Chapter 6

EFFECT OF VARIOUS TREATMENTS ON QUALITY

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6.7. References
6.1. Introduction

Owing to high ambient temperatures, post-mortem changes in fish captured from tropical regions occur more rapidly than in fish from temperate waters. Proper icing practices are fundamental to obtain high quality products. Freezing and frozen storage prolong the shelf life of seafood by retarding enzymatic and microbial activity. An alternative approach to extend the storage life of fish is, application of more than one treatment like addition of anti-microbial compounds, anti-oxidants, polyphosphates etc to improve the muscle quality. In the previous chapters, the effect of icing, super cooling, freezing and frozen storage as short term and long term preservative methods of two species of squids under study, Loligo species and Doryteuthis species and the importance of Good Manufacturing Practices, the processor has to adhere to, have been discussed. But it is important to have a better understanding of the effect of possible treatments in prolonging the frozen storage characteristics of the two species of squid. Hence a comparative study has been undertaken here with regard to the use of various treatments, and to recommend the most appropriate one to retain the physico-chemical, organoleptic and microbiological properties of the frozen product.

6.2. Review of Literature

Several work have been carried out on the effect of treatments on quality changes of different species of squid and other marine fishes during ice-storage and frozen storage.
6.2.1. Effect of Sodium Salts

Sodium chloride treatment is widely used in cephalopod processing. The firmness of the meat was regained by the use of sodium salts, especially sodium chloride. Sodium salts play an anti-oxidative/anti-microbial role in the meat processing (Rhee et al., 1997). The sensory quality was found to be increased when treated with sodium ascorbate, lactate and phosphate (Kulashekhra and Rhee, 1996). Hennigar (1988) studied the effect of washing and sodium chloride concentration on mechanical properties of fish muscle gel. NaCl is required to solubilise myofibrillar protein, which then repolymerise to form protein network responsible for the gel (Sakai, 1981). Chang et al., (1995) studied the effect of sodium acetate on catfish fillet (Ictalurus punctatus). Matlock et al., (1984) studied the increasing water holding capacity of salt treated sample through extraction of salt soluble protein and the enhanced juiciness of cooked meat. The texture of cooked mantle of squid (Illex argentinus) as influenced by the specimen characteristics and treatments was studied by Ilona et al., (1987).

The effect of salt in the range of 0% to 5% on the textural property of the minced squid product was studied by Pan et al., (1979). He found that the maximal breaking force was found at a concentration of 3%-4% salt, and 2.5% salt is the maximum concentration to give maximal elasticity. Scanning Electrons Microscopy (SEM) showed a structure of thread like bundles on mixing with salt for 10 minutes and prolonged mixing resulted in a cross linking network. It was suggested that uniformity and compactness of the protein matrix, played an important role in textural quality of minced fish product.
6.2.2. Treatment with Mild acid

Treatment with 2% salt and 0.2% citric acid was found to improve the overall quality of cuttlefish fillet on frozen storage (Sophia and Sherief, 2003). The NPN fraction and alpha amino nitrogen content were found to be higher in treated sample when compared to control and were organoleptically in good conditions up to 8 weeks of frozen storage.

