish precipitated by ethanol

Table V and Figures 13, 14 & 15 depict the purification scheme (protein content, protease activity, specific activity, purification fold and recovery %) of the protease enzyme isolated from the visceral organ wastes of Indian oil sardine (Sardinella *longiceps*) fish.

Protein content

From Table V and Figure 13 it is understood that the protein contents of ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated + Sephadex G-100 run protease enzyme samples from the visceral organ wastes of the selected fish decreased significantly ($P<0.05$) on comparison with the protein content of the crude enzyme extract (8.85 mg/ml).

Among the ethanol precipitated samples, the 50% ethanol precipitated sample registered the highest protein content (0.35 mg/ml) which was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by dialysis of the 50% ethanol precipitated sample gave a higher protein content (0.65 mg/ml), than purification by dialysis chromatography of the 50% ethanol precipitated sample (0.56 mg/ml).

Thus, it can be inferred from the values of protein content that purification of the ethanol precipitated enzyme sample by Sephadex G-100 recorded the maximum protein content (0.65 mg/ml) as compared to all the other samples.

TABLE – V

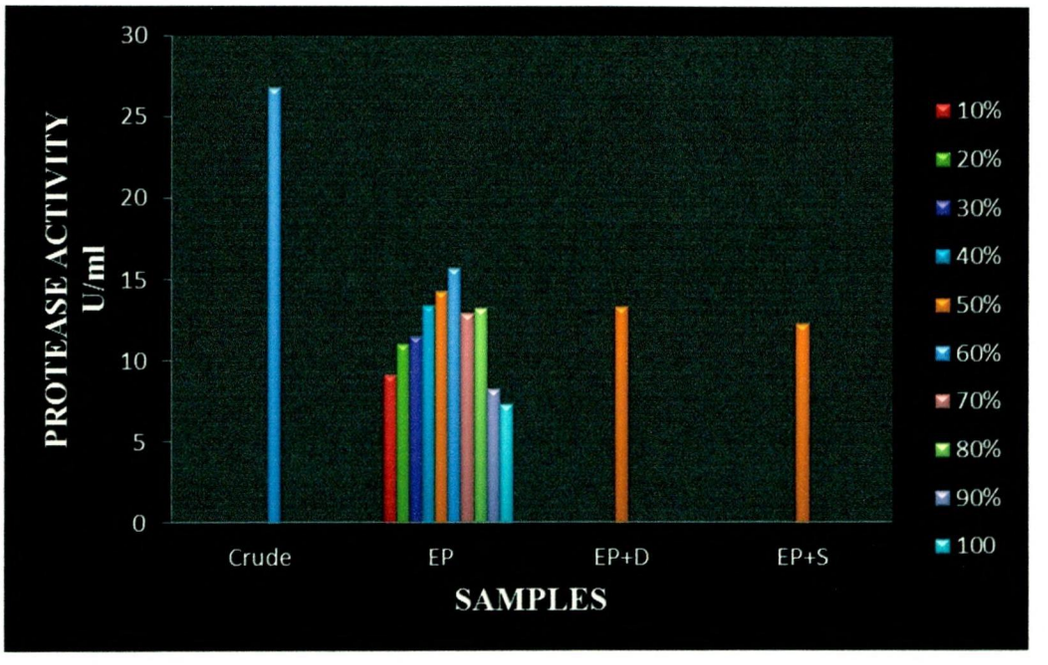
Purification scheme of protease from visceral organ wastes of Indian oil sardine *(Sardinella longiceps)* fish precipitated by ethanol

S.No	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	8.846±0.03	26.82±0.003	3.08±0.163	1±SD	100±SD
II	Ethanol Precipitated					
1	10%	0.22±0.03	9.14±0.077	41.05±2.41	13.83±0.92	39±0.073
2	20%	0.24±0.015	11.02±0.05	45.54±4.24	14.79±1.50	47±0.53
3	30%	0.25±0.02	11.52±0.04	46.75±1.01	15.16±2.10	49±0.50
4	40%	0.32±0.01	14.29±0.07	46.75±1.01	16.17±2.17	67±0.56
5	50%	0.35±0.03	15.73±0.06	55.84±1.38	18.12±2.67	57±0.81
6	60%	0.28±0.04	13.44±0.10	44.96±0.19	14.59±0.21	61±0.73
7	70%	0.27±0.01	12.91±0.04	40.68±4.72	13.19±1.4	56±0.45
8	80%	0.26±0.03	13.22±0.03	38088±0.10	12.62±0.16	55±0.49
9	90%	0.23±0.05	8.24±0.015	37.12±4.69	16.56±2.0	35±0.30
10	100%	0.2±0.026	7.34±0.012	32.5±5.8	12.04±1.35	31±0.74
III	Ethanol Precipitated +Dialysed	0.65±0.02	13.32±0.01	21.9±0.843	7.11±0.304	62±0.37
IV	Ethanol Precipitated + Sephadex G-100	0.56±0.098	12.3±0.126	20.72±3.61	6.73±1.24	52±0.90
CD(0.05)		0.248	0.270	0.403	5.27	3.42

All the three values are mean of triplicates

FIGURE- 14

Protease activity of ethanol precipitated samples from wastes of visceral organ Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated sample

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

Purification by dialysis of the 50% ethanol precipitated sample gave a significantly ($P<0.05$) higher protease activity (13.32 U/ml), than purification by Sephadex G-100 (12.3 U/ml).

