Chapter 3

QUALITY CHANGES DURING ICE – STORAGE

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3.1. Introduction

Being a highly productive marine species and due to the fact that squid are harvested all year round and after the harvest season, they are usually set for the market. Myofibrillar proteins are responsible for the spoilage of squid meat. Bacterial spoilage of squid meat is a very common and considerably less developed problem and is influenced by the bacterial genera *Loligo duvauceli* and *Loligo opalescens*. In addition, landings in Kerala are mostly detected during the third quarter of squids, which is the time when faulty processing is more common. The efficiency of processing and preservation methods increases the spoilage rate and the cost of preservation will make it a viable method for preservation. Moreover, the economical viability of the squid market is an important factor in the preservation of squid. In the case of squid preservation, it is recommended that high priority be given to limiting spoilage at each stage of processing.

Generally, spoilage bacteria grow at temperatures above 4°C. As the temperature is raised, the spoilage bacteria also grow, and in a few days, spoilage occurs. However, packing in ice is a common method of preservation to maintain a temperature below 4°C. This cooling, thus extends the shelf-life of the squid. Freezing and icing is the easiest way to improve the shelf-life of squid.
3.1. Introduction

Being a highly perishable item, squid must be preserved immediately after the harvest till it is ready for consumption. The main factors responsible for the spoilage at ambient temperatures are autolysis and bacterial spoilage. The rate and extend of the autolytic spoilage is considerably less than bacterial spoilage but, it plays a vital role in flavour development and the onset of bacterial spoilage. Two species of squid, *Loligo duvaucelii* and *Doryteuthis sibogae*, which dominate the squid landings in Kerala, are selected for the study. Due to the unique composition of squid muscle, it is prone to deterioration very easily. Careless handling, faulty processing or improper storage results in the quality deteriorations. The efficiency of the preservation methods depends on its ability to reduce the spoilage rate and thus increasing the shelf life and quality. The selection of a method for preservation depends on the nature and type of the product, economical viability of the method, intended use, adaptability and local tradition. In the case of squid, the handling immediately after harvest is of high priority because it determines, to a large extent, the quality at different stages of processing and the final product.

Generally, the rates of autolytic and microbial spoilage are dependent upon the temperatures at which the squid is stored. Deteriorative processes are retarded at reduced temperatures and when the temperature is low enough, spoilage can almost be stopped. Normally, to keep the squid cool, packing in ice is used, since ice storage is the first and easy method of preservation to maintain the native quality of the squid. Keeping the squid cool, thus extends the high quality life (HQL) of the squid. Even though icing is the easiest and the most economical method of preservation, various
factors like quality of ice, method of icing, and the storage time can affect the results. In this study the quality differences in both the selected species of squid under different conditions of storage in ice, were compared to suggest a better storage technique to maintain the intrinsic quality of the raw material to the maximum.

3.2. Review of literature

Many authors have carried out several investigations on various aspects of storage of Loligo duvaucelii, but no systematic study has been carried out till date on Doryteuthis sibogae.

The chemical, physical and the bacteriological parameters of the industrial samples of squid (Loligo species) and cuttlefish (Sepia species) were studied by Lakshmanan (1993). In the study of Joseph et al., (1977), the quality of the squid tube (Loligo duvaucelii) stored in ice has been studied in detail. Mathew et al., (1999a) has investigated the distribution of non-protein nitrogenous extractives in the muscles of 41 species of marine fish of India including squid Loligo duvaucelii. Sophia and Sherief (2003) have investigated the effect of treatments on the iced storage shelf life of cuttlefish (Sepia aculeate) fillets. In this, the changes in NPN and its fractions during iced storage and the effect of iced storage duration on frozen storage characteristics of cuttlefish fillets were studied. Paarup (2002) studied the sensory, chemical and bacteriological changes during ice storage of squid (Todaropsis eblanae). The effect of storage conditions on sensory properties, colour parameters and psychrotrophic bacterial counts of squid (Loligo plei) stored either in contact ice or in non-contact ice were studied by Lapa (2002). Civera (2000) has studied the chemical and microbial characteristics of squid and has investigated the effect of different compound involved in the storage process. Melaj et al., (1993) waters as a good for squid (Sepia shiokara) with reduced growth of inoculated.
Ice-storage time can affect the post mortem changes in the selected species of cephalopods. Sagedhal et al., (1998) have investigated the post-mortem changes in adenosine triphosphate (ATP) and related compounds in the mantle of squid Illex argentinus. The factors involved in the evaluation of the freshness of the squid were also studied by Melaj et al., (1998). The biological evaluation of sea squid found in Pakistan waters as a good source of protein was studied by Begum et al., (1994). Yamasaki et al., (1993) and Nishimura and Shinano (1992) have studied the effect of squid liver and Trimethylamine oxide on the squid product (Ikakshiokara) with respect to the micro flora and the chemical properties and the growth of inoculated Staphylococcus aureus.

Various handling and processing methods for Atlantic short finned squid (Illex illecebrosus) were studied by Ke et al., (1991) including the effect of contact icing and non-contact icing on the quality of squid. Baldrati (1990) studied the handling, marketing and processing of cephalopods in Italy and the importance of cephalopods (cuttlefish, squid and octopus) in the Italian seafood market. Longer storage of squid tubes and cuttlefish fillet in ice resulted in a noticeable decrease in NPN value (Regahunath, 1984; Joseph et al., 1977; and Joseph and Perigreen, 1988). Matsumotto, (1958) studied quality changes of squid when kept in contact with water and the decrease in PN content during washing. Tanikaka et al., (1954) observed mustiness in raw squid indicating the spoilage when TVN level exceeded 30mg/100g tissue.

There are extensive works carried out on iced storage characteristics of many tropical fishes of India (Nair et al., 1971; Nair and Danny, 1975; Guptha and Govindan, 1975; Bandyopadhyay et al., 1986; Joseph et al., 1988; and Sankar and Ramachandran, 2002). Nadia, (2002) investigated the
spoilage of mackerel during storage at ambient temperature and in ice. Jose and Reghunath (2002) studied the tissue proteinase activity of mackerel during iced storage. The biochemical and microbiological qualities of *Laboe gonius* stored in ice were studied by Lilabati and Viswanath (1999). The changes in K value and biogenic amines in Atlantic mackerel during iced storage were studied by Mathew (1999).