In addition to the use of acetic acid and acetates in anti-microbial preservation they are used as sequestrants, acidulants and flavouring agents. Spirit vinegar is made from distilled grain alcohol, which contained 4gms of acetic acid per 100ml (Desrosier, 1959). Six types of vinegar are recognised by USFDA and are cider, wine, malt, sugar, glucose and spirit vinegar (Furia, 1968). Acetic acid in the form of vinegar has been used in the food preservation since 5000B.C. (Lueck, 1980). The acid must penetrate the cell wall of microorganism and denature the protein. In order for acetic acid to accomplish this, it must be present in a concentration above 0.5% and acetic acid is 10 to 100 times powerful as preservative at a pH of 3.0 (Lueck, 1980). Gelation of shark myofibrillar proteins by weak organic acids was studied using acetic acid, citric acid, tartaric acid and hydrochloric acid, in which the water formed gel associated with an increase in viscosity was formed, when the pH was lowered to 4.5 using acetic acid (Venugopal, 1994). Venugopal et al., (1995) also reported that a thermo stable gel was prepared from shark myofibrillar protein by reducing the pH to 4.0 using acetic acid. Addition of 1.5 to 2.5 % of NaCl showed significantly higher gel strength as gelation enhancers (Gomez guillem, 1996). Drop wise addition of glacial acetic acid resulted in its slow thickening due to gelation dependent on viscosity increase. In this study the proteins were precipitated on heating the different protein extracts and the acetic acid and acetic acid and NaCl treated samples showed a significant increase in gel strength as compared to control and NaCl treated samples. Anti microbial properties of acetic acid and acetic acid and NaCl treated discs were studied by Liao (2003). Treated discs showed acetic acid and NaCl treated discs to have a significant reduction in bacterial count with 0.8 logs. Similar results were obtained in reducing fungal count on fruits or seeds with acetic acid and NaCl (Parnell and Hyder, 1995; Parnell and Hyder, 1995). Fish protein glaze with 2.5% acetic acid was studied by Lee, Rhee, 1996, Rhee, 1996. The acetic acid treated squid (Loligo pealei) showed reduction in shelf life. Ascorbic acid added after nine months of treatment could improve the shelf life.
on heating the dispersion either by increasing the pH to 6.0 or addition of salts (Venugopal et al., 1997). Venugopal et al., (2002) and Venugopal (2003) studied the physico chemical and rheological characterization of gel from shark treated with mild acid.

Anti microbial action of acetic acid on cut surface of apple slices was studied by Liao et al., (2003) and reported that washing with a mixture of acetic acid and H₂O₂ was most effective in removing salmonella from apple discs. Acetic acid is generally recognised as safe and has been approved for use as a food additive (FDA, 1982). Dickson (1992) reported that washing beef tissue with 2% acetic acid reduced the number of Salmonella by 0.5 to 0.8 logs. Similar effect of acetic acid on lamb carcass on reduction of bacterial count was also reported by Anderson et al., (1988). Treatment of fruits or seeds with acetic acid or vinegar has also been shown to be effective in reducing fungal decays and food borne pathogens (Shilberg and Gaunce, 1995; Parnell and Harris, 2003). The protection of quality loss by applying a fish protein glaze prepared by gelatin of fin protein by soaking in dilute acetic acid was studied by Kakatkar et al., (2004) and Smruti et al., (2004).

Ascorbic acid treatment inhibited lipid oxidation and preserved the desirable odour in meat products (Boles and Parrish, 1990, Kulshrestha and Rhee, 1996, Rhee et al., 1997). According to Selvaraj (1991), ascorbic acid treated squid (Loligo sp) sample was found to have improved quality and shelf life. Ascorbic acid treated sample developed no discolouration even after nine months of storing. Several studies showed that ascorbic acid treatment could improve the quality of seafood.
6.2.3. Treatment with Tripolyphosphates

Pre-dip treatments in sodium chloride (NaCl) or sodium tripolyphosphate (STPP) were found to reduce drip loss and maintain good quality of a number of species of fish during frozen storage (Kumta and Gore, 1970; Tanikava et al., 1963). Sawant and Patange (2002) investigated the usefulness of tripolyphosphates in enhancing the sensory qualities, and retarding progression of rancidity in frozen mussels. Polyphosphates also sequester transition metal ions such as copper and iron. These metal ions accelerate lipid oxidation, which leads to premature flavour deterioration. Phosphates have anti-microbial effect due to their ability to chelate metal ions, essential for microbial cell division (Davidson and Juneja, 1990). Although STPP had anti-microbial activity in laboratory culture media, its effect on microorganisms in meat products has been less conclusive (Molins et al., 1984 and Venugopal et al., 1984). STPP have no anti-microbial effect in temperature abused frozen raw ground beef (Molins et al., 1987) and refrigerated raw pork containing salt (Choi et al., 1987). Sodium tripolyphosphate in meat products increased water holding capacity and juiciness (Matlock et al., 1984 and Molins et al., 1991), prevented oxidative rancidity development (Choi et al., 1987; and Stoick et al., 1991) and showed anti bacterial effect. When judiciously applied to seafood, phosphate bind the inherent juices and to produce a tender juicy product (Henson and Karen, 1992). According to the author, the thaw – drip was reduced considerably in seafoods when dipped for two minutes in 12% STPP solution.

