Chapter 8

STUDIES ON TISSUE PROTEOLYTIC ENZYMES

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8.1. Introduction

Squids are known to have only one to two years of life span and some species of squid grow very fast from spring till autumn and die after spawning. In order to achieve its rapid growth, the protein turnover must be very fast which usually results from an active proteolytic system. Active proteases were found not only on the visceral part of the squid but also in the mantle muscle as well. On post-mortem, squid enters a state of uncontrollable protein degradation from both natural and bacterial sources. Rough handling and pressure due to icing apparently facilitates subsequent release of proteolytic enzymes. Proteins of squid muscle differ significantly from those of fish muscle with specific catheptic activity, approximately twice as high as that found in other finfishes. Proteolytic degradation is probably the single most important characteristic relating to the eating quality of the squid. Little information is known on the proteolytic activities in muscle of squids and other cephalopods from tropical waters. It is being noticed that the needle squid underwent softening even during thawing, while the loligo squid was rather stable. A comparison of their autolysis seemed to be a logical approach to look for the reasons for the above difference between the two squids.

8.2. Review of Literature

The squid mantle showed extensive protease activity and observed that squid mantle actomyosin was much less stable than those of carp and rabbit (Magita et al., 1958). The intact squid myosin was successfully isolated only when proteinase inhibitors like trasylol and soybean trypsin inhibitor, or ethylene diamine tetra acetic acid (EDTA) were added to the

extraction medium. Squids have been studied from the species Sotula pacifica in Alaska.

A comparison of changes in the squid and Falkland squid tissue has been found of a study by Haard (1980) and proteases and protease inhibitors in squid, protease activity and was studied by Schober et al. (1968) found that cathepsin B proteolytic enzyme activity was high and found that cathepsin B protease inhibitors proved to be effective. Characterization of protease inhibitors studied by Haard (1980) in the participation of milked and salmon caught in California. Williams (2002) suggested that enzyme levels in wash water. The role of protease inhibitors was described in the draft...
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A comparative study of autolysis of Argentina squid (*Illex argentinus*) and Falkland squid (*Martilina hyagesi*) was done by Lee and Bonnie, (1990) in which the optimum temperature for autolysis and the optimum pH have been found out. The visceral proteinase activity was studied by Hameed and Haard (1985). Doke and Ninjoor (1987) have studied the alkaline proteinases and exopeptidases from shrimp muscle. The effect of alkaline protease activity on some properties of comminuted squid (*Loligo forbesi*) was studied by Roger et al., (1984). Luten et al., (1992) studied the proteolytic enzyme activity as a measure of cooking of tropical shrimps and found that cooking time was inversely proportional to the enzyme activity. Schober et al., (1992) studied the isolation, characterization and application of proteolytic enzymes from fish viscera. Mateos et al., (2002) studied the thermal gelation profiles of frozen squid with added proteinase inhibitors. Visessanguan et al., (2000) suggested porcine plasma proteins as a surimi protease inhibitor and found effective on actomyosin gelation.

activity of squid muscle (*Todaropsis eblanae*) in the presence of various chemical inhibitors.

Aoki and Ueno (1999) explained the involvement of cathepsins B and L in the post-mortem autolysis of mackerel muscle. The cathepsin-D like proteinase has a maximum activity at pH 3.1 in the mantle muscle of *Ommastrephes sloani pacificus* (Sakai and Matsumoto, 1985). Cathepsin-D and E were present in the Atlantic short finned squid (*Illex Illecebrobus*) and long finned (*Loligo plealeii leseur*) (Leblanc and Gill, 1982). The effect of temperature on alkaline protease was studied by Deng (1981). Warrier et al., (1972) has studied the effect of cold storage on the proteolytic enzyme of fish muscle. Rodger et al., (1984) also studied the effect of alkaline protease activity on some properties of squid *Loligo forbesi*. The proteolytic activity of Atlantic croaker was studied in minced fish gel by Cheng et al., (1979) using casein as a substrate. Work on proteolytic inactivation using protease inhibitors to improve the quality of fish surimi has been carried out by Jiang et al., (2000). Cao et al., (1999) studied the proteolysis of a myofibril bound serine proteinase from *Cyprinus carpio* on myofibrillar proteins and their gel formation ability.