The bacteriology of loligo species was studied by Joseph et al., (1997) and the presence of bacteria before and after the treatment with ascorbic acid on *loligo duvaucelii* was studied by Selvaraj (1991). Various authors have also studied bacteriology of other species like Indian oil sardine stored in a mixture of seawater and ice (Shetty et al., 1992) and storage characteristics of catfish by Bhattacharyya and Chaudhuri (1990).

### 3.3. Materials and Methods

#### 3.3.1. Experimental Design

The samples of the two species of squid (*Loligo duvaucelii* and *Doryteuthis sibogae*) were collected from Munambam landing center as explained in Chapter 2. Collection was by random sampling of catch from the boat. The aseptically brought samples were segregated into three lots and separately iced.

Method 1-with GMP (Good Manufacturing Practices): In this flake ice was used in the ratio 2:1 with material and the ice and melt water was changed daily. Utmost care was taken to maintain the temperature at 0°C-2°C. The insulated boxes used for the study were with smooth interior and not to harbour any contaminants. The plastic boxes were kept clean throughout the study and were covered firmly with their plastic lids. For one sample two boxes were monitored, one box was kept empty and the other filled with ice and disinfectants. During this period, care was taken to avoid stagnation of melt water.

Method 2 -With Chilled Water (C.W.): Insulated boxes having holes to drain water were used. Block ice was crushed and used for icing the boxes which were kept either ice filled or replaced. Care was taken not to avoid stagnation of melt water.

Method 3 -Without Icing: To avoid icing, the direct contact of the material with air was avoided by using polythene cover. This was taken to avoid the contact of insoluble component with ice. The boxes were layered alternately with ice to three in order to maintain a uniform temperature below 10°C. The sample box was placed throughout the study to bio chemical and proximate analysis. The following parameters were measured in different storage materials.

#### 3.3.2. Proximate content

The analyses...
Ice-storage

Sample two boxes were used during the study. On the second day of storage, the box was emptied and washed thoroughly using permissible detergents and disinfectants. The washed box was kept for drying for the whole day. During this period, the second box was used for icing.

Method 2 - Without GMP In this, samples were kept in a clean box, having holes to drain the melt water off. In this sample block ice was used. Block ice was crushed in an ice crusher up to 2 to 3 inch size and this was used for icing the sample. Till the end of the study, the ice was not removed or replaced. Care was taken to maintain the temperature below 5°C and to avoid stagnant melt water.

Method 3 - With GMP, without direct contact with ice and water: In this, the direct contact of the squid tubes and the ice was prevented using a polythene cover. The method of icing was as in the Method 1. Immense care was taken to avoid breakage of polythene cover and leaching out of the soluble components of the squid muscle. In all the methods, ice and squid were layered alternatively and the number of layers of samples was limited to three in order to avoid crushing of the lower layer.

The three samples were kept in an area where the temperature was below 10°C. The samples were drawn daily for 8 days and tested for various biochemical parameters. The experiment was done in triplicate. The following parameters were analysed in both the species of squid with different storage methods.

3.3.2. Proximate composition

The analyses of moisture, protein, fat and ash were done as in 2.3.
3.3.3. pH

10 g of the muscle was macerated with 90 ml distilled water and the pH was determined using digital pH meter (pH-500) Cyber Scan. Temperatures of the storage media and the storage place were checked and recorded at every two hours interval.

3.3.4. Total Volatile base nitrogen (TVBN)

10gms of minced sample was weighed and ground well with 10% Trichloroacetic acid (TCA). The extraction was repeated 2-3 times and made up the volume of supernatant to 50ml.

Total volatile base nitrogen (TVBN) was estimated using micro diffusion method of Conway (1962) using TCA extract of the sample. Pipetted 1ml of N/50 H₂SO₄ into the inner chamber of the Conway diffusion apparatus. Fixed the ground glass cover of the unit in such a way that the inner chamber was completely covered and leaving a small portion of the outer chamber uncovered. Pipetted out 1ml of TCA extract into the outer chamber and added 1ml of saturated potassium carbonate solution. The cover glass was slid into position so that the entire unit was covered fully and ensured thorough mixing by rotating the unit. The entire unit was kept overnight at room temperature. Excess acid in the inner chamber was titrated against 1/50 N NaOH using Tachiro’s Indicator. A blank was also run simultaneously with 1ml of TCA instead of muscle extract.

\[
TVBN \text{ as mg\%} = \frac{0.28 \times (\text{blank} \text{ sample value}) \times 50 \times 100}{\text{Weight of the Mantle tissue (g)}}
\]

3.3.5. Trimethylamine nitrogen (TMA-N)

The procedure of extracting TVBN except that in the place of formaldehyde (neutralised with sodium carbonate) the TMA-N was extracted with N/50 H₂SO₄. The result was expressed as mg% of TMA-N as mg% of TMA-N.

3.3.6. Alpha Amino nitrogen (AAN)

Alpha amino nitrogen was determined by iodine method. The TCA extract was treated with 0.1% thymolphthalein indicator and a blue colour was obtained. The supernatant was made up to 100 ml and allowed to stand for 10 minutes. Filtered through Whatman no. 1 paper and a conical flask followed in acetone. It was then titrated with 1/50 N NaOH using starch as indicator.

The alpha amino nitrogen (AAN) was expressed as follows:

\[
\text{AAN} = \frac{X}{M \times 50 \times 100} \times 28 \times \text{sample weight (g)}
\]
3.3.5. Trimethylamine Nitrogen (TMA-N)

The procedure for determination of TMA-N was the same as that of TVBN except that in the outer chamber along with 1 ml of TCA extract, 1 ml of formaldehyde (neutralized with CaCO₃) was also added (Shewan 1971). The result was expressed as mg% of nitrogen

TMA-N as mg% of nitrogen = \( \frac{0.28 \times \text{blank sample value} \times 50 \times 100}{\text{Weight of the Mantle tissue (g)}} \)

3.3.6. Alpha Amino Nitrogen

Alpha amino nitrogen content of muscle in the TCA extract was determined by iodometric titration of Pope & Stevens, (1939). 10 ml of TCA extract was taken in a 100 ml standard flask, added 1-2 drops of thymolphthalin indicator and then neutralized with alkali till a faint blue colour was obtained. After adding 30 ml of cupric phosphate complex it was made up to 100 ml using distilled water, shaken well and kept for 30 minutes. Filtered the solution and 10 ml of the filtrate was pipetted out into a conical flask followed by 10 ml of glacial acetic acid to make the solution acidic. It was then titrated against N/100 Sodium thiosulphate (Na₂S₂O₃) using starch as indicator.

