Chapter 2

COMPOSITION OF SQUID MUSCLE WITH SPECIAL EMPHASIS ON PROTEINS

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2.1. Introduction

Cephalopods are mainly commercial in the Mediterranean countries and the Far East, but although consumption is much lower in the rest of the world, there has been a considerable increase in recent years. These species are normally processed both fresh and frozen. The percentage of the edible portion of the cephalopods is exceptionally high, between 60 to 80% of the total weight depending on species, size of specimen and sexual maturity, whereas in fish the percentages is only 40-70% (Sikorski & Kolodziejska, 1996)

The main edible portion of the squid is the mantle. A variety of processed food is made from squid as the raw material and each is appreciated for its special flavour and taste. The fresh squid has a sticky feeling, and is not easy to chew. It contracts following cooking and a round shape is assumed due to the shrinkage, especially the skin. The squid meat differs from fish muscle, structurally and biochemically.

The squid muscle is composed of several layers of fibres running transversally to each other and covered with several sheets of connective tissue. The muscle fibres are not only strong which differ from fish meat, but their arrangement is also very different. The protein present can be broadly categorized into myofibrillar protein composed of similar myofibrils as the muscle protein of other animals, sarcoplasmic proteins which fills the gaps of myofibrils and muscle based protein composed of connective tissue. Myofibrilar protein is the major constituent followed by sarcoplasmic protein and muscle based proteins.

The mantle of squid is low fat fishes and the edible portion of cephalopod is a rich source of proteins. The analysis of the muscle content shows that the muscle enzyme activity which include tendon and connective tissue have profound influence on the muscle enzyme activity group specific or differently. (Nishino, 1982). By comparison, the differences in content of a species of cephalopod and the role of water and the significance of salt on the muscle protein-based constituents will be discussed under the different conditions in order to find out the best processing conditions.

Micro-organisms present in the squid muscle, which is specially due to sea water, can reach the process easily with the spoilage caused by post processing. Therefore, the squid microbiology has shown the presence of a large bacterial load to spoil.

Every fish must be washed thoroughly with this native micro-organisms with the washing of sea fish. The post processing procedure used should be to generate the internal environment by saltwater.

The microorganisms in the squid muscule inhibited by saltwater.
The mantles of cephalopods are quite close in chemical composition to low fat fishes and white meat fish. The four major constituents in the edible portion of cephalopods are water, proteins, lipids and ash (minerals). The analysis of these four basic constituents is referred to as proximate analysis (Love, 1970) Different factors like environmental conditions, which include temperature, salinity, pressure and availability of food etc. have profound influence in the biochemical composition. There may be group specific or species-specific in the biochemical composition (Stansby, 1982). By comparing the composition of squid muscle with some selected species of cephalopods, an attempt is made to emphasize the nutritional significance of squid as a food item. Information of the biochemical constituents will also be helpful in defining optimum processing and storage conditions in order to preserve the quality to the maximum.

Micro-organisms play a vital role in the quality and safety of squid muscle, which is being handled by the workers from the point of catch till it reaches the processing plant. Sanitary significant bacteria are the major cause of post mortem changes in the squid muscle. The study of microbiology helps us to define the precautionary measures to reduce the bacterial load to get a premium quality product.

Every fish will have a native flora of micro-organism. The nature of this native microflora will largely depend upon the habitat of fish. Since the washing of seafood items in fresh water is inevitable, the quality of water used should be taken care of.

The microbes of fresh water are mostly a mixture of gram +ve and gram –ve organisms. They are less tolerant to salt; rather, they are killed or inhibited by salt concentrations above 0.5%. The fresh water microbes are
found to be a mixture of spoilage organisms and human pathogens. The presence of human pathogens can be traced to the intervention of human activities and fresh water availability.

2.2. Review of literature

2.2.1. Proximate composition

In order to emphasize the importance of squid and the quality deterioration during various processing steps, elaborate studies in food chemistry have been carried out. In the study done by Tze-kuei-chiou et al., (2000), changes in chemical constituents and quality of Argentina squid before and after drying during various seasons, were investigated. Lakshmanan and Balachandran (2000) in their study on various biochemical properties of squid and cuttlefish compared the proximate composition of fresh cephalopods available in Kerala coast. Lakshman et al., (1993) has done a detailed study on the quality of commercially frozen cephalopod product from India, where the selected species were loligo species of squid and sepia species of cuttlefish. The proximate composition and the sweet flavouring compounds like water extractable nitrogen (WEN), non-protein nitrogen (NPN) and alpha amino nitrogen content were studied in fresh and frozen stored cephalopods. Selvaraj (1991) has studied the biochemical changes of Loligo duvaucelii on frozen storage before and after treatment with ascorbic acid. Sikorski and Kolodziejska (1986) have studied the chemical composition, structure and properties of Loligo pealei squid meat. Sugiyama et al., (1980) has studied the structural details and the usage of squid. The yield of edible fleshy parts in the body of squid is exceptionally high, being 60% to 80% of the total weight, depending on the species, the size of the animal and the quality of the fish. Tsuda et al., (1972) have found that squid is high in iron and contains 15.5 mg of iron per 100g, whereas Varela et al., (1966) and Reis et al., (1986) have reported values of 8.7 to 83.7 for octopus.