6.2.4. Effect of Kiln-Curing on the Textural Quality of Fish

Since properties of the textural quality of cooked meat are determined. This is a three-stage process which consists of cooking yield was determined. The authors, squid slices were cut into 2 cm and cooked by sautéing for 2 minutes. The cooking yield was determined (Hinks, 1988). Cooking meat product, tenderness and

6.2.5. Effect of Salt Concentration on the Attrition of the Body of Squid and Fish

Yellow, oil body of squid and fish were placed in the ambient temperature and allowed to swim (Sugiya et al., 1980). The oil from tryptophan and ornithine in alkali (Vuillamont and others, 1983) and contraction state of the muscle fibres (Hinks, 1985). It causes the muscle fibres to disrupt and reorganise.
or sodium pyrophosphate to maintain good quality (Kunze and T最近日, 1992) investigated the effect of metal ions on sensory qualities, and found that phosphates also chelate metal ions, but not as effectively as some of these metal ions were able to participate in further deterioration. This ability of phosphates to chelate metal ions is significant (Juneja, 1990). When used in culture media, its effect is accelerated (Rothschild, 1983). In an microbial effect study, peptone (Rothschild et al., 1987) and lactose (Rothschild et al., 1987). Sodium pyrophosphate has a high chelating capacity and can help to prevent the chemically prevented oxidative reactions that occur during cooking (Sugiyama et al., 1991) and refrigerated storage (Kunze and T北, 1992). Seafood, especially squid, is a leading source of tender, juicy product that is easily digested. The by-product of squid - drip was present in the squid meat for 3 minutes in 12%

6.2.4. Effect of Cooking

Since properties and reactions of proteins are involved in determining the textural quality of cooked squid meat, composition and properties of the cooking medium (pH, ion composition, etc.) would also be needed to be determined. The cooking medium should quickly gelatinise the tunics, which consists of connective tissues, and should prevent excessive moisture loss from the muscle fibres (Ottewell and Hamann, 1979). According to these authors, squid should be cooked either very quickly, for example, by frying or sautéing for 2-3 minutes, or be simmered in a stew or Studies showed that cooking yield was not always sensitive to small differences in WHC (Trout, 1988). Cooking losses can also be influenced by the shape and size of the meat product, temperature profile cooking rate and pH.

6.2.5. Effect of Pigmentation on quality

Yellow, orange and violet – purple chromatophores are present on the body of squid and the chromatophores expand and contract in response to the ambient temperature, there by altering the colour of the body while swimming (Sugiyama et al., 1980). The pigment is produced in the squid from tryptophan as the starting material, can be divided into two molecules ommin and ommatin, in which former is strong in alkali, and latter is weak in alkali (Vuillaume, 1969). The market value of squid is related to the contraction state of its epidermal chromatophores named onnochromes (Hinks, 1985). Improper storage and handling result in chromatophores disruption and red discolouration of the meat.
6.3. Materials and Methods

Fresh samples of the two species of squid *Loligo duvaucelii* and *Doryteuthis sibogae* (needle squid) were used for the study. The dressed mantles were divided into 8 batches. In this study six methods were compared in order to find out the best treatment method. Additives were mixed in specific ratio to get a best combination. The systems used were:

a. Treatment control
b. Citric acid 0.3%
c. Citric acid 0.3% + Acetic acid 3%
d. Acetic acid 3%
e. STPP 3% + Acetic acid 3%
f. Ascorbic acid 0.3%
g. Lime Juice 3%

The above treatment media were mixed with NaCl (3% of the sample weight) and the sample was added along with ice in the ratio 1:2 and was kept at −1°C for 20 minutes with occasional stirring.

Immediately after the treatment, samples were kept in ice without direct contact for three days and analysed for TMA-N, TVN, alpha amino nitrogen, Total protein content, protein fractions and microbiological parameters. The treatment control contained no additives except NaCl. Fresh samples of both species of squids at zero day were also analysed for comparison of results. Samples were then taken for freezing and the biochemical, physico-chemical and organoleptic changes were studied for 120 days of frozen storage at an interval of 30 days.
Samples weighing approximately 2g were packed water tight in aluminium foil, and immersed in a constant temperature water bath and cooked at different temperatures (45°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C) as per the method of Paul, et al., (1966). The time required for the samples to reach the temperature of the water bath averaged 3 minutes and afterwards heated for 1 minute. These samples were organoleptically assessed for optimum temperature, which retained the best qualities, by six trained panellists on the basis of colour, appearance, texture and flavour, using a 10-point hedonic scale. The pH of the samples were also checked. Treatment and analyses were done in triplicate.