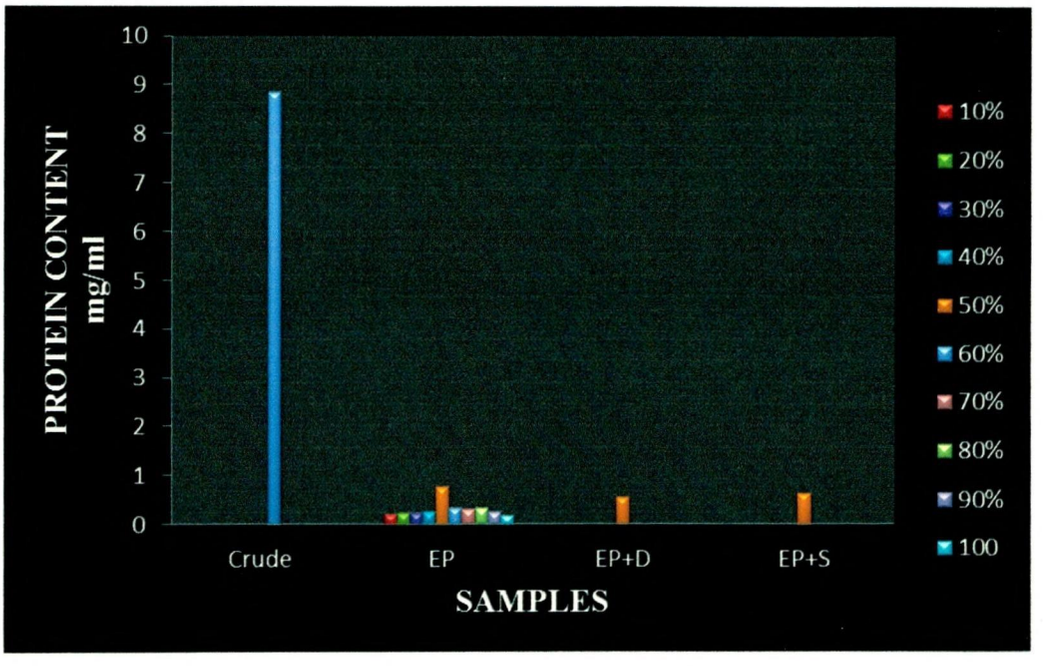
Thus it can be deduced from the study on protease activity that precipitation with 50% ethanol increased the protease activity of the fish waste samples to the highest level (15.73 U/ml)

Specific activity

From Table -V Figure-15 it is obvious that the specific activity of the enzyme isolated from the visceral organ wastes of Indian oil sardine fish,

FIGURE- 13

Protein content of ethanol precipitated samples of visceral organ wastes from Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated sample

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

Protease activity

Here again, the protease activity (Table V Figure-14) of the ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated + Sephadex G-100 run enzyme samples from the visceral organ wastes of fish showed significantly ($P<0.05$) lower values than that of crude enzyme extract (26.82 U/ml).

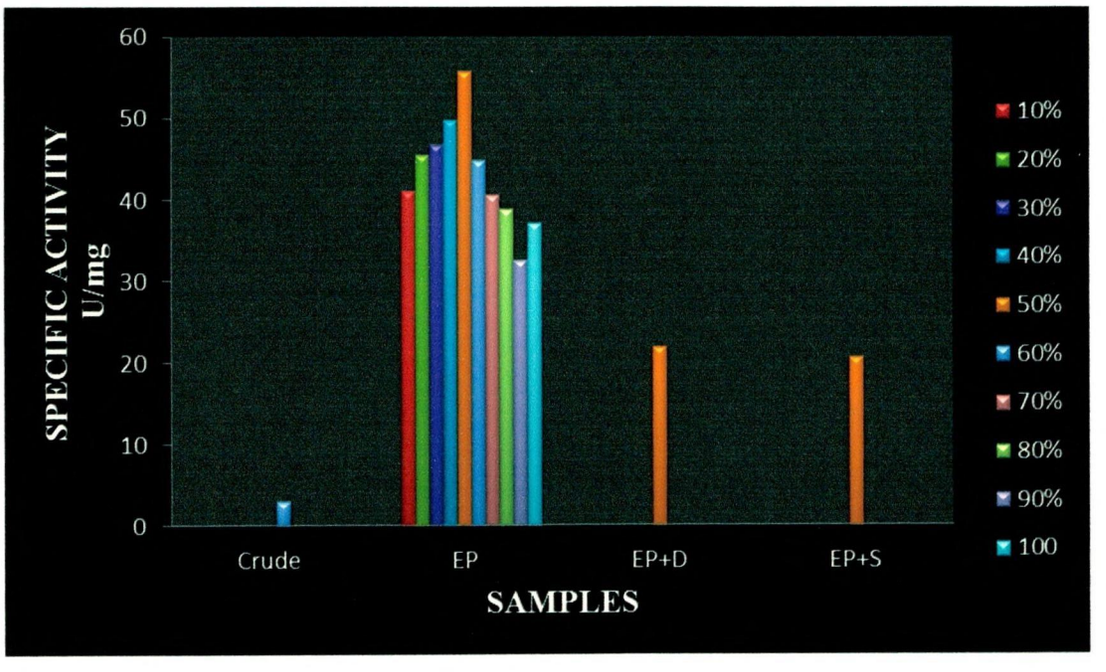
Here also, in the ethanol precipitated group, the 50% ethanol precipitated sample significantly ($P<0.05$) sample exhibited significantly the highest protease activity (15.73 U/ml), when compared to the samples of the same group.

recorded significantly higher values for specific activity in ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated + Sephadex G-100 run samples on comparison with the specific activity of the crude extract sample (3.08 U/mg).

Among the ethanol precipitated samples, the 50% ethanol precipitated sample registered significantly ($P<0.05$) the highest specific activity (55.84 U/mg) when compared to all the other samples of the same group.