The alpha amino nitrogen content was calculated using the following formula

\[
\text{Alpha amino nitrogen as (mg%) = } \frac{TV \times 0.28 \times 100 \times 50 \times 100}{10 \times 10 \times \text{weight of the sample (g)}}
\]
3.3.7. Fractions of Proteins

Various fractions of proteins – sarcoplasmic, myofibrillar, denatured and stroma proteins were analysed using the method as explained in Chapter 2 (Sub chapter 2.3).

3.3.8. Sensory Evaluation

Organoleptic parameters viz. colour, texture, odour and flavour of the samples were recorded in all the icing methods of both the species of squid. Trained panelists assessed these characteristics. Sensory tests and the degree of excellence were given by the hedonic scale (Herbert and Joel, 1985). The sensory score is the mean of all sensory characteristics. The sensory quality of the raw and cooked samples of squid and the criteria used in each case is given in Appendix C.0.

3.3.9. Bacteriology

The microbiological parameters like TPC, E-coli, staphylococcus, salmonella and vibrio were enumerated as per the procedure in 2.3.6.

3.3.10. Statistical tools

For the comparison of parameters between species, between storage days and between methods (with GMP, without GMP and without direct contact.), three way ANOVA was employed. The mathematical model used for the purpose was as follows:

\[ X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + E_{ijk} \]

where \( X_{ijk} \) is the value obtained on \( i \)th species using \( j \)th method of storage and \( k \)th day of storage. \( \mu \) is the overall effect and \( \alpha_i \) is the effect of the \( i \)th species. \( \beta_j \) is the effect of the \( j \)th storage and \( \gamma_k \) is the effect of the \( k \)th day.

3.4. Results

To compare the means, the days of storage and the days of storage using “3 way anova” were found to be significant and separated.

3.4.1. Proximate composition

Appendix C.1. The proximate composition of species of squid did not show significant differences in moisture content, but there were significant differences in fat content. The methods showed a significant difference in the moisture content, fat content and water, under identical storage conditions with or without GMP, had 10.44% and 10.17% respectively and for the raw and cooked fish respectively. In the control condition, the fish was higher than needle and quill. Further, the native quality showed a 1% increase in the percentage in needle.
effect and \( \alpha_i \) is the effect of \( i^{th} \) species, \( \beta_j \) is the effect of \( j^{th} \) method of storage and \( \gamma_k \) is the effect of \( k^{th} \) day of storage.

3.4. Results

To compare the effect of various methods of icing, between species and the days of storage, the results were subjected to statistical analysis using "3 way anova" (Snedecor & Cochran 1980). Wherever days were found to be significant, the LSD was worked out and the mean was separated.

3.4.1. Proximate composition

Appendix C.1 gives ANOVA table for moisture content of both the species of squid during ice storage. There was significant difference in moisture content, between species, between methods of icing and between days of storage. Between species, there was a significant difference and needle showed a significantly lower value than loligo (p< 0.001). There was significant difference between the species, and the squid kept under all the methods showed a gradual increase in moisture content. The rate of increase in the moisture content of loligo squid kept without direct contact with ice and water, under ideal condition with GMP and under conventional method without GMP, has shown an increase of 1.08%, 3.18% and 4.59% respectively and for needle the values were 0.68%, 1.68% and 3.3% respectively. In the loligo squid the rate of increase in moisture content was higher than needle squid. The samples without direct contact could maintain its native quality standards till the end of \( 8^{th} \) day of storage. The moisture percentage in needle squid kept under ideal conditions showed 80.43 ± 1.07,
in samples without GMP 81.28 ± 1.73 and without direct contact with ice and water 78.96 ± 0.38, but in loligo with GMP 79.55 ± 1.39, 77.66 ± 0.23 without direct contact with ice and without GMP 79.52 ± 1.24.

Appendix C.2. shows the ANOVA table for changes in fat content of both the species of squid during ice storage. The fat content of loligo sample, with GMP showed a decrease from 2.17% to 1.82%, without GMP showed significantly higher loss of fat during the storage period (2.2% to 2.0%) and in samples with non-contact ice showed a significantly lower value than other methods (2.2%-2.17%). All the values were significant at 5% level.

Appendix C.3. shows ANOVA of the changes in ash content of both the species of ice-stored squid. Ash content showed a significant difference between species, methods of icing and days of storage (p<0.001). Ash also showed a decrease of 1.5% to 1.12% in loligo and 1.50% to 0.5% in needle, for samples without GMP. The samples with GMP showed a decrease of 1.6%-0.9% (needle) and 1.56%-1.34% (loligo). The samples with non-contact ice showed a significantly lower loss of minerals during storage (1.55%-1.43% in loligo) and (1.37%-1.12% in needle). Between species, the needle squid showed a significantly lower value in the loss of fat and minerals during ice storage than loligo. Since fat and ash play a very insignificant role in determining the quality of squid, the study of fat and ash has been avoided in the rest of the work.

<table>
<thead>
<tr>
<th>Days</th>
<th>with GMP</th>
<th>w/o GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.9</td>
<td>14.2</td>
</tr>
<tr>
<td>2</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.42</td>
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</tr>
<tr>
<td>5</td>
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<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>14.2</td>
<td></td>
</tr>
</tbody>
</table>

3.4.2. Changes in Loligo

The results showed the squid of both the species showed a significant difference in days of storage in species storage in species respectively, having C.4. shows ANOVA of both species, needle showed GMP than loligo. Between method protein loss and with leaching. Among than the rest of the
Table 3.1. Changes of total protein during ice storage (%)