Simpson et al., (1991) have made a through review of these enzymes in fish. Endogenous proteinases in seafoods in particular have been reviewed exhaustively by Haard (1992).

### 8.3. Materials and Methods

#### 8.3.1. Sampling method

Squid mantle muscle of both the species of squid, *Loligo duvaucelii* and *Doryteuthis sibogae* were used for the study. For the study of proteolytic activity of various samples, the following were used:

- Fresh squid
- Frozen squid
- Sample preserved in refrigerated storage

#### 8.3.2. Preparation of samples

The samples were homogenized in chilled homogenization buffer. The homogenized samples were stored in ice. The homogenates were kept in refrigerated storage for further determination.

#### 8.3.3. Proteolytic activity

Enzyme activity was measured using the method described by Oh et al. (1981) using casein. The pH range, the following were used: 7.0, 7.5, 8.0, 8.3, and 3.0, acetic acid, a phosphate buffer, and a boric acid buffer at pH 9.5, 10.0.

1 mL of homogenized sample was used to determine the proteolytic activity. The samples were prepared by
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proteolytic enzymes, sampling was done from fresh stored and treated samples, the methods being given in the previous chapters.

➢ Fresh sample (2.3.)
➢ Samples ice-stored with GMP and without GMP (3.3.1.)
➢ Frozen stored samples (5.3.1.)
➢ Samples after treatment (6.3.)

8.3.2. Preparation of Enzyme extract

The samples of squid mantle tissue was cut into small pieces and homogenized in four volumes of cold distilled water using a high-speed homogenizer for 3-4 minutes, keeping the container in an external pack of ice. The homogenate was centrifuged at 15000g for 30 minutes in a refrigerated centrifuge at \(-5^\circ\text{C}\). The supernatant was used immediately for the determination of enzyme activity.

8.3.3. Proteolytic enzyme assay

Enzyme activity was assayed by the method of Leblanc and Gill, (1981) using 2% haemoglobin in appropriate buffers. To cover the test pH range, the following buffers were used: 0.2M glycine HCl buffer for pH 2.2 and 3.0, acetate buffer for pH 4.2 and 5.6, phosphate buffer for pH 6.0 and 7.0, boric acid borate buffer for pH 8.0 and 9.0 and glycine NaOH buffer for pH 9.5, 10.0 and 10.5 (Gomori, 1955).

1ml of aliquot of the extract was added to 2ml of buffer containing 2% hemoglobin and incubated for two hours at 37°C. The activity was terminated by adding 2ml of freshly prepared 10% TCA. Controls were prepared by the addition 10% TCA to the buffered Hb substrate. After
mixing, 1ml extract was added immediately and incubated for 2 hours at 37°C. These terminated mixtures were stored overnight at 0-4°C and then filtered through Whatman No.4 filter paper. The liberated TCA soluble peptides were determined by the method of Lowry et al., (1951). The enzyme activity was expressed in μg of tyrosine per ml per minute. The specific activity was expressed as in μg of Tyrosine per mg protein per minute. Enzyme assays were conducted in triplicate.

8.3.4. Optimization of pH, Temperature and Time

After determining the pH optima, a separate experiment was conducted to study the effect of in temperature of incubation (30°C-75°C) on the activity of proteases. Separate enzyme assay was carried out to optimize the time.

8.3.5. Enzyme assay in the presence of inhibitors

The inhibitors and their concentration in the incubation mixture were as follows:

PMSF (Phenyl Methane Sulphonyl Fluoride), 1 mM;
Soybean trypsin inhibitor, 100 μg/ml;
Iodoacetic acid, 1 mM;
EDTA (Ethylene diamine tetra acetic acid) 2 mM

1ml of the inhibitor solution was added to 1ml of extract and 2ml of buffered substrate haemoglobin and incubated for 2 hours at 37°C. The incubation tests were carried out at pH 3.0, 6.0 and 8.0/9.0 with the buffers described above. The activity was arrested by adding 2ml of 10% TCA.

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This was filtered and liberated acid soluble peptides were estimated by Lowry's method.