Figure- 15

Specific activity of ethanol precipitated samples from wastes of visceral organ Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated sample

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

Purification by dialysis of the 50% ethanol precipitated samples gave a better specific activity (21.90 U/mg), which was significantly ($P<0.05$) than purification by Sephadex G-100 run sample (20.72 U/mg).

It therefore be deduced that precipitation with 50% ethanol resulted in the highest specific activity (55.84 U/mg) which was very significant ($P<0.05$).

Purification fold

As regards the purification fold, ethanol precipitated + dialyzed sample and ethanol precipitated + Sephadex G-100 run samples of the isolated enzyme from the visceral organ wastes of fish recorded the highest values (7.11 & 6.73 respectively). On comparison with the crude sample (1.00), the purification fold of all the other groups of sample were significantly ($P<0.05$) higher.

Among the ethanol precipitated samples, the 50% precipitated sample registered the highest purification fold (5.12), the value of which was significant ($P<0.05$).

Purification by dialysis of the 50% ethanol precipitated sample gave a significantly ($P<0.05$) better purification fold (7.11), than purification by Sephadex G-100 (6.73).

Thus it is clear from the data on purification fold that precipitation with 50% ethanol produced the maximum (18.12)purification fold of the isolated fish waste enzyme compared to that of the crude enzyme.

Recovery %

The recovery % of all the samples recorded significantly ($P<0.05$) lower values than the crude (100%).

Then purification by dialysis gave an increase in the recovery % (41%), which was significant ($P<0.05$) than purification by Sephadex G-100 (31%).

Thus it is understood from the findings on recovery % that precipitation with 40% ethanol significantly ($P<0.05$) decreased the recovery % of the enzyme isolated from fish wastes (67%).

4.6 Purification scheme of protease from head and tail wastes of fish Indian oil sardine *(Sardinella longiceps)* fish precipitated by ethanol

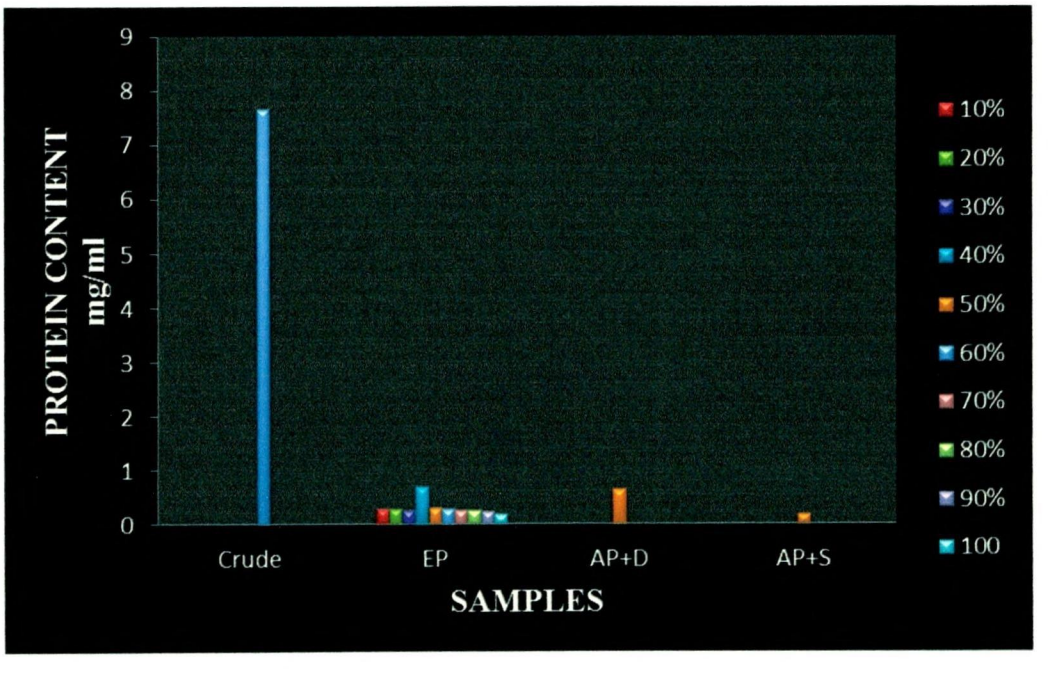
Table VI and Figures 16, 17 & 18 depict the purification scheme (protein content, protease activity, specific activity, purification fold and recovery %) of the protease enzyme isolated from the head and wastes of Indian oil sardine (Sardinella *longiceps*) fish.

Protein content

From Table VI and Figure 16, it is understood that the protein contents of ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated +

FIGURE- 16

Protein content of ethanol precipitated samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

TABLE – VI

PURIFICATION SCHEME OF PROTEASE FROM HEAD AND TAIL WASTES OF INDIAN OIL SARDINE *(Sardinella longiceps)* FISH PURIFIED BY ETHANOL PRECIPITATION

S.No	Samples	Protein content (mg/ml)	Protease Activity (U/ml)	Specific Activity (U/mg)	Purification Fold	Recovery %
I	Crude extract	7.65±0.03	23.56±0.03	3.0803±0.3	1±SD	100±SD
II	Ethanol Precipitated					
1	10%	0.3±0.015	11.92±0.81	39.34±0.09	12.97±0.28	44±1.18
2	20%	0.29±0.01	12.01±0.05	41.42±1.70	13.67±0.39	45±1.31
3	30%	0.26±0.01	14.26±0.03	52.93±0.12	17.76±1.41	53±2.15
4	40%	0.70±0.03	18.30±0.18	53.84±2.64	18.14±0.53	68±2.08
5	50%	0.31±0.02	15.31±0.02	51.86±0.06	17.11±1.40	57±1.79
6	60%	0.30±0.05	16.36±0.05	50.08±3.91	16.57±3.74	61±1.69
7	70%	0.27±0.01	15.31±0.03	48.05±9.99	15.87±1.43	56±1.59
8	80%	0.26±0.01	13.26±0.05	47.59±2.88	15.70±0.57	55±1.20
9	90%	0.25±0.01	8.43±0.012	33.77±1.70	11.14±0.54	35±0.79
10	100%	0.19±0.02	6.34±0.011	32.54±5.8	10.74±0.54	31±0.73
III	40% Ethanol Precipitated +Dialysed	0.65±0.11	8.45±0.620	44.47±0.33	14.67±0..33	41±0.73
IV	40% Ethanol Precipitated + Sephadex G-100	0.19±0.07	6.44±4.33	25.60±4.33	8.47±1.67	31±2.08
CD (0.05)		**0.22**	**1.391**	**4.88**	**1.391**	**6.27**