<table>
<thead>
<tr>
<th>Days</th>
<th>Loligo</th>
<th>Needle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with GMP</td>
<td>w/o GMP</td>
</tr>
<tr>
<td>1</td>
<td>20.9</td>
<td>20.84</td>
</tr>
<tr>
<td>2</td>
<td>18.5</td>
<td>16.42</td>
</tr>
<tr>
<td>3</td>
<td>17.2</td>
<td>14.98</td>
</tr>
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<td>4</td>
<td>16.42</td>
<td>14.02</td>
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<tr>
<td>5</td>
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<td>6</td>
<td>15.1</td>
<td>12.55</td>
</tr>
<tr>
<td>7</td>
<td>14.8</td>
<td>11.85</td>
</tr>
<tr>
<td>8</td>
<td>14.2</td>
<td>11.2</td>
</tr>
</tbody>
</table>

w/o GMP- without GMP, w/o DC-without direct contact with ice and water

3.4.2. Changes in total protein content

The results showed that there was a great loss of protein in processed squid of both the species during ice storage (Table 3.1.). The protein content showed a significant difference between species, methods of icing, and the days of storage in ice (p < 0.001). The F value for protein loss during ice storage in species, methods, and the days were 6.9, 87.95 and 26.3 respectively, having degrees of freedom (1,37), (2,37) and (7,37). Appendix C.4. shows ANOVA for changes in protein during ice storage. Among the species, needle squid showed a significantly higher protein loss than in loligo. Between methods, without direct contact with ice showed a very low protein loss and without GMP showed significantly higher value of protein leaching. Among days, day 1 showed a significantly higher protein content than the rest of the day and day 8 showed the least protein content.
The protein loss was significant in 8th day when compared with 1st, 2nd, and 3rd day of storage. There was no significant difference in 4th to 8th day of storage.

The samples of loligo had an average loss of 32.05%, 46.25% and 17.45% of total protein in samples with GMP, without GMP and without direct contact respectively. Repeated extraction of squid mantle muscle with water resulted in an increase in the amount of protein dissolution, in contrast to fish muscle, with more than 50% of total protein extracted in many cases. The needle squid kept under icing with GMP, without GMP and without direct contact had an average loss of 47.16%, 59.42% and 21.9% of protein respectively.

3.4.3. Total volatile bases
3.4.3.1. Total volatile base nitrogen

Figure 3.1 and 3.2 show the changes of TVBN of both the species during ice storage by the three methods of loligo and needle squid respectively.

![Figure 3.1. Changes in TVBN in loligo squid during ice storage](image)

Appendix C.5 shows the changes in TVBN of both the species during ice storage. There was no significant difference in the 1st, 2nd, and 3rd day (p < 0.001). Figure 3.1 shows a significantly lower value than the other methods. TVBN values were observed to be significantly lower in loligo at 2.9, 37.82, and 10.93 mg/g at 1st, 2nd, and 3rd day of storage respectively.

According to C.5, the TVBN value of 30 mg per kg has crossed 30 mg % only on the 8th day of storage. The TVBN value did not exceed the 30 mg % limit on any other day of storage.
Figure 3.2. Changes in TVBN of Doryteuthis sibogae during ice storage

Appendix C.5. gives ANOVA of changes of TVN values during iced storage. There was no significant difference between species. There was significant difference between methods (p < 0.001), and days of storage (p < 0.001). Between methods, icing without GMP showed significantly higher value than the other two methods. The samples without direct contact showed a significantly lower value. Between days, significantly higher values were observed on 8th day of storage. There was no significant difference between 7th and 8th day. Both the species of squid showed significantly lower value on 1st and 2nd day of storage. The F values were 2.9, 37.82, and 10.64 between species, between methods, and between days of storage respectively.

According to Woyewoda and Ke (1980) the samples exceeding TVN value of 30mg per 100g was not acceptable. In loligo without GMP, TVN crossed 30 mg % on 4th day of storage. But with GMP, it exceeded the value only on the 8th day of storage. The samples without direct contact with ice did not exceed the limit till 8th day of storage. In the case of needle squid,
TVN reached 30mg on 3rd day in the sample without GMP. It attained 30mg only on 6th day when GMP was followed. Samples without direct contact with ice remained within the limit of TVBN till 8th day of storage.

3.4.3.2. Trimethylamine Nitrogen (TMA N)

The changes in TVN of loligo and needle squid were given in ice storage. There was no significant difference between methods of ice storage. There was no significant difference among species needle squid and loligo. Among methods, with and without GMP methods, significantly higher values of TVN were observed. The lowest value was observed for loligo exceeding 3 to 10 GMP at 8th day of storage, with GMP crossed the limit of storage, with GMP of needle squid exceeding at 8th day of storage.

But in needle squid, the values exceeded 80 GMP at 8th day of storage without GMP and respectively.
The changes in TMA content during ice storage by three methods in loligo and needle squid are given in Figure 3.3 and 3.4, respectively.

Appendix C.6. gives the ANOVA of TMA in all the three methods of ice storage. There was significant difference between species (p < 0.01), between methods of icing (p < 0.001) and between days (p < 0.001). Among species needle squid showed significantly higher value than loligo squid. Among methods, without GMP gave a significantly higher value and without direct contact with ice a lower value. 8th day of storage showed significantly higher value when compared to 1 to 6 days. Highly significant lower value was observed in without direct contact with ice. The samples exceeding 3 to 10mg of TMA per 100g of sample is the limit of acceptability (Woyewoda and Ke, 1980). In loligo kept without GMP, TMA crossed the limit of 10mg% and became non acceptable on the 3rd day of storage, with GMP on 6th day of storage and without direct contact on 8th day of storage.

But in needle squid, the rate of increase in TMA was more than that in loligo, the values exceeding the acceptable level on 2nd, 5th and 7th day of ice storage without GMP, with GMP and without direct contact with ice respectively.
3.4.4. Alpha amino nitrogen

![Graph showing changes of alpha amino nitrogen of loligo during ice-storage](image1)

Figure 3.5 Changes of alpha amino nitrogen of loligo during ice-storage

![Graph showing changes of alpha amino nitrogen of needle squid during ice-storage](image2)

Figure 3.6 Changes of alpha amino nitrogen of needle Squid during ice-storage

3.4.5. Fractions of sarcoplasmic proteins

Table 3.2. gives the change of both the species of sarcoplasmic protein 54% and 65% in a contact respectively.

<table>
<thead>
<tr>
<th>Days</th>
<th>with GMP</th>
<th>w/o GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.88</td>
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<td>11.5</td>
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<tr>
<td>8</td>
<td>7.5</td>
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</tbody>
</table>
Figure 3.5 and 3.6 shows the gradual decrease in alpha amino nitrogen in loligo and needle squid respectively under different methods of ice storage. The leaching of alpha amino nitrogen was not significant at 5% level between days of storage and species (Appendix C.7). In the case of samples without direct contact, a steady increase in alpha amino nitrogen was observed during the storage period, retaining the sweet taste till the end of storage. But in the case of samples with direct contact, the alpha amino nitrogen reduced continuously and attained a very low level. There was a significant difference between days of storage, (p < 0.001). The value of retained alpha amino nitrogen in the muscle showed a significant lower value in samples without GMP. The leaching rate of alpha amino nitrogen in needle squid was higher than that in loligo.