6.3.1. Statistical analysis

Two-factor ANOVA was employed to compare the effect of each parameter like TMA-N, TVN, alpha amino nitrogen, leaching of protein, various fractions of proteins and TPC, between species and between treatments.

Mathematical model employed for the study was as follows: 

$$X_{ij} = \mu + \alpha_i + \beta_j + E_{ij}$$

Where, $X_{ij}$ is the observed value corresponding to the $i^{th}$ species on $j^{th}$ day of storage. $\mu$ is the overall effect, $\alpha_i$ is the effect of $i^{th}$ species and $\beta_j$ is the effect of $j^{th}$ day of storage and epsilon $ij$ ($E_{ij}$) is the random error.

The comparison of parameters between species and treatment was done for treatment study; $X_{ij}$ is the value obtained corresponding to $i^{th}$ species on $j^{th}$ treatment. $\mu$ is the overall effect, $\alpha_i$ is the effect of $i^{th}$ species and $\beta_j$ effect of $j^{th}$ treatment, $E_{ij}$ random error.
6.4. Results

6.4.1. Total volatile bases

6.4.1.1. Total Volatile Nitrogen

![Graph showing TVN content across treatments]

Figure 6.1 Effect of various treatments on TVN content

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

Figure 6.1 gives TVN content during various treatments where the trend was similar to TMA-N. Here also sample D showed the least content of TVN, (loligo 11.2mg% and needle 14mg%). The second best was sample C. Appendix F.1. revealed the ANOVA of TVN content. There was a significant difference (p<0.001) between both species and treatments.
6.4.1.2. Trimethylamine

![Figure 6.2. Effect of various treatments on TMA-N content](image)

Figure 6.2 gives the TMA content during various treatments and ANOVA table of TMA changes is given in Appendix F.2. There was a significant difference between species and between treatments (p<0.001). Similar trend in the changes of TMA-N due to various treatments was observed in both the species, with needle showing slightly higher value than loligo. The TMA-N content for the control group (without treatment) reached a high value of 7.7mg% and 11.2mg% in loligo and needle respectively. Minimum value was shown in the sample D, loligo showed 2.8mg% and needle showed 4.2mg%. All the samples were kept chilled with GMP. The next lower value of TMA was shown with sample C.
6.4.2. Alpha amino nitrogen

![Graph showing alpha amino nitrogen content for different treatments]

**Figure 6.2. Effect of various treatments on alpha amino nitrogen content**

Figure 6.3. shows the changes in alpha amino nitrogen content of both the species of squid with various treatments. Here the sample E showed the highest value of alpha amino nitrogen (loligo 98mg% and needle 77mg%). Sample D showed only 56mg% and 42mg% in loligo and needle squid respectively and among the treatments sample G showed the maximum leaching rate with alpha amino nitrogen content reaching 19.6mg% in loligo and 14mg% in needle squid. In sample A, where there was no treatment, the value reached 14mg% and 8.4mg% for loligo and needle respectively. There was a significant difference (Appendix F.3.) between species (p<0.01) and treatments (p<0.001).

6.4.3. Protein loss

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<tr>
<td>%</td>
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B.T. Before Treatment

3-Myofibrillar Protein Loss

Table 6.1. % of protein loss after various treatments and the species.
6.4.3. Protein loss

Table 6.1. % of protein retained during various treatments

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B.T. Before Treatment, 1- Total Protein, 2- Sarcoplasmic Protein, 3- Myofibrillar Protein, 4- Denatured protein, 5- Stroma Protein

Table 6.1. gives the leaching rate of the protein as a result of various treatments and the % of retained protein was calculated using the formula

\[
\text{Protein content in each fraction} \times 100 \over \text{Total protein (B.T.)}
\]
Sample E provided the best result in retaining the protein content (95% and 81% in loligo and needle squid). Sample D could hold 82% protein in loligo and 78% in needle squid. But in sample A, about 51% of the total protein was leached out in loligo and 54% in needle. Appendix F.4. shows that there was a significant difference between species and treatments (p<0.001). Between treatments, sample E showed a significantly higher value of protein while untreated sample showed the lowest value.