All the values are mean of triplicates

Sephadex G-100 run samples from the visceral organ wastes of the selected fish decreased significantly ($P<0.05$) on comparison with the protein content of the crude extract (7.65 mg/ml).

Among the ethanol precipitated samples, the 40% ethanol precipitated sample registered the highest protein content (0.70 mg/ml), which was significant ($P<0.05$) when compared to all the other samples of the same group.

Purification by dialysis of the 50% ethanol precipitated sample gave a higher protein content (0.19 mg/ml), which was significant ($P<0.05$) than purification by Sephadex G-100 (0.65 mg/ml).

Thus, it can be inferred from the values of protein content that precipitation with 40% ethanol and purification by dialysis recorded the highest (0.70 mg/ml) protein content when compared to all the other samples.

Protease activity

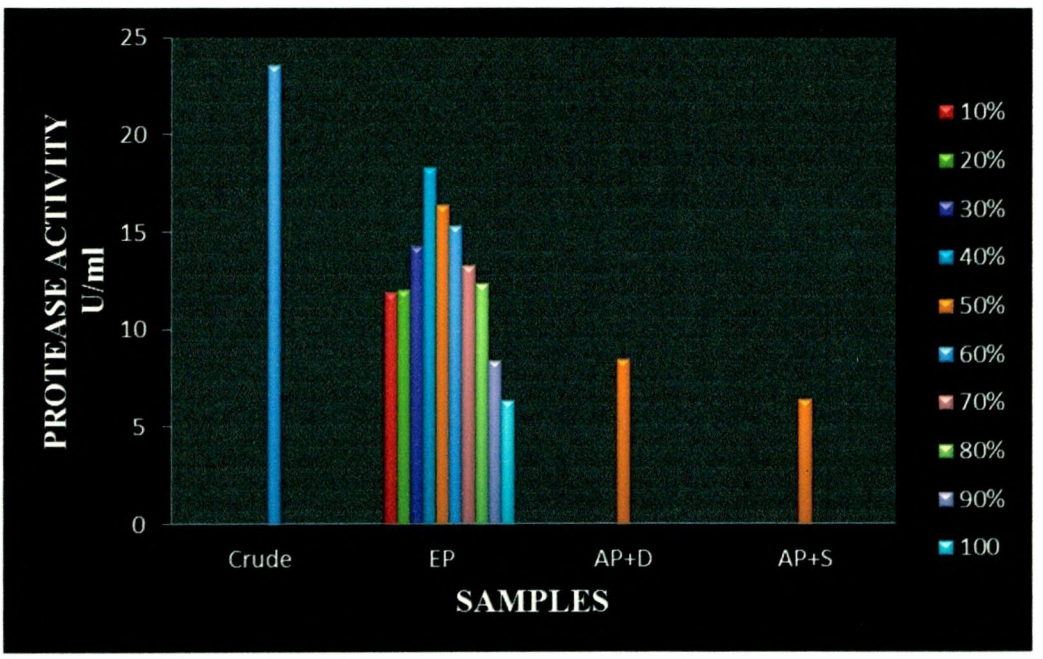
In case of protease activity (Table VI & Figure-17) also, the ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated + Sephadex G-100 run samples of the enzyme from the visceral organ wastes of the fish showed significantly ($P<0.05$) lower values than that of crude enzyme sample (23.56 U/ml).

Here also, in the ethanol precipitated group, the 40% precipitated sample exhibited the highest protease activity (18.30 U/ml). When compared to the other samples in the group which was very significant ($P<0.05$).

Purification by dialysis of the ethanol precipitated sample gave a higher protease activity (8.45 U/ml). which was significant ($P<0.05$) on comparison with the Sephadex G-100 run sample (6.44 U/ml).

FIGURE- 17

Protease activity of ethanol precipitated samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

Thus it can be deduced from the findings that the protease activity of the 40% ethanol precipitated gave the maximum (8.45 U/ml) protease activity.

Specific activity

Table V and Figure-15 it is understood from the specific activity of the enzyme isolated from the head and tail wastes of Indian oil sardine fish. ethanol precipitated, ethanol precipitated + dialyzed, ethanol precipitated + Sephadex G-100 run increased significantly ($P<0.05$) on comparison with the specific activity of the crude enzyme sample (3.08 U/mg).