3.4.5. Fractions of Proteins

Table 3.2. gives the changes in sarcoplasmic proteins during storage of both the species of squid in ice. According to the study, the percentage of sarcoplasmic protein fractions retained in the muscle tissue were 61%, 54% and 65% in samples with GMP, without GMP and without direct contact respectively.

3.4.5.1. Sarcoplasmic proteins

<table>
<thead>
<tr>
<th>TABLE 3.2. Changes in sarcoplasmic proteins during ice storage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>
There was significant difference between species, methods of icing and the days of storage (Appendix C.8.). In species, loligo showed a significantly higher value of protein content in the muscle than in needle tissue. Leaching rate of sarcoplasmic protein showed a higher value in samples without GMP. Among days, 1st day showed a higher protein content in the tissue than all other days. There was significant decrease in protein content from 1 to 6 days but on 7th and 8th day there was no significant difference.

3.4.5.2. Myofibrillar proteins

Table 3.3. gives the leaching rate of myofibrillar proteins during various methods of icing.

<table>
<thead>
<tr>
<th>Days</th>
<th>Loligo</th>
<th></th>
<th></th>
<th>Needle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with GMP</td>
<td>w/o GMP</td>
<td>w/o DC</td>
<td>with GMP</td>
<td>w/o GMP</td>
<td>w/o DC</td>
</tr>
<tr>
<td>1</td>
<td>8.6</td>
<td>8.3</td>
<td>9</td>
<td>9.3</td>
<td>9.04</td>
<td>9.5</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>7.7</td>
<td>8.6</td>
<td>8.7</td>
<td>8.5</td>
<td>9.2</td>
</tr>
<tr>
<td>3</td>
<td>7.2</td>
<td>7</td>
<td>7.8</td>
<td>8.3</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>6.9</td>
<td>6.7</td>
<td>7.4</td>
<td>7.8</td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td>5</td>
<td>6.3</td>
<td>6.1</td>
<td>7</td>
<td>7.4</td>
<td>7.2</td>
<td>8.5</td>
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<td>5.65</td>
<td>6.8</td>
<td>7.1</td>
<td>6.8</td>
<td>8.3</td>
</tr>
<tr>
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<td>5.2</td>
<td>5</td>
<td>6.6</td>
<td>6.7</td>
<td>6.5</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>4.8</td>
<td>6.4</td>
<td>6.4</td>
<td>6</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Minimum loss of water. Maximum loss
C.9.gives ANOVA for
significant differences so
(p<0.001 for all). Needle
myofibrillar proteins
significantly lower while

3.4.5.3. Denatured proteins

Table 3.4. showed
Needle showed a higher
Degree of denaturation
compared with other,
showed lowest degree
between all the methods.

<table>
<thead>
<tr>
<th>Days</th>
<th>with GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.12</td>
</tr>
<tr>
<td>5</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Minimum loss was found in sample without direct contact with ice or water. Maximum loss was reported in samples without GMP. Appendix C.9 gives ANOVA for myofibrillar protein during ice storage. There was significant difference between species, between methods and between days (p<0.001 for all). Needle squid showed higher value than loligo and the myofibrillar protein content in samples kept without GMP showed significantly lower value than the other two methods.

3.4.5.3. Denatured proteins

Table 3.4. shows the variation in denatured protein during ice storage. Needle showed a higher degree of denaturation when compared to loligo. Degree of denaturation was much higher in samples without GMP. When compared with other two methods, the samples without direct contact showed lowest degree of denaturation. There was significant difference between all the methods and between days (Appendix C.10).

**TABLE 3.4. Changes in denatured protein during ice storage (%)**

<table>
<thead>
<tr>
<th>Days</th>
<th>Loligo with GMP</th>
<th>Loligo w/o GMP</th>
<th>Loligo w/o DC</th>
<th>Needle with GMP</th>
<th>Needle w/o GMP</th>
<th>Needle w/o DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>0.13</td>
<td>0.08</td>
<td>0.1</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>0.18</td>
<td>0.09</td>
<td>0.13</td>
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<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>0.14</td>
<td>0.24</td>
<td>0.12</td>
<td>0.19</td>
<td>0.28</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>0.16</td>
<td>0.28</td>
<td>0.15</td>
<td>0.32</td>
<td>0.35</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>0.18</td>
<td>0.34</td>
<td>0.17</td>
<td>0.27</td>
<td>0.37</td>
<td>0.23</td>
</tr>
</tbody>
</table>
3.4.5.4. Stroma proteins

TABLE 3.5. Changes in stroma protein during ice storage (%)

<table>
<thead>
<tr>
<th>Days</th>
<th>Loligo with GMP</th>
<th>Loligo w/o GMP</th>
<th>Loligo w/o DC</th>
<th>Needle with GMP</th>
<th>Needle w/o GMP</th>
<th>Needle w/o DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>1</td>
<td>1</td>
<td>0.97</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>2</td>
<td>1.02</td>
<td>0.97</td>
<td>0.96</td>
<td>0.92</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
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<td>0.92</td>
<td>0.93</td>
<td>0.9</td>
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<tr>
<td>4</td>
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<td>0.92</td>
<td>0.87</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>5</td>
<td>0.9</td>
<td>0.88</td>
<td>0.9</td>
<td>0.83</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
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<td>0.86</td>
<td>0.87</td>
<td>0.8</td>
<td>0.78</td>
<td>0.76</td>
</tr>
<tr>
<td>7</td>
<td>0.8</td>
<td>0.82</td>
<td>0.85</td>
<td>0.77</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>8</td>
<td>0.75</td>
<td>0.74</td>
<td>0.8</td>
<td>0.71</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 3.5 gives a detailed picture of stroma protein fraction during ice storage.

Loligo showed a lesser leaching rate of stroma while needle exhibited higher leaching rate. Appendix C.11 shows the ANOVA for stroma protein content during ice storage. There was a significant difference between species, between methods and between days (p<0.001).