Maximum retention of sarcoplasmic protein was found in sample E followed by sample D. The LSD was calculated for treatments as 1.06. Appendix F.5. shows a significant difference in the sarcoplasmic protein between species and treatments (p<0.001). Among species, needle showed a higher rate of leaching when compared to loligo.

Here the sample E showed the least extractability of myofibrillar protein followed by sample D. Appendix F.7.6. shows there was significant difference myofibrillar proteins extractability between species and treatments (p<0.001).

Maximum denatured protein was found in sample E. Denaturation was minimum in control (zero time). Sample D also showed high level of denatured protein. Appendix F.7. shows a significant difference of denatured proteins between species and treatments (p<0.001).

With regard to stroma proteins, sample E in loligo showed maximum retention, while in needle squid sample D showed the maximum retention. There was no significant difference between species; while between treatments there was significant difference (p<0.001, Appendix F.8.). Minimum value of stroma protein was found in treatment control (A).

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<thead>
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<th>Needle</th>
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<td>F'</td>
<td>G</td>
<td>G'</td>
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6.4.4. Bacteriology

Table 6.2. Changes in total plate count during various treatments.

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<th>Needle</th>
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<td>2.2X10⁵</td>
</tr>
<tr>
<td>B</td>
<td>2.8X10⁴</td>
<td>3.3X10⁴</td>
</tr>
<tr>
<td>C</td>
<td>1.2X10⁴</td>
<td>1.5X10⁴</td>
</tr>
<tr>
<td>D</td>
<td>1.5X10⁴</td>
<td>1.7X10⁴</td>
</tr>
<tr>
<td>E</td>
<td>1.8X10⁴</td>
<td>1.9X10⁴</td>
</tr>
<tr>
<td>F</td>
<td>1.75X10⁴</td>
<td>2.01X10⁴</td>
</tr>
<tr>
<td>G</td>
<td>2X10⁴</td>
<td>2.4X10⁴</td>
</tr>
</tbody>
</table>

Table 6.2 shows changes in Total Plate Count (TPC) in samples after various treatments. Here sample C showed maximum reduction in the bacterial count. The second best treatment system was sample D alone, which could reduce the bacterial count to 10%. Appendix F.9. gave significant difference between species and treatments (p<0.001).

6.4.5. Organoleptic quality

Table 6.3. Organoleptic Quality changes and pH during various treatment systems

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>washed</th>
<th>Flavour</th>
<th>colour</th>
<th>Texture</th>
<th>Odour</th>
<th>grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loligo</td>
<td>A</td>
<td>7</td>
<td>7.3</td>
<td>P</td>
<td>Pink</td>
<td>Flabby</td>
<td>P</td>
</tr>
<tr>
<td>Needle</td>
<td>A'</td>
<td>7.3</td>
<td>7.5</td>
<td>P</td>
<td>Pink</td>
<td>Flabby</td>
<td>P</td>
</tr>
<tr>
<td>Loligo</td>
<td>B</td>
<td>6</td>
<td>6</td>
<td>G</td>
<td>W</td>
<td>Soft</td>
<td>G</td>
</tr>
<tr>
<td>Needle</td>
<td>B'</td>
<td>6.3</td>
<td>6.3</td>
<td>G</td>
<td>W</td>
<td>Soft</td>
<td>G</td>
</tr>
<tr>
<td>Loligo</td>
<td>C</td>
<td>4</td>
<td>4.4</td>
<td>G</td>
<td>W</td>
<td>Soft-Firm</td>
<td>G</td>
</tr>
<tr>
<td>Needle</td>
<td>C'</td>
<td>4.2</td>
<td>4.5</td>
<td>G</td>
<td>W</td>
<td>Soft-Firm</td>
<td>G</td>
</tr>
<tr>
<td>Loligo</td>
<td>D</td>
<td>4.6</td>
<td>4.8</td>
<td>E</td>
<td>W</td>
<td>Soft-Firm</td>
<td>E</td>
</tr>
<tr>
<td>Needle</td>
<td>D'</td>
<td>4.8</td>
<td>5.1</td>
<td>E</td>
<td>W</td>
<td>Soft-Firm</td>
<td>E</td>
</tr>
<tr>
<td>Loligo</td>
<td>E</td>
<td>4.9</td>
<td>5.3</td>
<td>S</td>
<td>SP</td>
<td>Soft</td>
<td>S</td>
</tr>
<tr>
<td>Needle</td>
<td>E'</td>
<td>6</td>
<td>6.2</td>
<td>S</td>
<td>SP</td>
<td>Soft</td>
<td>S</td>
</tr>
<tr>
<td>Loligo</td>
<td>F</td>
<td>6.4</td>
<td>6.7</td>
<td>S</td>
<td>SP</td>
<td>Soft</td>
<td>P</td>
</tr>
<tr>
<td>Needle</td>
<td>F'</td>
<td>6.8</td>
<td>7</td>
<td>P</td>
<td>SP</td>
<td>Flabby</td>
<td>P</td>
</tr>
<tr>
<td>Loligo</td>
<td>G</td>
<td>6.5</td>
<td>6.8</td>
<td>S</td>
<td>SP</td>
<td>Flabby</td>
<td>P</td>
</tr>
<tr>
<td>Needle</td>
<td>G'</td>
<td>7</td>
<td>7.3</td>
<td>P</td>
<td>SP</td>
<td>Flabby</td>
<td>P</td>
</tr>
</tbody>
</table>

