Chapter 10

SUMMARY AND CONCLUSION

The thesis on the topic “Influence of processing variables on protein quality and frozen storage stability of two commercially important species of squid (Loligo duvaucelii and Doryteuthis sibogae)” is presented in 10 chapters.

Chapter 1. This chapter gives a brief account of the present export status of seafood from India and the role played by squid in the export. This chapter also describes the significance of the research work undertaken and the objectives to which the study is focusing.

Chapter 2. The protein content of the squid was compared with other marine cephalopods and thereby to emphasize the nutritive value of squid. Proximate composition of both species of squid was compared with other cephalopods. The variations in protein during various months were also studied in different species. The fractions of proteins namely sarcoplasmic, myofibrillar, denatured and stroma proteins were extracted and analyzed. From the results, the squid was evaluated to be a good source of protein. The repeated washing resulted in the loss of proteins along with the other soluble substances, thus reducing the quality. The time in between the landing and the processing factory is found to be very crucial, since it directly reduces the quality of squid. Utmost care is to be taken to reduce the hazards. Thus practice of storing squid directly in slush ice at any point from landing to processing is not advisable due to the high dissolution of
myosin fraction and other proteins in water. The loss of proteins as well as Non-protein nitrogen (NPN) fraction could affect the organoleptic quality of squid in general. The sweet taste of squid is linked to the NPN fraction. The leaching rate of needle squid was found to be much higher than that of loligo squid. This leads to a considerable loss of nutrients and higher rate of degradation in needle squid compared to loligo squid. Hence further investigations were undertaken to reduce this nutritional loss and quality degradation. The microbiological study was done for total plate count, sanitary significant bacteria like *Escherichia coli*, *Coagulase positive staphylococcus* and pathogens like *Salmonella* and *Vibrio cholerae*.

Chapter 3. In this chapter various changes in quality parameters during different methods of ice-storage were studied in detail. The methods adopted for the icing were with GMP, without GMP and without direct contact with ice and water. In method-1- with GMP, (Good Manufacturing Practices) flake ice was used and the ice was changed with fresh ice daily. In method-2- without GMP, block ice was used for icing and samples were kept in slotted boxes, allowing the melt water to flow out. In this case, the ice was not changed daily but was replenished as required. In Method–3- without direct contact with ice and water, the samples were packed in polythene covers and kept in ice (with GMP) to avoid direct contact with ice and water. In all the three methods insulated boxes were used.

Moisture, fat and ash were calculated and statistically compared between methods, species and days of storage using students t test. The chemical indices like trimethyl amine (TMA), total volatile base nitrogen (TVBN) and alpha amino nitrogen were estimated using trichloro acetic acid extract. Organoleptic quality and pH of the tissue were monitored in all the above methods. The degree of external color changes were also monitored.

Needle Leaching rate is higher for needle squid than in loligo squid due to its leaching. As per the study, samples beyond 6 days of storage were considered non-contact samples.

Chapter 4. The temperature of the ice storage material was maintained in a chill room. 

A microbiological analysis for species, method and the samples with t test analyses. Results showed that the processing procedure and the process should be changed to avoid differences between species. Some significant differences were adopted a process.
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above methods. Organoleptic assessment was done by sensory test and the degree of excellence was given by hedonic scale. Microbiological quality changes were also studied during ice-storage.

Needle squid showed a faster rate of deterioration than loligo. Leaching rates of protein and alpha amino nitrogen were more in needle squid than in loligo. Non-contact ice-stored material showed a low rate of leaching. As per the results, the ice-storage was not found to be advisable beyond 6 days even with GMP. The storage time could be prolonged using non-contact ice.

Chapter 4. The material was kept at -2°C for 8 days. The temperature was maintained by mixing 3% sodium chloride with ice and the material was layered with the ice in the ratio 1:2 and the samples were kept in a chill store maintained at -10°C. The physical, chemical and microbiological indices were estimated and statistically compared between species, methods and days of storage using 3-way ANOVA. The results showed that the samples kept with GMP had a better quality than the samples without GMP.

A similar lot with GMP was frozen by IQF (individually quick frozen) and the samples were drawn at different points of the processing line for analyses. Results showed that the repeated washing of squid tube during processing should be avoided in order to minimize the nutrient loss during the process. The time of direct contact with ice during processing also should be reduced. Leaching of protein showed significant difference between species, steps and days of storage. Fractions of protein also showed significant difference in leaching. From the study, it is also suggested to adopt a proper treatment to reduce the leaching effect.
Chapter 5. Frozen samples were stored at -20°C to -25°C and samples were drawn at every 30 days for six months of frozen storage to evaluate physical, chemical and microbiological parameters. 2-way ANOVA was used for comparison of different quality parameters between species and days of storage. The variations in water soluble and salt soluble fractions of protein were very significant during the storage. But the difference in denatured protein was not significant between days and species. Among the various storage systems, the storage without direct contact with ice is proved better for short period preservation, while frozen storage is advised for longer periods.

Chapter 6. The effects of six treatments in the two species of squid were assessed by physico-chemical, microbiological and organoleptic studies. The systems used were (a) 0.3% Citric acid (b) 0.3% Citric acid + 3% acetic acid (c) 3% Acetic acid (d) 3% Sodium tripolyphosphate (STPP) + 3% acetic acid (e) 0.3% Ascorbic acid (f) 0.3% Lime juice. A control was also taken without any treatments. All the samples were mixed with 3% salt and material to ice in the ratio 1:2. These were kept for 20 minutes. After the treatment, the samples were kept in non-contact ice with GMP for 3 days and samples were drawn for various analyses. The results were compared using 2-way ANOVA.