3.4.6. Physical and organoleptic Characters

Samples kept under ideal conditions retained the native pH of the fresh tissue up to 8th day of storage where there was a slight increase (Table 3.6). The samples kept with GMP and without direct contact showed a slight increase in pH of both the species (6.2 to 6.8 and 6.2 to 6.7). The samples without direct contact with ice showed a superior organoleptic quality when compared with other two methods in both the species. Organoleptically the
samples of both the species were acceptable up to 6th day, which were kept under proper GMP, while the samples without GMP were rejected after two days of storage. The samples without direct contact with ice showed an acceptable standards till the end of the storage. Samples without GMP showed a higher rate of increase in pH with the storage days in both the species. They also showed an inferior sensory score, reaching the rejection level by the 3rd day of storage. In all the methods pH did not correlate significantly with sensory score.

Table 3.6. Changes in pH and Organoleptic score during ice-storage

<table>
<thead>
<tr>
<th>Storage period</th>
<th>WITH GMP</th>
<th>NEEDLE</th>
<th>WITH GMP</th>
<th>NEEDLE</th>
<th>WITH GMP</th>
<th>NEEDLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>pH</td>
<td>score</td>
<td>pH</td>
<td>score</td>
<td>pH</td>
<td>score</td>
</tr>
<tr>
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<td>7</td>
<td>6.2</td>
<td>7</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>7</td>
<td>6.5</td>
<td>5</td>
<td>6.2</td>
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<td>6.5</td>
<td>7</td>
</tr>
<tr>
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<td>6.5</td>
<td>5</td>
<td>7.5</td>
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<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
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<td>6.6</td>
<td>5</td>
<td>7.8</td>
<td>2</td>
<td>6.7</td>
<td>6.5</td>
</tr>
<tr>
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<td>6.8</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>6.7</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage period</th>
<th>WITH GMP</th>
<th>LOLIGO</th>
<th>WITH GMP</th>
<th>LOLIGO</th>
<th>WITH GMP</th>
<th>LOLIGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>pH</td>
<td>score</td>
<td>pH</td>
<td>score</td>
<td>pH</td>
<td>score</td>
</tr>
<tr>
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<td>6.2</td>
<td>7</td>
<td>6.2</td>
<td>7</td>
<td>6.2</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>6.2</td>
<td>7</td>
<td>6.3</td>
<td>6</td>
<td>6.2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>7</td>
<td>6.5</td>
<td>5</td>
<td>6.2</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
<td>6.5</td>
<td>6.8</td>
<td>4</td>
<td>6.3</td>
<td>7.5</td>
</tr>
<tr>
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<td>6.4</td>
<td>6</td>
<td>6.9</td>
<td>3</td>
<td>6.3</td>
<td>7.5</td>
</tr>
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<tr>
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<td>5</td>
<td>7.4</td>
<td>2</td>
<td>6.5</td>
<td>7</td>
</tr>
</tbody>
</table>
### 3.4.7. Bacteriology

**Table 3.7. Changes in TPC during ice storage (counts/g)**

<table>
<thead>
<tr>
<th>Days</th>
<th>LOLIGO w/o GMP</th>
<th>LOLIGO w GMP</th>
<th>LOLIGO W/o DC</th>
<th>NEEDLE w/o GMP</th>
<th>NEEDLE w GMP</th>
<th>NEEDLE W/o DC</th>
</tr>
</thead>
<tbody>
<tr>
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<td>$4.21 \times 10^4$</td>
<td>$3.5 \times 10^4$</td>
<td>$5.3 \times 10^5$</td>
<td>$2.85 \times 10^4$</td>
<td>$3.57 \times 10^4$</td>
</tr>
<tr>
<td>2</td>
<td>$1.76 \times 10^5$</td>
<td>$2.2 \times 10^4$</td>
<td>$2.28 \times 10^4$</td>
<td>$1.85 \times 10^5$</td>
<td>$1.85 \times 10^4$</td>
<td>$1.97 \times 10^4$</td>
</tr>
<tr>
<td>3</td>
<td>$1.82 \times 10^5$</td>
<td>$2.25 \times 10^4$</td>
<td>$2.3 \times 10^4$</td>
<td>$2.02 \times 10^5$</td>
<td>$2.0 \times 10^4$</td>
<td>$2.05 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
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<td>$2.45 \times 10^4$</td>
<td>$2.22 \times 10^5$</td>
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</tr>
<tr>
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<td>$2.62 \times 10^4$</td>
<td>$2.28 \times 10^5$</td>
<td>$2.31 \times 10^4$</td>
<td>$2.35 \times 10^4$</td>
</tr>
<tr>
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<td>$2.85 \times 10^4$</td>
<td>$2.29 \times 10^5$</td>
<td>$2.41 \times 10^4$</td>
<td>$2.46 \times 10^4$</td>
</tr>
<tr>
<td>7</td>
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<td>$2.93 \times 10^4$</td>
<td>$2.35 \times 10^5$</td>
<td>$2.48 \times 10^4$</td>
<td>$2.65 \times 10^4$</td>
</tr>
<tr>
<td>8</td>
<td>$2.22 \times 10^5$</td>
<td>$2.62 \times 10^4$</td>
<td>$3 \times 10^4$</td>
<td>$2.46 \times 10^5$</td>
<td>$2.75 \times 10^4$</td>
<td>$2.79 \times 10^4$</td>
</tr>
<tr>
<td>9</td>
<td>$2.25 \times 10^5$</td>
<td>$2.65 \times 10^4$</td>
<td>$3.2 \times 10^4$</td>
<td>$2.50 \times 10^5$</td>
<td>$2.86 \times 10^4$</td>
<td>$2.9 \times 10^4$</td>
</tr>
<tr>
<td>10</td>
<td>$2.28 \times 10^5$</td>
<td>$2.75 \times 10^4$</td>
<td>$3.25 \times 10^4$</td>
<td>$2.68 \times 10^5$</td>
<td>$2.97 \times 10^4$</td>
<td>$3 \times 10^4$</td>
</tr>
<tr>
<td>11</td>
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<td>$2.80 \times 10^4$</td>
<td>$3.37 \times 10^4$</td>
<td>$2.89 \times 10^5$</td>
<td>$3.08 \times 10^4$</td>
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<tr>
<td>12</td>
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<td>$3.42 \times 10^4$</td>
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<td>$3.28 \times 10^4$</td>
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</tr>
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<td>13</td>
<td>$2.55 \times 10^5$</td>
<td>$3 \times 10^4$</td>
<td>$3.5 \times 10^4$</td>
<td>$3.4 \times 10^5$</td>
<td>$3.34 \times 10^4$</td>
<td>$3.39 \times 10^4$</td>
</tr>
</tbody>
</table>

The analysis of variance of total bacterial count (TPC) after conversion to the logarithmic values showed no significant difference between species. TPC was assessed up to 13 days (Table 3.7). But between days and the methods there were significant differences ($p < 0.001$). Among the methods the icing without GMP showed significantly higher growth rate than the other two methods. The method with GMP showed a lower growth than the other two. There was a reduction of TPC in the first two days and then the number gradually increased with storage days (Appendix C.12). The sanitary significant bacteria like *E.coli* and *Staphylococcus* showed a higher rate of growth.