The muscle content of squid is similar to fish and mammals, and the muscle content is related to the proximate composition and the quality of the flesh. Several authors (Pacheco et al., 2001; Pacheco et al., 2001; Pacheco et al., 2001)

2.2.2. Fractionation

There are no studies on the extraction of muscle from squid. However, studies are of interest to the food industry. The muscle content is related to the quality of the flesh. Borderias, 1983; Ibarz, 1983; Ibarz, 1983

The myofibrillar muscle is similar to fish and meat. The protein content of squid muscle is high, and the protein yield is high. The extraction with water is the most efficient, and the protein can be more susceptible to myofibril disintegration, and more easily inactivated. Swanson et al., (1978). Tsuchiya et al., (1980). The yield of protein after various treatment methods is not the same.
Composition of squid

The muscles of squid mantle differ in structure from the muscles of fish and mammals (Sikorski and Kolodziejska, 1986). Several studies on the proximate composition of various marine species have also been done by several authors (Panchavannam et al., 2003; Smuruthi et al., 2003; Chand et al., 2001; Pacheco-Aguilar et al., 2000; Kher-un-Nisa et al., 1995).

2.2.2. Fractionation of protein

There are many publications concerning muscle protein solubility in moderate ionic-strength saline solutions in the various myosystems. These studies are of interest in that many of the functional properties of fish muscle are related to the solubility of the constituent Proteins (Jimenez and Borderias, 1983; Hultin et al., 1995).

The myofibrillar proteins of cephalopods are highly water soluble, unlike fish and mammalian proteins. Nearly 85% of the total protein in squid muscle could be solubilized with distilled water by exhaustive extraction with water (Matsumoto, 1958). Squid myosin is also reported to be more susceptible to trypsin and myosin ATPase and is reported to be more easily inactivated by heating than that of fish and mammals (Tsuchiya et al., 1978). Tsuchiya et al. (1977) has isolated and purified the squid actin of Todarodes pacificus. A detailed study on the solubility of rabbit muscle protein after various time temperature treatments was carried out by Paul et
al., (1966). Kitabayashi et al., (1954) and Matsumoto et al., (1959) have compared the fraction of squid with other cephalopods in *(Todarodes pacificus)* proteins.

Lakshmanan and Balachandran (2000) have made a comparative study of the protein fractions of frozen squid and cuttlefish. Raghunath (1984) has also carried out experiments on *Loligo duvaucelii* tubes to determine the rate and quantum of the leaching of WEN and NPN when stored in slush ice which showed a high content of water soluble nitrogen fraction. The fractions of protein in various species of squid were studied by Sikorski and Kolodziejewska (1986). Tsuchiya et al., (1978) has extracted and purified the squid myosin of *Todarodes pacificus*. Purity of each preparation obtained was checked by SDS polyacrylamide gel electrophoresis. The same author has worked on the physico-chemical properties of squid myosin. A characteristic feature of sarcoplasmic fraction is the high activity of proteases, reported in fresh unfrozen muscle, which brings about extensive degradation of the myofibrillar proteins during the course of fractionation. Horie et al., (1975) and Iguchi et al., (1981) has studied the fractionation of squid protein in various species.

### 2.2.3. Bacteriology

Seafood products pass through physical, chemical and bacteriological hazards at various areas of landing, transportation and processing. Most important of all these is the handling of the material by the workers, which directly influence the quality of the seafood by the contamination due to bacteria. Antony et al., (2003) has studied the presence of sanitary significant bacteria like *Escherichia coli*, *coagulase positive staphylococcus*, *faecal streptococcus*, diversified conventional fishes and considered as a factor.
faecal streptococcus, and salmonella on Tuna-mas and mas based diversified convenience products. The presence of E. coli, which is considered as a faecal indicator organism, was studied in the deep-sea water fishes by Madhusudana Rao & Surendran (2003). Nazeem Beena et al., (2002) has studied the growth of cross contaminating bacterial pathogens in fish muscle at different temperatures of storage of 5°C, 20°C and 35°C, to mimic the prevailing conditions in the retail fish markets. The samples at 35°C showed maximum growth of all the microorganisms. Sudha (2002) in her work, explained prevalence of Vibrio species in fish from pelagic and demersal habitats. Iyer (2000) has detected presence of various serotypes of salmonella from various seafoods in Cochin, Bombay and Calicut. Lilabati and Viswanath (1999) have studied the changes in the bacterial and fungal quality during storage of smoked Esonus danricus. Occurrence of Vibrio cholerae non-01 and their dispersion phenomena in the coastal waters of Mangalore was investigated by Sreeja and Raveendran (1999). The near-shore stations recorded comparatively higher incidence of these organisms, while in deep waters the occurrence showed a gradual decrease. Selvaraj (1991) has studied the microbiological quality of ascorbic acid treated Loligo duvaucelii before and after treatment. Shetty and Shetty (1990) studied the bacteriological changes of Sardinella longiceps during the chilled seawater storage.

Fishing from polluted waters or washing in polluted coastal water are also responsible for bacterial contamination. Studies conducted in this respect showed that salmonella was present only in 1.86% of the samples from frozen cuttlefish (Varma et al., 1985). Lakshmanan et al., (1984) reported that 94% of the samples were free from salmonella. Lakshmanan et al., (1993) detected
only 5.5% of salmonella in whole squid, 6% in process cuttlefish and nil in processed squid and whole cuttlefish.