Another set of treated samples were immediately frozen and stored for 6 months at -20°C to -25°C and analyzed for physico-chemical, microbiological and sensory evaluation at thirty days of interval. The results were analyzed using 3-way ANOVA. Even though the leaching rate was minimum in STPP acetic acid mixture treated sample, its bacteriological and organoleptic qualities were inferior to that of the sample treated with acetic acid alone. Storing at 40°C to 100°C for 6 months did not affect the sample, and no changes were observed. Among all the treatments, both the species stored at 40°C to 100°C were avoided.

PAGE was carried out using with GMP (with GMP) as well as kept at chill stored conditions. All the samples stored and treated with GMP were stored and treated with GMP, and therefore sodium dodecyl sulphate was the appropriate method.

The leaching of myofibrillar molecular was confirmed by electrophoresis. The myofibrillar molecular was also carried out for the extraction of myofibrillar molecular. Leaching of myofibrillar molecular was confirmed by electrophoresis. The treated samples were analyzed, suggesting...
acid alone. The effect of cooking was studied at various temperatures from 40°C to 100°C. Even though STPP treated sample reduced the drip loss in the sample, the organoleptic quality was found to be lost while cooking. Among all the treatments, acetic acid treated sample was most acceptable in both the species. The treatments like citric acid treatment, STPP treatment were avoid due to the poor quality standards of the products.

Chapter 7. Aqueous extract of mantle was used for the study and PAGE was done using disc electrophoretic apparatus according to the method of Laemmli. The samples selected for this study included ice stored (with GMP, without GMP and without direct contact with ice and water), chill stored (with and without GMP), from various processing steps, frozen stored and the treated sample. PAGE was carried out in the presence of sodium dodecyl sulphate (SDS). It was found that 10 % gel strength was appropriate for a distinct separation of squid proteins.

The bands obtained were characterized by comparing with protein molecular weight markers. The extractability of the proteins in water, phosphate buffer and borate buffer were also compared. Characterization of the proteins in samples from various storage systems and treatments were also carried out. From the protein profile, it was observed that the rate of extraction of proteins from needle squid was more compared to loligo. Leaching of protein in the sample kept in direct contact with ice and water was confirmed by the loss of protein bands. Myosin, which is a component of myofibrillar protein, was also solubilised in aqueous extract. Mild acid treated sample showed feeble bands of Myosin Heavy Chain (MHC) suggesting possible disappearance due to acid-induced gelation.
Chapter 8. Squids are known to have only one to two years of life span. In order to achieve this rapid growth, the protein turnover rate must be very fast which usually results from an active proteolytic enzyme activity in the squid muscle. In this chapter tissue proteolytic enzyme activity of squid muscle was determined using haemoglobin as substrate. The effect of pH, temperature and the period of incubation time on proteolytic enzyme activity was studied and arrived at the optimum conditions with respect to specific proteases present. The influence of GMP and various treatments on the proteolytic enzyme activity was studied in both the species. The protease enzymes in squid mantle were also characterized by studying the effect of various inhibitors like phenylmethylsulphonyl fluoride (PMSF), soybean trypsin inhibitor, iodo-acetic acid and ethylenediaminetetraacetic acid (EDTA). The proteolytic activities were considerably high at pH 3.0, 6.0 and 8.0. Maximal proteolytic activity in squid muscle extract at physiological pH was observed at 40°C. Between the two species the needle squid exhibited a higher proteolytic activity compared to loligo.

Chapter 9. A detailed study was done on lysosomal enzyme activity of squid muscle as an index of autolysis. The lysosomal stability of both the species of squid under various processing variables adopted was studied in detail. The enzyme acid phosphatase was used as an indicator for lysosomal stability. The lysosomal activity of the drip from freezing and thawing procedure was done. The results showed that the lysosomal activity was higher in needle squid than in loligo. Among the various treatments acetic acid treatment reduced the lysosomal activity considerably. The presence of cathepsin D like proteases of lysosomal origin is involved in the protein degradation in squid mantle muscle. Treatment is inevitable in the case of squid in order to enhance the stability of lysosomal enzymes and to prevent various treatments like exposure to acetic acid and proteolytic enzyme. The difference in the stability and activity of proteases in squid muscle matrix. This chapter also describes the respective studies.

If fishes in the aquatic environment affect sea fish, and marine food products, the quality factor of the marine product could be significantly increased. It is essential to maintain good sanitary conditions, good fish handling, and water management in order to maintain a healthy aquatic environment.
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Squid in order to maintain the storage quality. In the present study, the level of lysosomal protease enzyme released into the medium was reduced by various treatments. Reduced amount of lysosomal enzymes in the drip of acetic acid treated sample and their low activity in the drip confirm the ability of acetic acid to maintain the inter-protein network and retain proteins including water soluble enzymes in the gel matrix to the maximum. The difference in the rates of release of enzymes could be due to the difference in the binding of these enzymes to the lipoprotein membrane matrix. This is very well supported by the increased bound activity in the respective samples.

If fishermen, processor and retailers understand the factors, which affect seafood quality and make a conscientious effort to control these quality factors, problems associated with autolytic and microbial changes could be significantly minimized. Every stage of handling from harvest to consumption affects quality. In the processing plants and at retail outlets good sanitary conditions and strict temperature conditions must be maintained so as to provide high quality products to the consumer. The time-temperature management throughout the process line should be strictly followed.