Flavour/Odour: P-Poor, G-Good, E-Excellent, S-Satisfactory
Colour: W-White, SP-Slight Pink
Table 6.3. describes the organoleptic quality and the pH of the mantle after various treatment systems. The pH of sample D was about 4.6 to 4.8 whereas in the treatment media pH was 4.2. In the case of sample C the pH was below 4 and developed a severe sour taste creating an unpleasant flavour when cooked.

In table 6.4. the organoleptic quality of both the species in cooking at various temperatures is shown. The sample E was very tough and rubbery after cooking. The cooking temperature was optimised for 1 minute at 70°C. The temperature more than 70°C gave a yellowish colour to the product and a slightly tougher texture, which in turn gave the panel members a dislike while tasting it.

6.4.5.1. Cooking

Table 6.4. Organoleptic Quality changes and pH during various treatment systems after cooking at different temperatures

<table>
<thead>
<tr>
<th></th>
<th>45°C</th>
<th>50°C</th>
<th>60°C</th>
<th>70°C</th>
<th>80°C</th>
<th>90°C</th>
<th>100°C</th>
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<tbody>
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<td>5</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Needle</td>
<td>A'</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5.5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Loligo</td>
<td>B</td>
<td>6</td>
<td>6.5</td>
<td>7</td>
<td>7.5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Needle</td>
<td>B'</td>
<td>6</td>
<td>6.5</td>
<td>7</td>
<td>7.5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Loligo</td>
<td>C</td>
<td>6</td>
<td>6.5</td>
<td>6.5</td>
<td>7.5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Needle</td>
<td>C'</td>
<td>6</td>
<td>6.5</td>
<td>6.5</td>
<td>7.8</td>
<td>7.5</td>
<td>7</td>
</tr>
<tr>
<td>Loligo</td>
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<td>7.5</td>
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<tr>
<td>Needle</td>
<td>D'</td>
<td>6.5</td>
<td>7</td>
<td>7.5</td>
<td>8</td>
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</tr>
<tr>
<td>Loligo</td>
<td>E</td>
<td>4.5</td>
<td>5.5</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
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<tr>
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<td>E'</td>
<td>4.5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>4.5</td>
<td>3</td>
</tr>
<tr>
<td>Loligo</td>
<td>F</td>
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<td>5.5</td>
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<td>6</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
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<td>F'</td>
<td>5</td>
<td>5.5</td>
<td>5.5</td>
<td>6</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Loligo</td>
<td>G</td>
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<td>6.2</td>
<td>4.5</td>
<td>4</td>
</tr>
<tr>
<td>Needle</td>
<td>G'</td>
<td>5</td>
<td>5</td>
<td>5.8</td>
<td>4.5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

The sample was mixed sweet and sour.
The needle squires organoleptically good.