Throughout the study, in Needle samples. There was no difference observed between keeping samples without GMP in the ice or in Needle samples. But in Loligo there was a significant decrease in the growth of *Staphylococcus* in Needle samples. This might be because the Needle samples remained at a significantly lower temperature than the Loligo samples for a longer period of time.

Since there is no direct evidence that Loligo samples were exposed to a hypothermic environment, it remains almost same until the end of the storage period.

### 3.5. Discussion

Chemical and physical changes are common phenomena during the freezing and thawing process, which largely depend on the rate and extent of the process. The freezing rate can be influenced by various factors, such as the temperature difference between the product and the surrounding environment, the rate of heat transfer, and the size and shape of the product. Fast freezing rates can lead to the formation of small ice crystals, which can cause cell disruption and damage. Slow freezing rates, on the other hand, can lead to the formation of large ice crystals, which can cause less damage to the cells. However, the formation of large ice crystals can also be problematic as they can be difficult to remove during thawing.

Flake ice avoids damaging the surface layers of fish, thereby causing less damage to the quality of the fish as it melts, with the small ice crystals coming from ice to water, which is sufficient to cool about 30% of the water and 70% of the flesh.
higher rate of growth in the samples without GMP irrespective of species. Throughout the study *salmonella* and *vibrio* were not detected in the samples. There was no significant difference between samples with GMP or without GMP in the growth of *Staphylococcus* in needle (t = 1.043, df = 7). But in loligo there was a significant difference between the methods in the growth of *Staphylococcus* (t = 4.686, df = 7, p < 0.01). Without direct contact with ice showed no significant growth of *E-coli* and *Staphylococcus*, since there is no direct contact with ice and water. The number of colonies remains almost same till the end of the storage.

### 3.5. Discussion

Chemical and physical qualities play vital role in grooming the quality of a product. Since the spoilage of squid starts immediately after the death which largely dependent on the temperature conditions, the sooner the squid can be cooled the better will be the quality and shelf life. In ice storage, it is not only enough that there is sufficient quantity of ice to preserve the squid, but the effectiveness of icing depends on how well the ice is dispersed among the squid. The smaller the size of ice, the greater will be the contact between fish and ice. The rate of heat removal depends on the contact area. Flake ice avoids damaging the squid. The large pieces exerts point forces thereby causing damage which in turn increase the rate of leaching of protein in crushed ice. The major advantage of using ice for chilling fish is that it has a high latent heat of fusion, which can remove large amount of heat as it melts, without changing the temperature at 0°C. During transition from ice to water, 1 kg of ice absorbs 80 kcal of heat and this will be sufficient to cool about 3 kg of fish from 30°C to 0°C. Hence theoretically, ice about 30% of sample weight can bring down the temperature of the
sample from ambient conditions to 0°C. But lot of other factors like surrounding temperature, type of box used for icing, length of time fish need to be kept chilled, thickness of the fish etc., have to be taken into consideration in calculating the amount of ice needed to chill fish. Hence in tropical conditions 1:2 fish to ice ratio is ideal for ice storage.

Dressed squid mantles in seafood industry are stored in crushed ice and its meltwater, in non-perforated containers prior to freezing. It has been observed that the melt water becomes turbid and white mainly due to the leaching of NPN and soluble protein from the squid.

The changes in the moisture content in packed and unpacked samples of *Chanos chanos* stored in ice for 25 days were studied by Subrata and Imam (1985). The moisture content of unpacked sample showed a slight decrease up to three days of ice storage and then a gradual increase till 19th day after which there was a fall. In the case of packed sample, without direct contact, with ice, showed a gradual decrease in the moisture throughout the storage period. According to Joseph et al., (1977) the moisture content showed a gradual increase during the ice storage of squid tubes. Moisture content of the *Loligo duvaucelli* tubes increased from 78.33% to 83.08% at the end of 8th hour of ice storage (Reghunath, 1984). The present study also shows a similar trend. The gradual rise in moisture in samples stored in direct contact with ice is due to the absorption of ice meltwater by the muscle. But studies with prawn stored in ice showed a weight increase in the 1st two days without loss of nutrients, but by prolonging the storage period both solids and water were lost resulting in gradual decrease in weight (Mathen and Thomas, 1988). The moisture content in needle squid reached a difference of 1.68% under ideal conditions. But in the

According to Matsumoto (1958) the cuttlefish *cuttlefish* (Lakshmanan, 1984) observed that a barrier, which prevents the protein fractions retentive samples with GMP, comparable with the conventional method in samples with non-skinned product owing to a barrier, which prevents the proteins from being dissolved in water. The advantages of using GMP, comparable with the conventional method in samples with non-skinned product owing to a barrier, which prevents the proteins from being dissolved in water. The advantages of using GMP, comparable with the conventional method in samples with non-skinned product owing to a barrier, which prevents the proteins from being dissolved in water. The advantages of using GMP, comparable with the conventional method in samples with non-skinned product owing to a barrier, which prevents the proteins from being dissolved in water. The advantages of using GMP,
of other factors like
length of time fish need
to be taken into
chill fish. Hence in
ice storage.

ice stored in crushed ice
without freezing. It has been
observed mainly due to the

unpacked samples
studied by Subrata and
sample showed a slight
increase till 19th
day. Sample, without direct
moisture throughout the
moisture content
in squid tubes. Moisture
was 83.33% to 83.08% at
the present study also
samples stored in
meltwater by the
weight increase in
along the storage
and gradual decrease in
needle squid
determinations. But in the

conventional method, the difference of moisture content reached 3.3% while
samples with non-contact ice showed only an increase of 0.68%. Joseph et al., (1977) have studied the quality of the squid tube stored in ice and
found that ice stored tubes were not acceptable after 5 days.