8.4. Results

![Figure 8.1. Proteolytic activity of both species of squid at various pH](image)

Figure 8.1. shows the proteolytic activity at various pH in both the species of squid. The optimum pH was determined from the above figure. Both the species showed a high degree of proteolytic activity. But needle showed a higher activity than loligo at all the pH range. A high activity was found in the acid pH range with a maximum at pH 3.0 in both the species (loligo 0.95 units and needle 1.61 units). In loligo, there were two peaks for enzyme activity, at pH 3.0 and 8.0. At pH 6.0 also showing a slight increase in the activity was observed. A significant observation in this study was that at pH 4.2, there was a sharp dip in the activity of both the species with
loligo showing least activity of 0.28 units. The pH, 4.2 is same as the effective pH of the tissue in acetic acid treated squid mantle.

Figure 8.2. Proteolytic activity of both species at pH 3.0 and various temperatures

Figure 8.3. Proteolytic activity of both species at pH 6.0 and various temperatures

The optimum pH for both the species was observed (40°C) at pH 6.5°C (Figure 8.2).

pH optimum for enzyme activity.

To detect, incubated at a temperature to reach maximum decrease in this.
Figure 8.4. Proteolytic activity of both species at pH 8.0/9.0 and various temperatures

The optimum temperature for protease activity was found to be 40°C for both the species at pH 6.0. At pH 3.0 and pH 9.0/8.0 two peaks were observed (40°C and 65°C), where the maximum activity was obtained at 65°C (Figure 8.2, 8.3 and 8.4).

pH optimization was repeated at 40°C and 65°C. The trend in the enzyme activity was found to be same in both temperatures.

To determine the optimum time for incubation, the reaction media was incubated at 40°C for various time intervals (pH 3.0). The minimum time to reach maximum activity was found to be 2 hours after which, a gradual decrease in the activity was observed (Figure 8.5).
Figure 8.5. Optimization of time of incubation for protease assay in both species of squid at pH 3.0

Figure 8.6. Proteolytic activity of both species of squid ice-stored with GMP/without GMP with varying pH.

The proteolytic activity of both the species ice stored with GMP and without GMP at various pH were studied (Fig 8.6.). In the case of loligo, with...
GMP and without GMP, the enzyme activity was found to be minimum at pH 4.2. In all the cases the activity showed a maximum pH 3.0. In both the species, the pattern of proteolytic activity changes in the pH range were specific, with samples without GMP, showing higher activity than with GMP.

![Graph showing proteolytic activity over storage days](image)

**Figure 8.7. Proteolytic activity of both species of squid during frozen storage**

Figure 8.7 shows the proteolytic activity of loligo and needle squid mantle tissue during frozen storage. The protease activity showed a gradual decrease during the period of 8 months frozen storage. The change in the activity in both the species were almost equal.

**Table 8.1. Proteolytic activity in the drip of frozen stored samples**

<table>
<thead>
<tr>
<th>Storage Months</th>
<th>Needle E.A.</th>
<th>Loligo E.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.95</td>
<td>26.09</td>
</tr>
<tr>
<td>2</td>
<td>11.10</td>
<td>8.03</td>
</tr>
<tr>
<td>3</td>
<td>9.53</td>
<td>6.86</td>
</tr>
<tr>
<td>4</td>
<td>7.40</td>
<td>6.40</td>
</tr>
<tr>
<td>5</td>
<td>3.63</td>
<td>3.17</td>
</tr>
<tr>
<td>6</td>
<td>2.45</td>
<td>0.17</td>
</tr>
</tbody>
</table>
E.A. – Enzyme activity

The drip of frozen stored sample was collected by thawing the frozen samples. In both the species, the enzyme activity showed a decreasing trend and the drip from six months stored sample, showed a very low activity (Table 8.1).

**Table 8.2. Effect of treatments on proteolytic enzyme activity**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Needle</th>
<th></th>
<th></th>
<th>Loligo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.A.</td>
<td>Protein mg/ml</td>
<td>S.E.A.</td>
<td>E.A.</td>
<td>Protein mg/ml</td>
</tr>
<tr>
<td>A</td>
<td>90.76</td>
<td>1.63</td>
<td>55.82</td>
<td>78.27</td>
<td>1.95</td>
</tr>
<tr>
<td>B</td>
<td>9.86</td>
<td>3.01</td>
<td>3.27</td>
<td>4.96</td>
<td>3.80</td>
</tr>
<tr>
<td>C</td>
<td>27.36</td>
<td>2.33</td>
<td>11.76</td>
<td>21.16</td>
<td>2.93</td>
</tr>
<tr>
<td>D</td>
<td>26.42</td>
<td>3.01</td>
<td>8.78</td>
<td>16.96</td>
<td>3.54</td>
</tr>
<tr>
<td>E</td>
<td>18.03</td>
<td>2.38</td>
<td>7.59</td>
<td>16.43</td>
<td>2.79</td>
</tr>
<tr>
<td>F</td>
<td>30.49</td>
<td>1.98</td>
<td>15.39</td>
<td>24.96</td>
<td>2.17</td>
</tr>
</tbody>
</table>