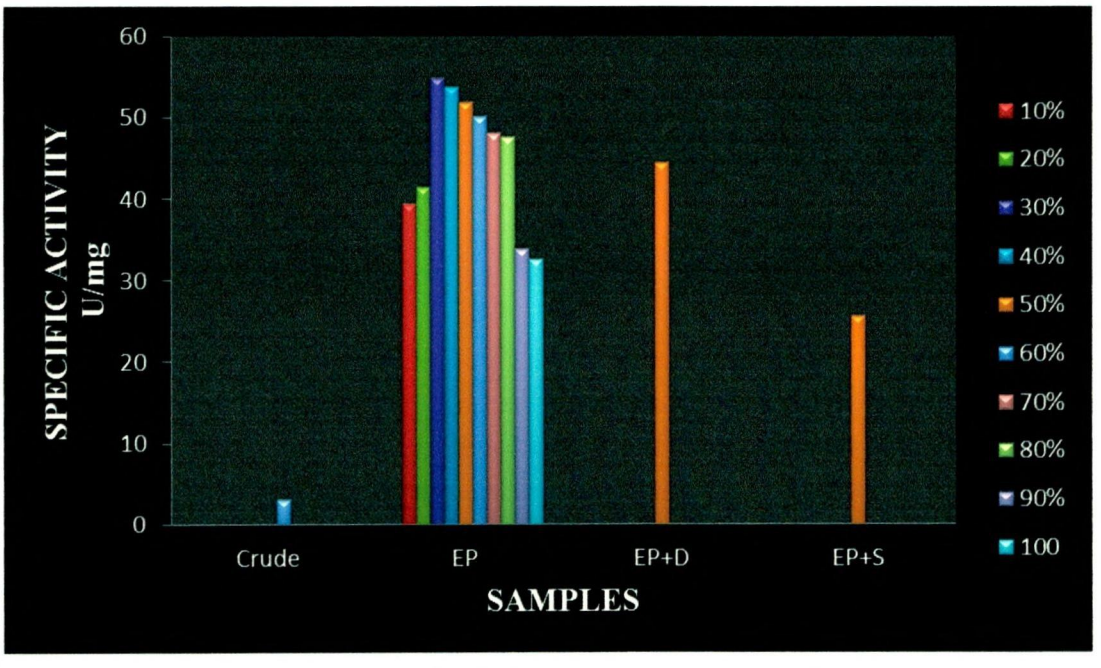
Among the ethanol precipitated samples, the 40% sample significantly ($P<0.05$) registered the highest specific activity (53.84 U/mg) when compared to all the other samples of the same group.

Purification by dialysis of the 40% ethanol precipitated sample gave a higher specific activity (44.47 U/mg), which was significant ($P<0.05$) when compared to purification by Sephadex G-100 (25.60 U/mg).

Thus, it can be concluded from the study on specific activity that precipitation with 40 % ethanol revealed the highest specific activity (53.84 U/mg) of the protease enzyme isolated from fish wastes.

FIGURE- 18

Specific activity of ethanol precipitated samples from head and tail wastes of Indian oil sardine (*Sardinella longiceps*) fish



EP – Ethanol Precipitated sample

EP + D –Ethanol Precipitated + Dialysed

EP + S – Ethanol Precipitated + Sephadex G-100

Purification fold

From Table VI, it is clear that the ethanol precipitated, ethanol precipitated + dialyzed sample followed by ethanol precipitated + Sephadex G-100 run samples from the head and tail wastes of the selected fish recorded

significantly ($P<0.05$) higher purification fold. on comparison with thecrude sample (1.00).

Among the ethanol precipitated samples, the 40% precipitated sample registered the highest purification fold (18.14), which was significant ($P<0.05$).

Dialysis of the 40% ethanol precipitated sample recorded a significantly ($P<0.05$) higher purification fold (14.67) when compared to purification on column (8.47).

Thus it is clear from the data on purification fold that precipitation with 40% ethanol registered the maximum purification fold (18.14) of the isolated fish waste enzyme compared to that of the crude.

Recovery %

The recovery % of all the samples from the head and tail wastes of fish were decreased significantly ($P<0.05$) on comparison with that of the crude enzyme.

Among the ethanol precipitated samples, the highest recovery was recorded by the 40% ethanol precipitated sample (68%), and this was significant ($P<0.05$).

Purification by dialysis of the ethanol precipitated samples gave an increase in the recovery % (62%), which was significant ($P<0.05$) than purification by Sephadex G-100 (50%).

When compared to crude sample, Sephadex G-100 purified protease showed 6.4 folds purification. These results were supported by Kishimura *et al* 2005 for characterizing the trypsin isozymes from the viscera of Japanese Anchovy, stated that gel filtrated sample yielded 38 folds purification when compared to the crude sample. This shows that the Sephadex G-100 purified protease has high purification fold.

Thus it is understood from the findings on recovery % that precipitation with 40% ethanol decreased the recovery % of the enzyme isolated from fish wastes to the maximum content (68%).

4.7GEL FILTRATION PATTERN OF PROTEASE ON SEPHADEX G-100 COLUMN CHROMATOGRAPHY

The elution profile of protease on Sephadex G-100 column chromatography from visceral organ wastes and head and wastes of ammonium precipitated samples is depicted in Figure-19 and Figure-20.

FIGURE- 19

Elution profile of protease from visceral organ wastes of Indian oil sardine (*sardinella longiceps*) fish of ammonium precipitated sample by Sephadex G-100 column chromatography