Studies conducted by Varma et al., (1985) and reported that all the samples of cuttlefish were free from Vibrio cholerae. Lakshmanan et al., (1993) observed in his study 11.1% of Vibrio cholerae non-01 in frozen whole squid, 23.8% in processed squid, 6.4% in whole cuttlefish and 13.6% in processed cuttlefish. All the samples were free from Vibrio cholerae -01. Since water is used in large quantities during processing, it can be a major source of contamination of Vibrio cholerae. (ICMSF 1978).

The objectives of the present study are

- to compare the protein content of various other cephalopods with selected species of squid.
- to evaluate the nutritional significance of squid as a food item with reference to the other species of cephalopods.
- to compare the extractability of various fractions of proteins of squid with other cephalopods.

2.3. Materials and Methods

Fresh samples of Loligo duvaucelli, Dorytheuthis sibogae, Sepia pharaonis and Octopus globosus, were caught from the offshore waters of Munambam for the study. The samples immediately after catch were transferred to clean insulated boxes and iced indirectly by separating the samples from ice by a thin layer of polythene sheet. The samples were transported to the processing laboratory where the treatment and processing of the samples were done. The chilled samples were skinned, cleaned and taken in triplicate. The moisture content was determined following like moisture content, fat content and various fractions of proteins of squid.

2.3.1. Determination of Moisture Content

The moisture content was determined by drying at 103°C in a drying oven. The samples were taken in pre-weighed tins and dried at 103°C until they become constant. The moisture content was determined from the weight loss.

The percentage moisture content was calculated from 100.

2.3.2. Determination of Fat Content

Fat content was determined by extraction technique (Guarnerio, 1990). About 2 grams of the fat were weighed into an extraction thimble and connected to a pre-chilled, pre-cooled Petroleum ether (B.P. 30-60°C). The solvent was allowed to boil over a water bath until the solvent boiled.
Composition of squid

Taken in triplicate for the study. Samples were drawn for various analyses like moisture content, crude fat, ash content, crude protein and various fractions of protein.

2.3.1. Determination of Moisture

The moisture content was estimated by the method of AOAC (1990). The moisture content was determined by drying 10gms of the sample was dried at 103°C in thermostatically controlled hot air oven. The samples were taken in pre-weighed glass dish with cover and kept in oven till the weight become constant. The weight was checked for constant weight by repeatedly heating and then cooling the sample in a desiccator. The percentage solid was determined from the above experiment by using the formula

\[
\text{Percentage solid} = \frac{\text{Weight of dry sample}}{\text{Weight of wet sample}} \times 100
\]

The percentage moisture was calculated by subtracting solid weight % from 100.

2.3.2. Determination of Crude Fat

Fat content of the moisture free sample was determined by extracting the fat by using a suitable solvent by soxhlet extraction method (AOAC 1990). About 2 grams of the sample was accurately weighed into an extraction thimble, and was placed in the extractor. The extractor was connected to a pre-weighed dry receiving flask and a water condenser. Petroleum ether (B.P.40-60°C) was used as the solvent. The unit was heated over a water bath and the temperature was controlled at 40°C-60°C so that the solvent boiled continuously and siphoned 5 to 6 times per hour.
Extraction was continued till the solvent in the extractor became colourless and fat free. The solvent in the receiving flask was evaporated completely and weighed for fat content.

Percentage of fat = \( \frac{\text{Weight of fat}}{\text{Weight of sample}} \) \times 100

### 2.3.3. Determination of Ash Content

Ash content was determined by the incineration of the sample (AOAC 1990). 2 grams of sample was taken in a pre-weighed silica crucible and the sample was charred on low heat. Then it was then kept at 550°C in a muffle furnace to get a white ash, which was cooled in a decicator and weighed.

Percentage of ash = \( \frac{\text{Weight of ash}}{\text{Weight of sample}} \) \times 100

### 2.3.4. Determination of Crude Protein

1 gm of homogenized sample was used for determining the crude protein content using Micro Kjeldahl method (AOAC 1990). About 1 gram of sample was accurately weighed into a digestion tube. About 2gms of digestion mixture (CUSO₄ and K₂SO₄ as a catalyst in the ratio 1:8) and 10 ml of concentrated H₂SO₄ (AR) were added to the sample taken in a digestion tube. The samples were digested to a clear solution in a Kel Plus digestion unit. 50 ml of distilled water was added to the cooled tube slowly till no heat was generated on adding water. The solution was made up to 100 ml. Pipetted out 5 ml of the prepared sample into Kjeldahl micro distillation apparatus. The bottom end of the condenser was fitted to a delivery tube, which was immersed in 10 ml of 2% boric acid solution with added

Tachiro’s indicator unit to make it alkaline. It was absorbed into alkali and titrated against standard H₂SO₄. The titration was determined the nitrogen content of the sample multiplied by a factor of 6.25.