E.A. = Enzyme activity (μg tyrosine/ml/hr)
S.E.A. = Specific enzyme activity (μg tyrosine/mg protein/hr)

A-Control  C-Acetic acid citric acid mixture
B-Acetic acid alone  D-STPP acetic acid mixture
E-Ascorbic acid  F-Lime Juice

The effect of various treatments (as described in 6.4.6) on the proteolytic activity is given in the table 8.2. The samples treated with acetic acid showed the minimum activity in both the species while the untreated sample showed maximum activity. Among all the treatments, limejuice treated sample showed maximum activity. The specific enzyme activity also showed a minimum value in acetic acid treated sample of loligo and needle squid.
Table 8.3. Effect of inhibitors on the residual protease activity (%) of autolysis of the mantle muscle of both species of squid at 40°C

<table>
<thead>
<tr>
<th></th>
<th>Needle</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 3.0</td>
<td>pH 6.0</td>
<td>pH 9.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMSF</td>
<td>15.73</td>
<td>45.83</td>
<td>12.98</td>
<td>34.09</td>
<td>24.08</td>
<td>48.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy try</td>
<td>15.19</td>
<td>44.26</td>
<td>25.59</td>
<td>67.20</td>
<td>18.22</td>
<td>36.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodo acetate</td>
<td>26.40</td>
<td>76.94</td>
<td>19.90</td>
<td>52.26</td>
<td>20.16</td>
<td>40.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>31.61</td>
<td>92.13</td>
<td>10.39</td>
<td>27.29</td>
<td>9.62</td>
<td>19.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.31</td>
<td>100</td>
<td>38.08</td>
<td>100</td>
<td>49.54</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                      | Loligo       |          |          |          |          |          |          |
|                      | pH 3.0       | pH 6.0   | pH 8.0   |          |          |          |          |
| PMSF                 | 9.20         | 40.68    | 8.27     | 37.06    | 17.01    | 57.78    |          |          |
| Soy try              | 9.63         | 42.56    | 11.88    | 53.26    | 16.04    | 54.50    |          |          |
| Iodo acetate        | 10.99        | 48.61    | 11.76    | 52.70    | 17.51    | 59.49    |          |          |
| EDTA                 | 26.54        | 117.37   | 3.20     | 14.34    | 4.29     | 14.58    |          |          |
| Control              | 22.61        | 100      | 22.31    | 100      | 29.43    | 100      |          |          |

S.E.A. Specific enzyme activity
R.E.A. Residual enzyme activity – Value represent the percentage relative to the activity of control

Table 8.4. Inhibitors and inhibiting enzyme

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Proteinases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSF and Soy bean trypsin inhibitor</td>
<td>Inhibit Serine proteinases</td>
</tr>
<tr>
<td>Iodo acetate, PCMB and Leupeptin</td>
<td>Inhibit Thiol proteinases</td>
</tr>
<tr>
<td>Pepstatin</td>
<td>Inhibit Carboxyl proteinases</td>
</tr>
<tr>
<td>EDTA</td>
<td>Inhibit Metalloproteinases</td>
</tr>
</tbody>
</table>
The proteolysis at pH 3.0, pH 6.0 and pH 8.0/9.0 was carried out in the presence of inhibitors, to investigate what kind of proteinases function at those pH. Based on the essential catalytic group of the enzymes, Hartley (1960) classified proteinases in the four classes namely Serine, Thiol, Carboxyl and Metalloproteinases. Barrett (1977) has suggested that the class assignment can be done on the basis of different sensitivities to different inhibitors. The inhibitors and inhibiting enzymes are given in the Table 8.4 by different inhibitors. At an optimum temperature of 40°C, the inhibition of various inhibitors of the protease enzymes varied with pH (Table 8.3).