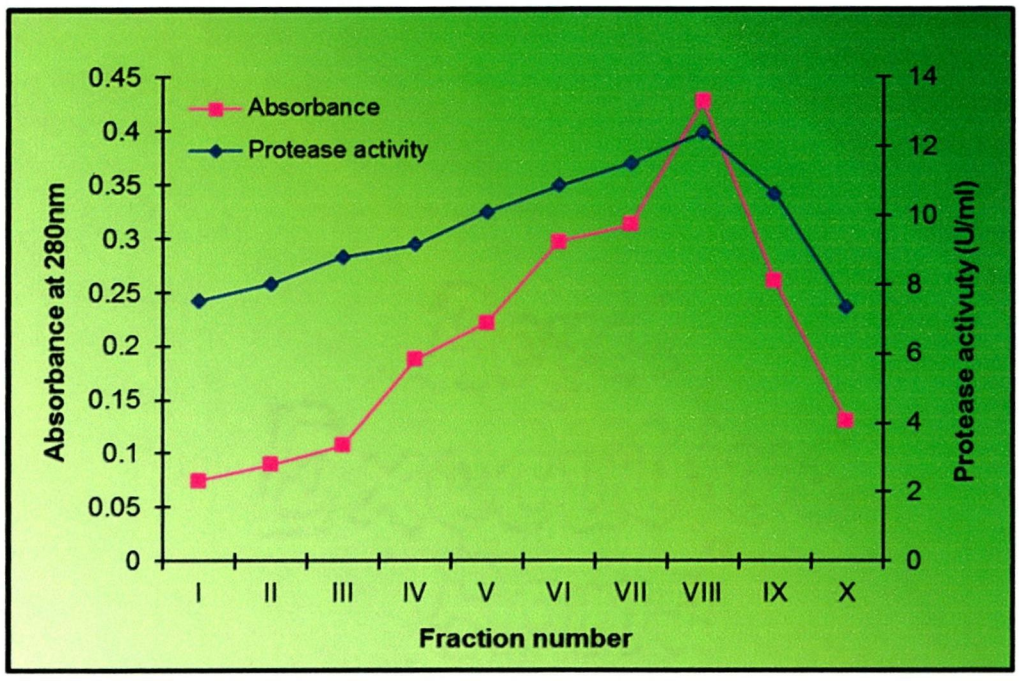
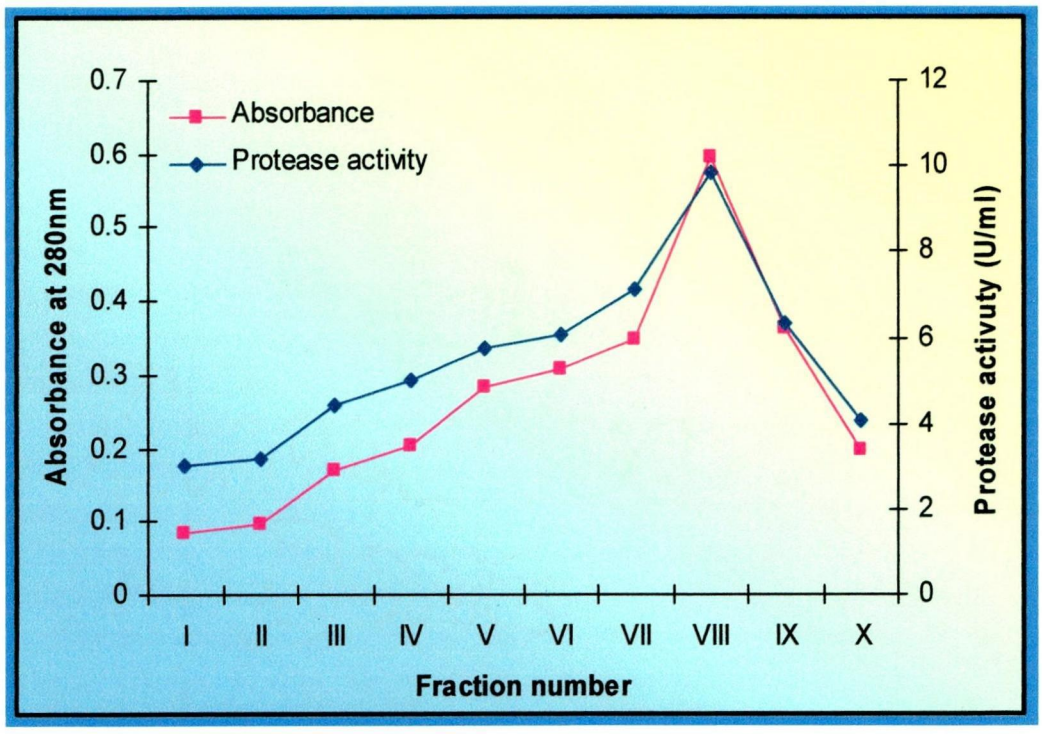


FIGURE- 20

Elution profile of protease from head and tail wastes of Indian oil sardine (*sardinella longiceps*) fish of ammonium precipitated sample by Sephadex G-100 column chromatography



The elution profile of protease on Sephadex G-100 column chromatography from visceral organ wastes and head and wastes of acetone precipitated samples is depicted in Figure-21 and Figure-22.

FIGURE-21

Elution profile of protease from visceral organ wastes of Indian oil sardine (*sardinella longiceps*) fish of acetone precipitated sample by Sephadex G-100 column chromatography