### 2.3.5. Fractionation of Proteins

The protein in the leaf was separated (1966). A detailed description of the extraction scheme is presented. The stroma and stroma fraction, were separated at speed homogenizer (Gomori 1955), were taken for determination and from the two extracts were taken for determination. Separation into fractions following precipitation with 30% trichloroacetic acid extract is designated as sarcoplasmic propean, the strength 0.6 and protein.
Tachiro’s indicator. 40% NaOH was added to the sample in the distillation unit to make it alkaline. The ammonia thus produced on steam distillation was absorbed into the boric acid solution. The distillate collected was back titrated against standard N/70 H2SO4 using Tachiro’s indicator and determined the nitrogen content. The nitrogen content thus obtained was multiplied by a factor 6.25 to obtain the crude protein content of the sample.

\[
\text{% Protein} = \frac{V \times 1 \times 100 \times 100 \times 6.25}{5 \times 5 \times \text{weight of the sample}}
\]

2.3.5. Fractionation of protein

The protein of the muscle was fractionated by method of Paul et al., (1966). A detailed scheme of the procedure is shown in Figure 2.1. The extraction scheme was based on separation into sarcoplasmic, myofibrillar and stroma fractions. The samples were disintegrated with a Virtis high-speed homogenizer in 50 ml of KCl- Borate buffer prepared according to Gomori (1955), with an ionic strength of 0.05 and pH 7.5, then centrifuged at 1200 X G for 10 minutes. The supernatant was decanted, and the residue was extracted again with 35 ml of KCl – Borate buffer. The supernatants from the two extractions were combined and made to volume; Aliquots were taken for determination of total sarcoplasmic nitrogen, and for the separation into nonprotein and protein nitrogen of fractions and by precipitation with 10 % trichloroacetic acid. The total nitrogen of this extract is designated as sarcoplasmic protein. The residue from the sarcoplasmic protein extraction was treated with KI- Borate buffer of ionic strength 0.6 and pH 7.5, to obtain the myofibrillar proteins.
Two extractions were performed, followed by centrifugation at 0°C throughout the extraction and centrifugation process. The residue after 0.5 M KI borate buffer extraction was analyzed for nitrogen content. The mixture with 5 M KI borate buffer was centrifuged before centrifugation at 0°C. These extractions were repeated on the extracts and TCA precipitate. The proteins were analyzed for nitrogen content.

2.3.6. Bacteriological Examination

2.3.6.1. Total Plate Count

Both Nutrient Broth (NPB) and Nutrient Agar (Nutrient extract agar) were used to prepare samples for bacteriological examination. A petri dish containing agar was inoculated with the sample with the aid of a spreader. Nutrient broth was used to maintain the sample, which was considered as 1 g of the inoculum into 9 g of agar inoculum from 1 g of the inoculum. The plate was then streaked and incubated.
Two extractions were made using 50ml and 35 ml of buffer solution followed by centrifugation at 1200 X G for 10 minutes. KI rather than KCl was used to minimize the gelation. Disintegration of the original sample, extraction and centrifugation in KCl and in KI solutions were carried out at 0°C throughout the process, in order to minimize the denaturation of proteins. The residue after extraction of myofibrillar proteins in KI-borate buffer extraction was further extracted with 50ml and 20ml of 0.1N NaOH at room temperature with gentle stirring to remove the denatured protein. The mixture with 50ml NaOH was kept at room temperature for 45 minutes before centrifugation and the second for thirty minutes. The residue from these extractions was designated as stroma fraction. All the above said extracts and TCA precipitated sarcoplasmic and stroma residues were analyzed for nitrogen content by micro Kjeldahl method.

2.3.6. Bacteriological Analyses
2.3.6.1. Total Plate Count

Both Nutrient agar (NA) and TGBE agar (Tryptone glucose beef extract agar) were used to determine the total plate count (TPC). The samples for bacteriological analysis were taken in a sterile stainless steel dish containing about 10gms of the sample aseptically transferred Blended the sample with 90ml of sterile phosphate buffer. Acid washed sterile sand was used to make the slurry homogeneous. The resulting dilution was considered as 10⁻¹. Prepared decimal dilutions by transferring 1ml of inoculum into 9ml of buffer solution taken in test tubes. Poured 1ml of inoculum from 10⁻⁴ and 10⁻² to sterile petridishes. Pour about 20ml of TGBE agar and the plates were rotated slowly on a flat surface to ensure uniform
blending and the agar was allowed to solidify. Incubated the petridish at 37±1°C for 48 hours in the inverted position to prevent the condensation of moisture on the surface of the agar medium during incubation. The dilutions having 30 to 300 numbers of colonies were selected. TPC per gram as calculated using the formula

$$\text{TPC per gram} = \frac{\text{Number of colonies} \times \text{reciprocal of the dilution}}{\text{Weight of the sample}}$$

The sampling and the analysis of TPC were done by the method (APHA, 1998). The medium used was of Hi-media. The remaining portion of the inoculum at $10^1$ dilution was used for isolation and identification of *E.coli* and *staphylococcus aureus*.

### 2.3.6.2. *Escherichia coli*

*Escherichia coli* was isolated from the homogenate using Turgitol 7 agar (APHA, 1998). 0.5 ml was taken and streaked to the medium using a bend glass rod. The plates were incubated for 18–24 hours at 37 °C and were observed for yellow, flat, smooth, circular, non-mucoid colonies with pin pointed pink center. The suspected colonies were taken for further confirmation tests. The suspected colonies from T7 agar was taken and inoculated to Mac-Conkey broth and incubated at 44 ± 1°C. Indole test, Voges proskeur, Methyl red test, and citrate utilization tests were done. Number of *E.coli* per gram of the sample were calculated by the formula

$$\frac{\text{Number of colonies on T7} \times \text{reciprocal of the dilution}}{\text{Weight of the sample} \times 0.5}$$

### 2.3.6.3. Coagulase positive

Coagulase positive Agar (B.P) (APHA, 1998), containing yolk protein and a known concentration of calcium chloride, in the presence of potassium carbonate for the coagulation. Coagulase positive was confirmed only when the sample showed the coagulation of the sample was confirmed.