The residual activity in loligo was found to be very low when PMSF and soy trypsin inhibitors were added. In other words, at pH 3.0 the cathepsin D like proteinases (with an optimum pH 3.0) was very likely to be present in the aqueous extract of both the species studied. At physiological pH, (6.0-7.0) PMSF inhibited the proteolysis to a residual activity of 37.06% in loligo and 34.09% in needle. The inhibition due to soy trypsin inhibitor was not much effective at this particular pH for both the species. A chymotrypsin – like serine proteinase was likely to be present in the muscle of both the species. At this pH, EDTA was more effective in suppressing the protein degradation. Iodoacetic acid inhibition was not significant when compared with inhibition due to EDTA. To some extent, iodo acetic acid inhibited the proteolysis in both the species (Needle squid –52.26% and Loligo –52.70%) indicating the presence of a thiol proteinases. EDTA was found to be a very effective inhibitor at neutral and alkaline pH. Suggesting that, there exist highly active metalloproteinases.

In contrary to this, at pH 3.0, EDTA did not inhibit but rather increased the activity (117% of residual activity in loligo and 92.3% in needle squid).

8.5. Discussion

The activity of proteinases of Loligo opalescens exhibited a high hydrolysis activity, both in neutral and alkaline pH, 55°C to 60°C. Sakai and Makino (1973) studied the mantle muscle of A. japonicus at 55°C to 60°C. Drabikowski (1974) studied the muscle of varied fish species.

The activity of proteinases in Loligo opalescens by Matsumoto, (1975) was for carboxyl proteases and the carboxyl proteinase activity of Loligo opalescens by Barrett, 1977 was 25.15% in acid and 23.15% in neutral pH. This contributes much to the defense system in this pH. Since proteolysis was almost 60% in Loligo, the activity in the acid range against the protease and protein degradation. The cathepsin B like proteinases of pH 3.0 was also found to be active. EDTA which was inhibited in this enzyme may also be inhibited by other inhibitors. Iodoacetic acid may be capable of inhibiting the activity in the squid muscle. Further studies are necessary to find cathepsin B in the muscle.
8.5. Discussion

The activity - pH profiles of squid mantle muscle aqueous extract, exhibited a high proteolytic activity in the acid pH range, with a low activity in neutral and alkaline pH ranges. A similar observation was reported by Sakai and Matsumotto (1980) in squid *Omnastrephes sloani pacificus*. In the mantle muscle of Falkland squid, optimum temperature for autolysis was 55°C to 60°C and at 25°C, optimal pH was 6.8 (Lee and Bonnie, 1990). Drabikowski et al., (1977) has observed a similar profile in the skeletal muscle of various vertebrates.

The activity at pH 3.1 was strongly inhibited by pepstain (Sakai and Matsumotto, 1980, Lee and Bonnie, 1990), which was specific to the carboxyl proteinases such as pepsin and cathepsin D (Aoyagi et al., 1972, Barrett, 1977). Thus the results indicate that a cathepsin D like proteinase contributes mainly to the protein degradation in the squid mantle muscle at this pH. Since cathepsin D is believed to constitute the major proteolytic activity in the lysosomes, (Barrett, 1972), lysosomes may be involved in protein degradation of the squid mantle muscle. The autolytic activity at pH 3.0 was also inhibited by other inhibitors to some extent, a thiol proteinases was inhibited by Iodo acetic acid. cathepsin B is thiol proteinase, which can be inhibited by Iodo acetic acid. The inhibition of activity by Iodo acetic acid may be due to a presence of an identical enzyme other than cathepsin B in the squid mantle. Inaba et al., (1978, 1976) established presence of cathepsin B in various species of squid.

In fish skeletal muscle, several of these proteinases such as cathepsin D (Doke et al., 1980), neutral proteinases (Makinodan et al., 1983), cathepsin B (Chen and Zal, 1986), alkaline proteinase (Makinodan et al.,
1983) and some peptidases (Osnes and Mohr, 1985) have been identified and characterized. Among these, alkaline proteinases seemed to mediate changes in the muscle texture when processed at 50°C to 70°C (Makinodan et al., 1985) apparently due to its heat stability. A comparative result was obtained in the present study, where a significantly active enzyme was observed in both squid at pH 8.0 with optimal temperature of 65°C.