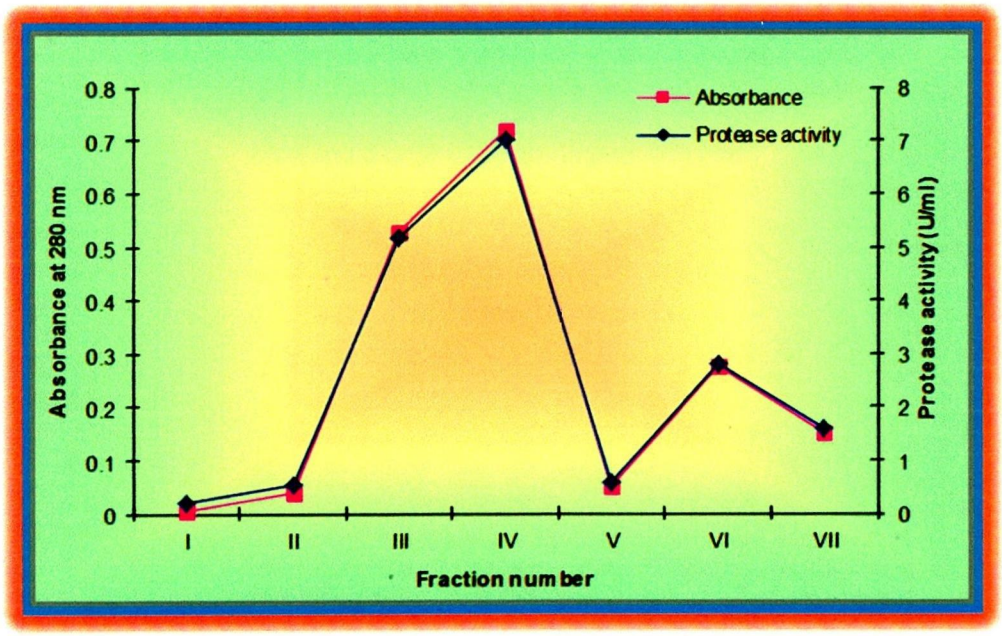


FIGURE -22

Elution profile of protease from head and tail wastes of Indian oil sardine (*sardinella longiceps*) fish of acetone precipitated sample by Sephadex G-100 column chromatography

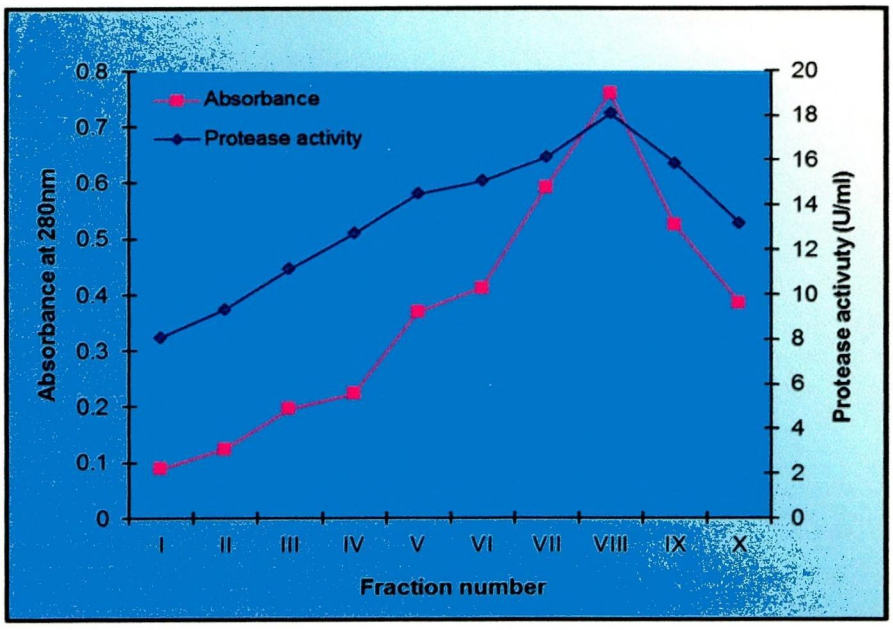


FIGURE-23

Elution profile of protease from visceral organ wastes of Indian oil sardine (*sardinella longiceps*) fish of ethanol precipitated sample by Sephadex G-100 column chromatography

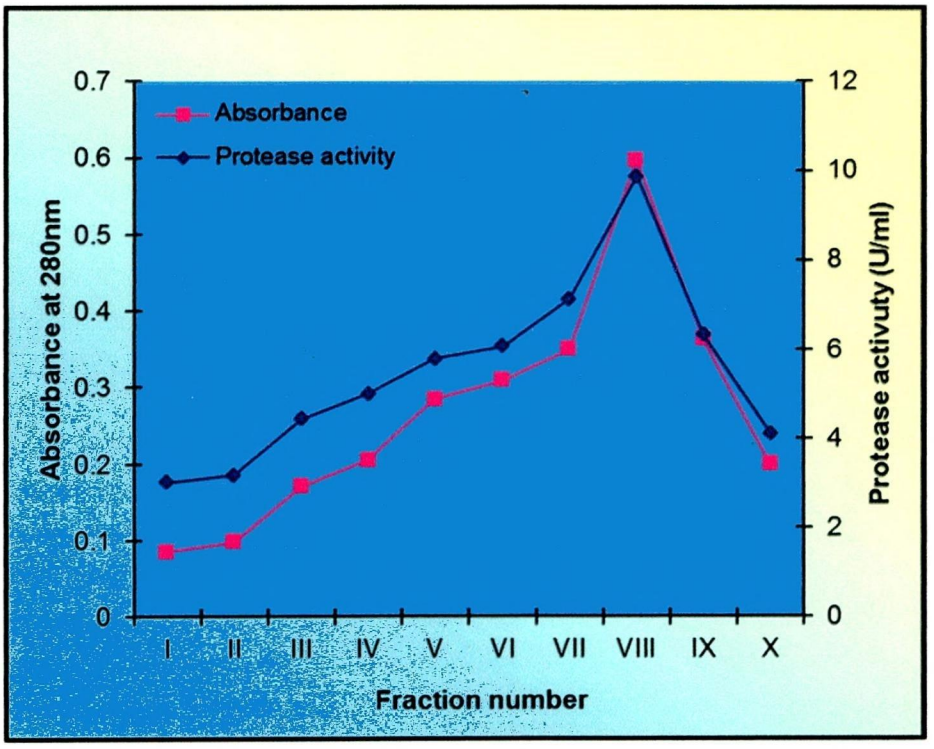
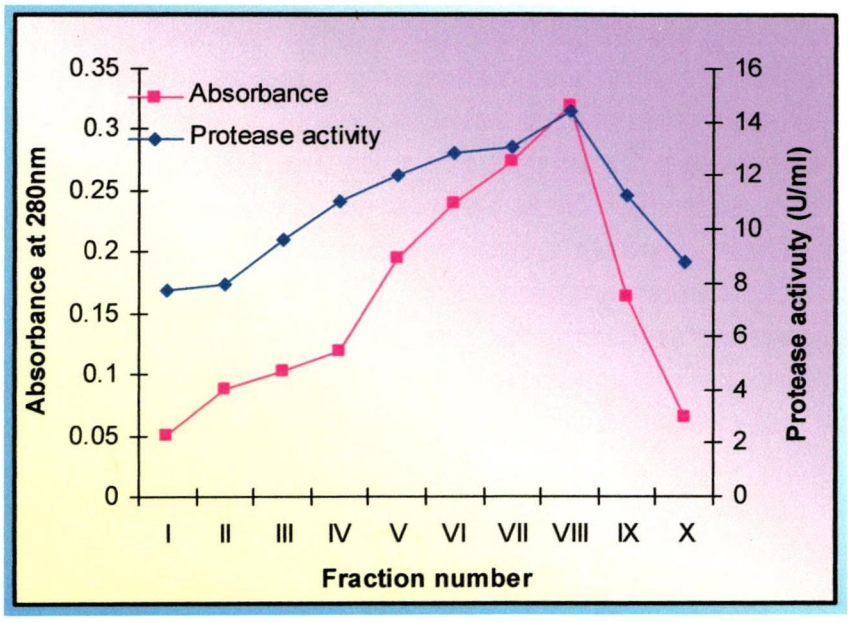