### 2.3.6.4. Salmonella

Salmonella was isolated by the selective media (APHA, 1995) method. Trypticase soy broth was streaked for 24 hours at 37°C. In ferrous sulphate and thiosulphate broths, selenite cystine broth and cholesertin were added. The recovery was reduced to selective medium and the number during 8 hours to 24 hours was multiplied from zero to 24 hours. After 8 hours of incubation, if the brown colonies appear in the medium, confirmatory test, per US FDA method.

### 2.3.6.5. Vibrio cholera

25g of sample (APW) for 24 hours at 37°C.
Composition of squid

2.3.6.3. Coagulase positive staphylococcus

Coagulase positive staphylococcus culture was done in Baird Parker Agar (B.P) (APHA, 1998). The staph, a proteolytic bacteria utilizes the egg yolk protein and a clear zone was formed surrounding the colony. Due to the presence of potassium tellurite, black colonies appeared on the medium. Coagulase positive staph can coagulate rabbit blood plasma. The organism was confirmed only after the coagulase test. Number of bacteria per gram of the sample was calculated using the formula:

\[
\frac{\text{Number of colonies on BP} \times \text{reciprocal of the dilution}}{\text{Weight of the sample} \times 0.5}
\]

2.3.6.4. Salmonella

Salmonella was pre-enriched using lactose broth following US FDA (1995) method. Taken 375g sample in 3750ml of lactose broth. It was kept for 24 hours at 37°C and the selective enrichment was done in two separate broths, selenite cystine broth, and tetra thionate broth. In SCB the selenite was reduced to selenium by salmonella and colon bacilli decreased in number during 8 to 12hrs and thereafter increased rapidly. But salmonella multiplied from zero hour onwards. The samples were taken between 6 and 8 hours of incubation. In tetra thionate broth, salmonella reduced thionate to thionite and flourish while other faecal bacteria were inhibited. Further confirmatory test, like bio-chemical and serological test were carried out as per US FDA method (1995).

2.3.6.5. Vibrio cholerae

25g of sample was pre-enriched in 225ml of alkaline peptone water (APW) for 24 hours at 37°C. After incubation 1ml sample was taken from
just below the surface, since *vibrio cholerae* is micro aerophilic, and secondary enrichment was done in 9ml APW. This was incubated for 6-8 hours at 37°C and was streaked on Thiosulphate citrate bile salt sucrose agar (TCBS). Further confirmatory tests were carried out as per US FDA method (1995).

2.4. Results

2.4.1. Proximate composition

The proximate composition of the selected two species of squid and other cephalopods are given in the table 2.1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Moisture%</th>
<th>Fat %</th>
<th>Ash %</th>
<th>Protein%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loligo</td>
<td>79.26</td>
<td>1.99</td>
<td>0.96</td>
<td>21.72</td>
</tr>
<tr>
<td>Needle</td>
<td>81.27</td>
<td>2.01</td>
<td>0.99</td>
<td>20.32</td>
</tr>
<tr>
<td>Octopus</td>
<td>77.25</td>
<td>1.2</td>
<td>0.9</td>
<td>17.49</td>
</tr>
<tr>
<td>Sepia</td>
<td>78.21</td>
<td>2</td>
<td>0.7</td>
<td>17.3</td>
</tr>
</tbody>
</table>

The needle squid showed the highest value for moisture content when compared to other species (81.27%). Loligo squid also had higher moisture content when compared with others (79.26%). The moisture content is higher in the case of needle when compared with loligo, which indirectly helps for the faster disintegration. The higher moisture content provides a strong substratum for the bacteria also.

Ash content of all the samples taken, have almost same value throughout the study. The fat content and ash content showed no significant changes in the selected species, but the moisture content and the protein content varied considerably. Fat and ash content in all the species is comparatively less and less susceptible to oxidation changes.

<table>
<thead>
<tr>
<th>Total nitrogen</th>
<th>Sarcoplasmic</th>
<th>Myofibrillar</th>
<th>Denatured</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loligo</td>
<td>3.16</td>
<td>1.83</td>
<td>1.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Needle</td>
<td>3.34</td>
<td>1.89</td>
<td>1.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Octopus</td>
<td>3.42</td>
<td>1.92</td>
<td>1.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Sepia</td>
<td>3.26</td>
<td>1.76</td>
<td>1.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
The tissue content when dried is higher and higher moisture content is preferred, which indirectly shows that protein content provides a good source of food.

The most same value showed no significant differences in protein and the protein content in all the species is expressed as % nitrogen.