Although alkaline proteinase has been purified from the muscle fish varieties such as Anatic krill (Osnes and Mohr, 1985), Atlantic croaker (Lin and Lanier, 1980), White croaker (Busconi et al., 1984) and carp (Iwata et al., 1973), information on the enzymes from crustacean’s species is scarce. While some investigators (Lin and Lanier, 1980, Busconi et al., 1984) suggested that the enzyme is sulphhydryl dependent, others showed to be either a metallo or a serine proteinase (Kozlovskoya and Elvakova, 1975; Busconi et al., 1984). Tissue proteinases have been implicated as adversely affecting the quality of stored muscle foods due to the sustained action of endopeptidases and exopeptidases that are involved in the complete breakdown of tissue proteins (Goll et al., 1983).

In this study also, an attempt was made to assay the tissue proteolytic enzyme of squid mantle at various pH and to characterize these proteases by using specific inhibitors. Between the loligo and needle squid, a distinct variation in the enzyme activity of various proteases was observed which might be due to the species-specific differences. The result obtained for the protease activities in the samples with and without GMP, frozen-stored sample and treated sample are comparable with the pattern of protein bands in SDS-PAGE (Chapter 7). The fluctuation in the endogenous muscle proteinase content and their activity observed at various storage conditions are affected by different factors. The release of this characteristic activity as a function of storage time is the focus of this chapter.

Subjected to the same conditions of stored squid, acetic treated samples showed a higher activity of proteinase. An initial activity of 15.3 units reduced to 4.5 units when the temperature lowered to 4°C. The effect of pH on the activity at pH 4.0–10.0 was found to be in agreement with the studies of others (Mclean et al., 1974). In addition, results of the inactivated samples are presented as follows.

8.6 Conclusion

Optimum activity of proteinase at pH 3.0, pH 6.5, and pH 9.0 in the case of both the species were found to be minimum at 13°C and maximum at 40°C at pH 6.5. The temperature range for the enzyme to maintain activity was found to be

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are affected by post-rigor condition and consequent softening of muscle. The release of lysosomal proteases in the squid at acidic pH and their activity as a result of various treatments, will be discussed in the next chapter.

Subjecting to various pre-treatments to extent the quality of frozen stored squid, had significant difference in the proteolytic activity of which, acetic treated sample showed a very low protease activity compared to all other treatments. Venugopal et al., (1994) had reported that acid protease activity of shark protein dispersion at pH values of 3.8, 6.0 were 13.6 and 15.3 units respectively, which decreased to 8.5 units when the pH was lowered to 4.5 by addition of acetic acid. The sharp decrease in the protease activity at pH 4.2 observed in the squid mantle, specifically in loligo, is in agreement with the above observation. The acetic acid treated sample were thus found to be organoleptically and physico-chemically of the best quality (6.4). In addition, the presence of proteases of microbial origin could also be inactivated due to the anti-microbial effect of acetic acid.

8.6. Conclusion

Optimal pH of the autolysis was found to be at three different Points pH 3.0, pH 6.0 and pH 8.0 in the case of loligo and pH 3.0, pH 6.0 and pH 9.0 in the case of needle. In loligo squid, the protease activity was found to be minimum at pH 4.2 (acetic acid alone treated sample pH). Optimum temperature for protemase activity was found to be 40°C at pH 6.0 and 65°C and 40°C at both pH 3.0 and pH 8.0/9.0. The optimum time of incubation for the enzymal assay was found to be 2 hours.
Between the two species studied, needle squid showed higher protease activity than loligo. But samples iced with GMP showed a lesser activity than samples without GMP. During frozen storage a gradual reduction in the activity was observed and it may due to freeze denaturation of the enzymes. The quantity of protein was gradually reduced in the drip, which also indicated the denaturation of proteins during frozen storage. Among treatments, acetic acid treated sample was found to be the best, which offers minimum proteolytic activity and high degree of protein retention. The proteolytic activity was strongly inhibited by specific protease inhibitors, suggesting the presence of serine proteinase, thiol proteinase, cathepsin D and metallo proteinase in the squid mantle tissue.

8.7. References


8.7. References


Nathan, O. Kapalan. McCollum-Pratt Institute, Baltimore, Maryland.


*Not referred in original