FIGURE-24

Elution profile of protease from head and tail wastes of Indian oil sardine (*sardinella longiceps*) fish of ethanol precipitated sample by Sephadex G-100 column chromatography



From the Figure 19, 20, 21, 22, 23and 24 it is understood that the elution profile of all the samples except the acetone precipitated visceral organ waste samples showed maximum absorbance peak and protease activity for fractions VIII.

Hence from the findings of the above study it can be stated that protease enzyme from the visceral organ and head and tail wastes of the selected fish Indian oil sardine (*Sardinella longiceps*) can best be isolated by 40-50% ammonium sulphate 50-60% acetone or 40-50% ethanol and purified by dialysis.

Table -VII

Comparison of purification schemes of ammonium sulphate precipitated samples from visceral organ waste and head and tail waste of Indian oil sardine (*Sardinella longiceps*) fish

SI. No	Samples	Visceral organ wastes					Head and Tail Wastes				
		Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %	Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %
1	**Crude**	8.846±0.03	26.82±0.03	3.03±0.03	1±SD	100±SD	7.65±0.03	23.56±0.03	3.08±0.03	1±SD	100±SD
2	**Ammonium Sulphate precipitated 30-40%**	0.54±0.01	19.66±0.43	22.15±0.3	7.4±0.50	72±0.02	0.33±0.01	19.42±0.06	58.33±0.49	19.24±0.1	75±0.004
3	**Ammonium Sulphate Precipitated + Dialysed**	0.27±0.04	11.13±3.74	37.99±1.5	12.47±0.01	64±0.01	0.61±0.04	15.26±0.44	23.31±3.74	27.69±0.15	60±0.0177
4	**Ammonium Sulphate Precipitated + Sephadex G-100**	0.87±0.02	10.13±0.30	32.77±0.2	11.69±0.01	55±0.003	0.66±0.02	14.28±0.01	23.73±0.30	27.83±0.25	55±0.0003

Figure - VIII

Comparison of purification schemes of acetone precipitated samples from visceral organ waste and head and tail waste of Indian oil sardine (*Sardinella longiceps*) fish

SI. No	Samples	Visceral organ wastes					Head and Tail Wastes				
		Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %	Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %
1	Crude	8.846±0.03	26.82±0.03	3.03±0.03	1±SD	100±SD	7.65±0.03	23.56±0.03	3.08±0.03	1±SD	100±SD
2	Acetone precipitated	0.54±0.011	16.84±0.05	52.85±1.8	7.07±1.14	62±2.04	0.86±0.020	18.26±0.03	31.08±1.15	10.51±0.25	67±2.47
3	Acetone Precipitated + Dialysed	0.49±0.07	15.34±0.07	31.58±5.8	8.27±1.35	57±1.93	0.56±0.005	16.39±0.01	42.32±1.43	20.38±0.31	9.57±1.76
4	Acetone Precipitated + Sephadex G-100	0.45±0.01	12.580.34	23.30±0.6	7.87±0.34	47±2.08	0.25±0.25	15.24±0.12	23.3±1.41	10.08±0.16	3.61±1.92

Table - IX

Comparison of purification schemes of ethanol precipitated samples from visceral organ waste and head and tail waste of Indian oil sardine (*Sardinella longiceps)* fish

SI. No	Samples	Visceral organ wastes					Head and Tail Wastes				
		Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %	Protein Content mg/ml	Protease Activity U/ml	Specific Activity U/mg	Purification Fold	Recovery %
1	Crude	8.846±0.03	26.82±0.03	3.03±0.03	1±SD	100±SD	7.65±0.03	23.56±0.03	3.08±0.03	1±SD	100±SD
2	Ethanol precipitated 30-40%	0.35±0.03	15.73±0.06	55.84±1.38	4.98±2.6	57.081	0.70±0.03	18.30±0.18	53.84±2.6	18.14±0.53	68±2.08
3	Acetone Precipitated + Dialysed	0.65±0.02	13.32±0.01	21.9±0.843	7.11±0.3	62±0.37	0.65±0.11	8.45±0.620	44.47±4.7	14.67±0.33	62±0.73
4	Acetone Precipitated + Sephadex G-100	0.56±0.098	12.3±0.126	20.72±3.61	6.73±1.24	52±0.09	0.19±0.07	6.44±4.33	25.60±4.3	8.47±1.67	50±2.08