<table>
<thead>
<tr>
<th>Protein%</th>
<th>21.72</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.32</td>
<td></td>
</tr>
<tr>
<td>17.49</td>
<td></td>
</tr>
<tr>
<td>17.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Total nitrogen content of different species of cephalopods during various months

<table>
<thead>
<tr>
<th></th>
<th>Loligo</th>
<th>Needle</th>
<th>Octopus</th>
<th>Sepia</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>3.48</td>
<td>3.25</td>
<td>2.80</td>
<td>2.77</td>
</tr>
<tr>
<td>November</td>
<td>3.32</td>
<td>3.06</td>
<td>2.64</td>
<td>2.62</td>
</tr>
<tr>
<td>December</td>
<td>3.15</td>
<td>3.00</td>
<td>2.54</td>
<td>2.56</td>
</tr>
<tr>
<td>March</td>
<td>3.23</td>
<td>3.09</td>
<td>2.51</td>
<td>2.54</td>
</tr>
<tr>
<td>April</td>
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<td>2.64</td>
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<tr>
<td>May</td>
<td>3.48</td>
<td>3.29</td>
<td>2.67</td>
<td>2.80</td>
</tr>
<tr>
<td>August</td>
<td>3.23</td>
<td>3.11</td>
<td>2.64</td>
<td>2.61</td>
</tr>
<tr>
<td>September</td>
<td>3.46</td>
<td>3.17</td>
<td>2.56</td>
<td>2.59</td>
</tr>
</tbody>
</table>

In Table 2.2 the total protein content of various cephalopod species during various months was given. Throughout the study, there were no much changes in protein content in all the cephalopod species. In the case of sepia and octopus protein content ranges from 17-18%, but in the case of squid it ranges from 18-22%, which clearly shows that the squid is rich in protein in all seasons.

2.4.2. Fractions of Protein

Table 2.3. Changes in the extractability of proteins expressed as % nitrogen

<table>
<thead>
<tr>
<th></th>
<th>Loligo % N</th>
<th>% of protein fraction on total N</th>
<th>Needle % N</th>
<th>% of protein fraction on total N</th>
<th>Sepia % N</th>
<th>% of protein fraction on total N</th>
<th>Octopus % N</th>
<th>% of protein fraction on total N</th>
<th>Denatured % N</th>
<th>% of protein fraction on total N</th>
<th>Stroma % N</th>
<th>% of protein fraction on total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td>3.48</td>
<td>100.00</td>
<td>3.30</td>
<td>100.00</td>
<td>2.87</td>
<td>100.00</td>
<td>2.84</td>
<td>100.00</td>
<td>0.01</td>
<td>0.40</td>
<td>0.07</td>
<td>2.00</td>
</tr>
<tr>
<td>Sarcoplasmic</td>
<td>1.84</td>
<td>52.97</td>
<td>2.21</td>
<td>66.97</td>
<td>1.15</td>
<td>40.00</td>
<td>1.10</td>
<td>38.70</td>
<td>0.01</td>
<td>0.40</td>
<td>0.07</td>
<td>2.00</td>
</tr>
<tr>
<td>Myofibrillar</td>
<td>1.56</td>
<td>44.97</td>
<td>0.99</td>
<td>29.98</td>
<td>1.44</td>
<td>50.32</td>
<td>1.59</td>
<td>56.02</td>
<td>0.01</td>
<td>0.40</td>
<td>0.07</td>
<td>2.00</td>
</tr>
<tr>
<td>Denatured</td>
<td>0.01</td>
<td>0.40</td>
<td>0.02</td>
<td>0.50</td>
<td>0.25</td>
<td>8.72</td>
<td>0.14</td>
<td>4.81</td>
<td>0.07</td>
<td>2.00</td>
<td>0.07</td>
<td>2.00</td>
</tr>
<tr>
<td>Stroma</td>
<td>0.07</td>
<td>2.00</td>
<td>0.07</td>
<td>2.00</td>
<td>0.03</td>
<td>1.03</td>
<td>0.02</td>
<td>0.83</td>
<td>0.07</td>
<td>2.00</td>
<td>0.07</td>
<td>2.00</td>
</tr>
</tbody>
</table>
Table 2.3.1 Changes in the extractability of fractions of sarcoplasmic proteins expressed as % nitrogen

<table>
<thead>
<tr>
<th></th>
<th>Loligo %</th>
<th>% of protein fractions on total sarcoplasmic N</th>
<th>Needle %</th>
<th>% of protein fractions on total sarcoplasmic N</th>
<th>Sepia %</th>
<th>% of protein fractions on total sarcoplasmic N</th>
<th>Octopus %</th>
<th>% of protein fractions on total sarcoplasmic N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1.84</td>
<td>100.00</td>
<td>2.21</td>
<td>100.00</td>
<td>1.15</td>
<td>100.00</td>
<td>1.10</td>
<td>100.00</td>
</tr>
<tr>
<td>NPN</td>
<td>1.71</td>
<td>92.97</td>
<td>2.15</td>
<td>96.96</td>
<td>0.95</td>
<td>82.57</td>
<td>0.92</td>
<td>83.78</td>
</tr>
<tr>
<td>PN</td>
<td>0.11</td>
<td>5.99</td>
<td>0.07</td>
<td>2.96</td>
<td>0.15</td>
<td>13.29</td>
<td>0.15</td>
<td>13.23</td>
</tr>
</tbody>
</table>

NPN = Non protein nitrogen
PN = Protein nitrogen

Table 2.3 and Table 2.3.1 gives the detailed picture of various fractions of proteins in various species. The sarcoplasmic protein, which was extracted by 0.05 M KCl borate buffer, contributed about 53% of the total proteins in loligo, 67% in needle squid, 40% in sepia and 38% in octopus. Myofibrillar proteins, which were extracted by 0.6 molar KI borate buffer, contributed only 30% (needle) to 45% (loligo) of the total protein content. Denatured protein contributed 0.4 (loligo) to 0.5% (needle) and stroma 2% to 3% in loligo and needle squid respectively. Protein nitrogen (PN) in loligo as per the fractionation method showed a very low content of about 6 to 7% only and non-protein nitrogen (NPN) contributed the rest (93-94%) of the total sarcoplasmic fraction. But in the case of needle squid, PN contributed about 3% and the rest by NPN. NPN was found to be very high in the cephalopods, especially in squids.