6.4.6. Frozen Storage

6.4.6.1. Total vol.

<table>
<thead>
<tr>
<th></th>
<th>25</th>
<th>20</th>
<th>15</th>
<th>10</th>
<th>5</th>
<th>0</th>
</tr>
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</table>

Figure

A-Control
B-Acetic acid alone
E-Ascorbic acid
The sample D cooked at 70°C gave a juicy, soft and firm texture and a mixed sweet and sour taste. This was ranked best by all the panel members. The needle squid developed a pink discolouration on sample E and was organoleptically graded unacceptable.

6.4.6. Frozen Storage

6.4.6.1. Total volatile nitrogen

<table>
<thead>
<tr>
<th>Treatments</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
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<td>0 days</td>
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<td>6</td>
<td>6</td>
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<td>6</td>
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<tr>
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<tr>
<td>90 days</td>
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<td>120 days</td>
<td>6</td>
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<td>6</td>
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<td>6</td>
</tr>
</tbody>
</table>

Figure 6.4. Changes of TVN of needle squid on frozen storage of treated samples

<table>
<thead>
<tr>
<th>A-Control</th>
<th>C-Acetic acid citric acid mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Acetic acid alone</td>
<td>D-STPP acetic acid mixture</td>
</tr>
<tr>
<td>E-Ascorbic acid</td>
<td>F-Lime Juice</td>
</tr>
</tbody>
</table>
Figure 6.5. Changes of TVN of loligo squid on frozen storage of treated samples

Figure 6.4 and 6.5 reveal the changes of TVBN during frozen storage after various treatments. Even though there was no much noticeable difference in the TVBN content in the sample of both the species of squid sample B showed lowest content among all the samples.

6.4.6.2. Trimethylamine

Figure 6.6. Changes of TMA of needle squid on frozen storage of treated samples

Alpha amino leaching during storage, which is avoided in the treatments. TMA value there is 140 in loligo.
Figure 6.7. Changes of TMA of loligo squid on frozen storage of treated samples

Figure 6.6 and 6.7 show the changes in TMA content of loligo squid on frozen storage of treated sample, where sample B showed a minimum TMA value throughout the storage period (below permissible limit - Loligo-5.6 & Needle squid –7). Sample B showed better results than other treatments. The sample D and F exceeded the limit after 120 days of storage, which was almost same as the control. Citric acid treatment was avoided in the further studies as it showed an inferior quality.

6.4.7. Alpha amino nitrogen

Alpha amino nitrogen content showed a maximum rate (180 in needle and 140 in loligo) in the sample D, while sample A showed maximum rate of leaching during treatments (Figure 6.8. and 6.9).
6.4.8. Protein Treatments

The lea...

Figure 6.8. Changes of alpha amino nitrogen of needle squid on frozen storage of treated samples

Figure 6.9. Changes of alpha amino nitrogen of loligo squid on frozen storage of treated samples

6.5. Discussion

(Outwell 1982) signified the softening of tough (toughness) during the first five minutes of the mantle reaction gelatinise the protein. The pH of raw meat between 20% protein (Paul...
6.4.8. Protein loss

The leaching of protein was found to be low in sample B (19.71 to 17.2 in needle squid and 20.71 to 18.53 in loligo) as well as in sample D (19.71 to 18.23 in needle squid and 20.71 to 19.23 in loligo). The sarcoplasmic proteins were found to be comparatively stable during freezing and frozen storage (needle squid-11.4 to 10.4 and loligo-11.91 to 10.32), but the myofibrillar protein content reduced considerably and the denatured protein content increased. The leaching rate was higher in needle squid than loligo. There was a decrease in toughness at temperatures below 100°C.

6.5. Discussions

(Otwell & Hamann, 1979b) and heating at 60-80°C could result in the softening of the meat and in retaining the juiciness of the product. Such temperatures are widely applied in cooking tender muscles to avoid severe toughness (Bykowski & Kolodziejski, 1985). These findings agree with the results of this study, in which cooking temperature of 70°C produced products with highest organoleptic score. According to Stanley and Hultin (1982) significant changes in texture of the squid mantle took place only during the first 8 minutes of the cooking. The cooking times in excess of five minutes would limit the advantage of heat tenderisation by decreasing the mantle moisture (Otwell and Hamann, 1979a). Otwell and Hamann (1979b) suggested the application of a cooked medium best suited to rapidly gelatinise the tunics of connective tissue in squid to produce tender product. The pH of rabbit muscle tissue increased while cooking. The tenderness of the meat between 65°C -75°C was mainly due to the changes in myofibrillar protein (Paul et al., 1966). The squid (Loligo duvaucelii) treated with 0.5%
ascorbic acid for 10 minutes found to improve the quality and shelf life when compared to the control (Selvaraj et al., 1991). The thaw drip increased gradually on cooking, which may be due to the protein denaturation and consequent decrease in water holding capacity (Joseph et al., 1985). Sodium acetate treated sample of cat fish fillet resembled the order and appearance of the fresh fillet up to six days and, could reduce the bacterial load to a great extent (Chang et al., 1995). Effect of NaCl in the range 0.1% to 5% on the textural property of minced squid meat showed that 2.5% gave the maximum elasticity and, prolonged mixing resulted in a cross linking network, Uniformity and compactness of protein matrix played an important role in the textural quality.