The total nitrogen also reduced considerably on the 5th and 6th day.
According to Matsumoto, (1978) 77 to 85% of the total protein of the squid
is water-soluble. Similarly, the non-protein nitrogen also showed a sharp
decrease when stored in ice. It was noticed that the squid meat contained
high percentage of NPN (Joseph et al., 1977) and Brongstrong, 1965). Matsumoto (1958) has also reported high myosin fraction in squid protein.
The average value of WEN and NPN were higher in whole squid and
cuttlefish (Lakshmanan, 1993). There was a great loss of WEN and NPN in
skinned product owing to the leaching effect of this component. Skin acts as
a barrier, which prevents the leaching effect to a greater extent. Raghunath
(1984) observed that WEN and NPN reduced considerably in squid mantles
stored in crushed ice and meltwater after 8 hours. These results are
comparable with this study. In this study the percentage of sarcoplasmic
protein fractions retained in the muscle tissue were 61%, 54% and 65% in
samples with GMP, without GMP and without direct contact respectively.

According to Lapa (2002), the non-contact ice-storage offers no
benefits over contact ice-storage in terms of preserving squid quality. But
other studies showed that squid kept in contact icing were decolourised in
less than 12 hours and could not be considered acceptable for food for more
than two days, but non-contact ice stored squid could hold the freshness for
a longer time (Kc, 1991). A considerable loss of dry matter in squid due to
leaching on contact with water has also been reported by Berg (1974). In
this study, it was noticed that the non-contact ice stored squid showed a better quality when compared with the other methods of icing. Water extractable nitrogen, NPN and alpha amino nitrogen gradually decreased and squid lost their characteristic sweetness and finally became bland in taste (Lakshmanan et al., 1993). Tanikava et al., (1954) observed mustiness in raw squid, which indicated the spoilage when TVN reached 30mg/100g. Lakshmanan et al., (1993) have stated that samples exceeding TMA value of 5mg/100gm lost their culinary characteristics. In loligo without GMP, TVN exceeded 30 mg % on 4\textsuperscript{th} day of storage while storage with GMP and without direct contact TVN remained within the limit even up to 8 days of storage. Almost a similar trend was shown in needle squid also.

Alpha amino nitrogen content of squid tubes stored in ice showed a very low value after two days of ice storage, while total volatile base nitrogen content showed a gradual increase (Joseph et al., 1977). Comparable results were obtained in this study and it is concluded that the squid mantle cannot be stored in ice after six days even with GMP. The changes in TVBN and alpha amino nitrogen showed a gradual increase in iced stored fish species were also reported by Bandyopadhyay (1986) and Sankar and Nair (1988).

From organoleptic evaluation, it is observed that the icing without direct contact was always more acceptable than the one with direct contact with ice even up to 8 days of storage. The flavour was better in the former one, as leaching of flavour compounds was minimum. At any particular stage of storage, the texture and colour were also more acceptable in the case of samples with GMP and without direct contact with ice. The samples kept with GMP and without direct contact showed a slight increase in pH in

both the species (6.2). Contact with ice showed a marked increase with other two methods of iced squid is likely due to the changes. TPC increased and eventuated the quality of cephalopods. Pseudoalteromonas dominated (Bandopadhyay, 1986) in both packed and frozen fish. steady increase in bacterial count was observed. decrease in TPC was due to the domination of Pseudomonas (Bandyopadhyay, 1986) and slight increase in bacterial count was observed. Pseudomonas was the microorganism responsible for getting the undesirable compound. 

3.6. Conclusion

In a developing country like India, frozen fish in domestic supermarkets are highly popular, but the quality of these fish is a major concern. The average consumer only cares about the taste and quality of the fish, but they are not aware of the degree of spoilage. The present work has established that the nature of the fish, and the method of freezing play a significant role in the quality of the fish. The results of the present study can be used to improve the quality of frozen fish, and to reduce the level of spoilage.
Both the species (6.2 to 6.8 and 6.2 to 6.7). But the samples without direct contact with ice showed a superior organoleptic quality when compared with other two methods in both the species. It is suggested that the spoilage of iced squid is likely to result from a combination of autolytic and bacterial changes. TPC increased with increasing ice storage time and decreased the quality of cephalopods (Civera, 2000). Shewanella putrefaciens, Pseudoalteromonas sp and Pseudomonas sp dominated in spoiled gutted squid (Paarup, 2002). There was an initial reduction in TPC, which later increased and eventually surpassed the original counts. Initial reduction in TPC was due to leaching and cold shock after which psychrophiles dominated (Bandyopadhyay, 1986; Joseph et al., 1977). In a study of icing of both packed and unpacked samples of Chanos chanos, initially a definite decrease in TPC was observed due to sudden chilling effect of ice on bacteria. This increased very slowly up to 17th day and then growth became faster up to 21 days of ice storage (Subrata, 1985). From 4th to 13th day, a steady increase in bacterial load was seen. There was a significant leaching effect of ice meltwater on bacterial population.

3.6. Conclusion

In a developing tropical country like India, where distribution of frozen fish in domestic market is not popular due to lack of cold chain facility, short term preservation of fish by icing has gained importance. Average consumers prefer fresh fish to iced fish even if both show the same degree of spoilage. This is probably due to the leaching of components responsible for good organoleptic characteristics, along with some undesirable components of spoilage. The extent of leaching during iced storage of different species of squid and its effect on overall quality has
been studied in detail. Needle squid were more susceptible to spoilage than loligo and leaching rate was also found to be more in the case of needle squid. Hence it is recommended to avoid direct contact with ice when transported from landing centers to the processing units and while storing in ice. Needle squid should be handled more carefully from the point of catching till the final stage of processing, since the post-mortem changes were much faster than in loligo. Ice storage is not advisable after 6th day even where GMP is followed. The samples with GMP and without direct contact with ice were acceptable till 8th day of storage in both the species of squid. The chemical indices as well as organoleptic score showed favourable results in samples without direct contact with ice. Alpha amino nitrogen, which contributes to the sweet flavour of meat, was retained in this method of icing, while in the other two methods, the alpha amino nitrogen as well as WEN were lost and a bland taste was obtained. Finely crushed block ice or flake ice should be used and layering of ice and squid is preferably done in flat boxes. Extended contact with ice and melt water should be avoided. The method recommended to the fishermen is to ice squid without direct contact with ice by using an intervening plastic sheet. Thus under similar conditions of icing (with GMP), storage of squid without direct contact with ice and melt water will have longer shelf life and give more acceptable product than those stored directly in ice.
3.7. References

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* Not referred in original