2.4.3. Bacteriology

Table 2.4. Values of Microbial Cell Count

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid loligo</td>
<td>1.8</td>
</tr>
<tr>
<td>Squid needle</td>
<td>2.0</td>
</tr>
<tr>
<td>Sepia</td>
<td>1.7</td>
</tr>
<tr>
<td>Octopus</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The different samples of *V. staphylococcus*, with counts ranging from 1.4 to 1.8, showed count ratios which were different in all the samples studied. *Coagulans* varied from 1.7 to 2.0.

2.5. Discussion

Over the years, the microbiology of cephalopods have been partially understood. Need for proximal and distal regions was studied to determine the changes occurring during the processing. The differences found were vast, but informative analysis was made.
2.4.3. Bacteriology

Table 2.4. Microbiology of various species of Cephalopods

<table>
<thead>
<tr>
<th></th>
<th>TPC</th>
<th>Ecoli</th>
<th>staph</th>
<th>Vibrio cholerae</th>
<th>salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid loligo</td>
<td>1.87X10^5</td>
<td>185</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Squid needle</td>
<td>2.03X10^5</td>
<td>225</td>
<td>325</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Sepia</td>
<td>1.78X10^5</td>
<td>175</td>
<td>310</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Octopus</td>
<td>1.82X10^5</td>
<td>200</td>
<td>305</td>
<td>nil</td>
<td>nil</td>
</tr>
</tbody>
</table>

The different types of bacteria enumerated were *E-coli*, *staphylococcus*, *vibrio cholerae* and *salmonella* along with the Total Plate Count. The results are given in table 2.4. Irrespective of the species, TPC in the raw material seemed to be in higher range of 1.875X10^5. *E-coli* also showed count ranging from 175-225 in all the species of cephalopods studied. *Coagulate positive staphylococci* were present in both the samples of squid, sepia and octopus. *Salmonella* and *vibrio cholerae* were absent in all the samples studied.

2.5. Discussion

Over the years, the efforts to establish proximate composition of fish have been partially successful, but many gaps in our knowledge still exist. Need for proximate composition of fish and other seafoods is currently greater than in the past because of its nutritional significance and the changes occurring in each chemical components during various stages of processing. The data regarding chemical composition of cephalopods is also vast, but information regarding a species of squid, *Doryteuthis sibogae*, which is scarcely utilized for consumption and in this study a comparative analysis is made with other species of cephalopods. The moisture content of
various species of cephalopods studied was in the range 75-84% (Suyama and Kobayashi, 1980, Tse-Kuei-Chiou, 2000, Lakshmanan & Balachandran, 2000, Selvaraj, 1991, Jose Joseph et al., 1977 and Lakshmanan et al., 1993). The crude protein content varied from 17-22% in the cephalopod species studied with squids showing highest level. The work of Suyama and Kobayashi (1980) showed that the protein content of various species of cephalopods varied from 15-23% and moisture content had an inverse relationship with proteins. The work done on the mantle tissue of fresh *Loligo duvauceli* in tropical waters also showed some variations of 17.5-22% (Selvaraj, 1995, Jose Joseph et al., 1988 and Raghunath, 1984). Studies of also confirmed that the squid muscle has higher total nitrogen than white lean fish (Sujiyama, 1980). The variations in the value of protein in the same species observed by various workers might be due to the difference in handling, affecting the leaching rate of proteins. In this study no significant variations were observed in total nitrogen in the different cephalopods species during various months of the year. Kawada and Takaschi (1955) had a comparable result showing no significant changes in protein and moisture content of squid muscle (*Todarodes pacificus*) throughout the year. The fat content showed values of 2% and less than 2% among the cephalopods studied. The ash content showed the mineral content in the muscle, which varied from 0.7 to 0.99%.

Although we correlate proximate composition to factors like geographical area or season of the year at which the fish is caught, the ultimate cause of the variation is due to the feed intake and other related factors such as metabolic efficiency of individual fish and the energy expenditure due to more or less active movement of the fish. The cephalopods under this category (under 5%) and both of them are found in this category.

### 2.5.1. Protein Sources

Proteins of fish have higher solubilities, water content, and total protein of squid than total protein of another cephalopod was 73% and 20%. Sikorski and Hamann (1979) studied the composition of protein with higher proteins in water. The skinned product usually faster following the effect of these conditions. The work of Joseph et al. 1977 on cephalopods showed the high content of non-protein nitrogen with the earlier finding in the presentation of this report with high nutritive value.
cephalopods under study can be categorized to a group, with low oil content (under 5%) and high protein content (15-20%). Most of the food fishes are found in this category.