The findings of this study confirms that a dip treatment with dilute acetic acid could retain the sarcoplasmic and myofibrillar fractions of proteins in both Loligo and needle squid which otherwise get leached out (control). On cooking also, acetic acid treated samples were organoleptically ranked highest.

Venugopal et al., (1997) prepared a stable gel from shark myofibrillar proteins by reducing pH to 4.0 by acetic acid. The stability of the protein in dispersion was dependent on the pH. In the presence of acetic acid, the positively charged protein molecules may repel among themselves and cause solubilisation, to form a stable gel without any aggregation and precipitation. In the presence of acetic acid, myosin heavy chain could breakdown with the formation of a fragment having molecular weight 160kDa (Chawla et al., 1995). The repulsion between protein molecules at low pH can be so strong as to hold the proteins in solution even at high

temperatures of fish and the sample was considered stable.

Triopolyphosphate and actomyosin complex may help in this helps to retain juices of seafood. The retention of protein and gelled texture. A decrease in discoloration in the deterioration of fish due to bleeding of pigment is interesting that the two species during S77a. In this study name limejuice which Acetic acid treatment stored mantle tissue.

6.6. Conclusion

Due to high process have been employed. Chilling alone has used in chilling with other methods of mixtures, has been used. Acetic acid showed...
temperatures of cooking. The microbial count in the acetic acid treated sample was considerably lowered due to its antimicrobial action.

Tripolyphosphates act on skeletal muscle proteins and can split actomyosin complex into extract myosin. Extracted myosin binds water and this helps to retain water-soluble proteins, minerals and vitamins and natural juices of seafood. Thus, this imparts a favourable effect in the texture and flavour. Here though STPP acetic acid mixture treatment showed the highest retention of proteins, cooking resulted in the development of a rubbery, gelled texture. Also, STPP acetic acid mixture treatment develops a pink discolouration in needle squid on storage. This may be due to the structural deterioration of the Omnochrome membrane in alkaline pH leading to bleeding of pigments, which downgrades the quality of the product. It is interesting that this type of discolouration was not prominent in loligo species during STPP treatment. Compared to the other four treatments used in this study namely citric acid, acetic acid + citric acid, ascorbic acid and lime juice which are being commonly used in the seafood industry, the Acetic acid treatment is most acceptable for extending the quality of chilled stored mantle tissue before going for freezing.

6.6. Conclusion

Due to highly perishable nature of squid and squid products, various processes have been developed for shelf life extension of the mantle tissue. Chilling alone has limitation in extension of shelf life. But combination of chilling with other treatments with permitted food additives, as single or in mixtures, has been applied to further augment the shelf life. Treatment with acetic acid showed the best chemical and microbial characteristics, like high
retention of total proteins, sarcoplasmic proteins and myofibrillar proteins, in both the species of squid. On cooking at 70°C, the acetic acid treated mantles showed best organoleptical quality while STPP treated samples developed a rubbery gelled texture. Even though STPP treated samples showed maximum protein retention, during storage they developed a pink discoulouration in needle squid. Compared to the other four treatments namely citric acid, citric acid + acetic acid, ascorbic acid and lime juice, which are being used in the seafood industry, the acetic acid treatment is most acceptable for extending the quality of chilled stored mantle tissue before going for freezing. Other aspects of the treatment like tissue protease activity, lysosomal activity and pattern of protein by SDS-PAGE will be dealt in detail in the proceeding Chapters.

6.7. References


6.7. References


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Chapter 6


*Not Referred in Original