2.5.1. Protein Solubility

Proteins of fish muscle are divided into three classes based on their solubilities, water soluble, salt soluble and insoluble proteins. 77-85% of the total protein of squid is water-soluble and can extract more than 80% of the total protein by repeated washing (Matsumoto, 1958). According to Lakshmanan and Balachandran (2000) the myofibrillar protein content in cephalopod was 77-85% and the sarcoplasmic protein showed a value of 12-20%. Sikorski and Kolodziejska (1985) and Kolodziejska et al., (1987) have studied the composition of squid meat and characteristic features of squid protein with high protease activity and high solubility of myofibrillar proteins in water. There are also reports about the changes in the solubility of myofibrillar protein and collagen in squid, *Loligo pealei* (Otwell and Hamann, 1979). The rates of extractable protein nitrogen decrease were usually faster following pre-process holding (Connel and Howgate, 1971). The skinned products showed a greater loss of NPN owing to the leaching effect of these components during washing (Lakshmanan et al., 1993). The work of Joseph et al., (1977) arrived at a similar result showing decrease of non-protein nitrogen during washing. They also noticed that there was a high content of non-protein nitrogen in squid meat. The results also agree with the earlier findings of Japanese scientists (Borgstrom, 1965). Proper presentation of the squid mantle tissue can obtain good quality products with high nutritive value.
2.5.2. Bacteriology

The quality of a seafood product mainly depends upon the number and type of microorganisms present. The toxic metals and pathogens in food items have great public health significance. The bacterial quality of cephalopods is shown in table 2.4. Enumeration of TPC is designed to provide an estimate of the total number of aerobic organisms in a particular food. It reflects the microbiological quality of the food and is useful for indicating the potential spoilage of the perishable food products. It is also an indicator of the sanitary conditions under which the food was produced and/or processed and also of the level of Good Manufacturing Practices (GMP) adopted during the processing. But in raw frozen food, uncontrolled destruction of the organism might have taken place during freezing which makes the above assumption baseless to a certain extent. Despite the above limitations, TPC can be taken as a valuable indicator of the effectiveness of any type of processing or chemical disinfections such as cooking, freezing and chlorination. Offshore waters do not contain E-coli, but natural water gets contaminated with E-coli either by direct contact or by mixing up with terrestrial sewage. The predominant aerobic bacterial flora of the large intestine of the human being and animals is composed of non-spore forming non-acid fast, Gram- negative bacteria. They exhibit general morphological and biochemical similarities and are grouped together in large and complex family of Enterobacteriaceae. Members of the coliform, including faecal coliforms are referred to as indicator organisms, since their presence in certain numbers may indicate the potential presence of pathogens in foods.

Lakshmanaraya
Cochin Fisheries unacceptably high crushed ice and Madhusoodana Rao contained high counts which was not due to temperature abuse borne infections in raw fish was clear that they were not carefully to avoid bad health

The studies carried out showed that 74.2% of the samples contained E-coli at 20/g. The studies by the cuttle fish fillet had shown that is mainly due to the role of poor handling (Liston, 1965 and Cann, 1974).

The role of Staphylococcus is a useful indicator of personal and present above room temperature presence of Staphylococcus indicator of personal...
Composition of squid

Lakshmanan et al., (1984) has studied the quality of fish landed at Cochin Fisheries Harbour. Of the total samples 8.5% were considered unacceptable based on TPC and 26.4% of the samples contained E-coli. The crushed ice and the platform had high bacterial load. In the study of Madhusoodana Rao and Surendran (2003) showed that the deep-sea fishes contained high count of E-coli and coliphages in the landing center samples, which was not due to the deep-sea fish or deep-sea waters but could be only from terrestrial sources. According to Nazeem Beena (2002) the temperature-abused contaminated fish could act as a potent vehicle of food borne infections in the country. From the above results and the references it was clear that the handling of any perishable item should be done very carefully to avoid health hazards.

The studies conducted by Iyer et al., (1986) on fresh water fish samples showed that 74.2% of the samples had E-coli less than the acceptable limit i.e. 20/g. The studies by Varma et al., (1985) have reported that 0.93% of frozen cuttle fish fillet had E-coli above the prescribed limit. The incidence of E-coli is mainly due to the external contamination during handling and processing (Liston, 1965 and Cann, 1977).

The role of Staphylococcus aureus as a food poisoning organism is a useful indicator of personal hygiene in the process involving human handling (Cann, 1977; ICMSF, 1978; Liston, 1980; Hobbs, 1982). The organism can multiply vigorously and produce toxin at temperatures near and above room temperature (Iyer, 1986). According to Hobbs (1983) the presence of Staphylococcus aureus, even in small numbers is an excellent indicator of personal hygiene.
Salmonella finds its entry into the product during handling in preprocessing and processing. The best method to avoid contamination with salmonella is to implement strict sanitary measures during preprocessing and processing.

2.6 Conclusion

Squid was found to be a good source of protein when compared with other seafood items. 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 has to be taken to reduce the hazards. Thus practice of storing squid in slush ice, at any point from landing to processing area, does not lead to a decrease in the physical yield of the product, but rather an increase. But as the loss of proteins as well as NPN fraction could affect the organoleptic quality of squid in general, and squid’s sweet taste in particular is linked to the NPN fraction. From the findings it is clear that the nutritive value of the squid is more susceptible to loss by leaching. In some particular circumstances even up to 80% of the squid protein can be extracted by repeated washing with water and seems to be more in skinned and processed squid. 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 studies are needed to reduce this nutritional loss and quality degradation.

2.7 References


2.7. References


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