II. REVIEW OF LITERATURE

2.1. Octopus biology and farming

The octopus belongs to:

- Phylum: Mollusca
- Class: Cephalopoda (Cuvier, 1797)
- Order: Octopoda (Leach, 1818)
- Family: Octopodidae (Orbigny, 1840)
- Genus: Octopus (Cuvier, 1767)
- Species: Octopus membranaceus (Quoy and Gaimard, 1832)

Common names: Four-eyed bird, Webfoot octopus etc.

Octopus is characterized by their eight arms, usually with sucker cups on them. They have soft bodies with no internal skeleton. A beak is their only hard part. Octopus has three defensive mechanisms viz., ink sacs, camouflage and autotomising limbs. Most of the octopus species can eject a thick blackish ink in a large cloud to aid in escaping from predators. They also have specialized skin cells both for color changing (chromatophores), light reflection and refraction (iridophores and leucophores). They use this ability to blend into the environment to hide, communicate with other octopus or warn.

The octopus is a carnivore, generally feeds on crabs, shrimps, shellfish and fish. The larger species of octopus have also been known to hunt small sharks and dogfish. They trap their prey in their tentacles and drag it towards their powerful beak-like jaws. Once it has bitten its prey, the octopus injects with poisonous saliva to kill. They can also use their tentacles to break shells apart once they have caught their prey. They bite chunks off with their sharp, parrot-like beak.

Octopus has a relatively short life span and some species live for as little as six months. Larger species, such as the North Pacific giant octopus, may live for up to five years if they do not reproduce. However, reproduction is a cause of death: males can only live for a few months after mating and females die shortly after their eggs hatch.

They crawl by walking on their arms, usually on many at once, on solid surfaces, while supported in water. They swim by expelling a jet of water from a contractile mantle and aiming it via a muscular siphon.

*Octopus membranaceus* mantle varies from saccular to very elongate with little differentiation between the mantle and head in the neck region. Arms are medium to long with the lateral arms longest. The funnel organ is W-shaped with stout to moderately slender limbs, 7-8 filaments to each demibranch of the gill, two cirri or warts over each eye. The most distinctive feature of this species is the conspicuous ocellus or ringed spot.
on each side of the head in front of and below the eyes. This consists of a dark brown splotch containing a bluish or greenish ring. The animal live in holes on flat bottoms, comes out during feeding. At present, information on large scale farming of octopus is not available. However, efforts are being made by Nabhitabhata (1995) to mass culture of big fin squid, *Sepioteuthis lessoniana*; spineless cuttlefish, *Sepiella inermis*; and pharaoh cuttlefish, *Sepia pharaonis*. He has reported the culture method, biology and behavior of cephalopods. Recently an attempt has been made by Chandage *et al.*, (2006) and successfully bread octopus in captivity at Dr.B.S. Konkan Krishi vidyapeeth, Dapoli.

### 2.2. Distribution

Cephalopods are an ancient group that appeared some time in the late Cambrian several million years before the first primitive fish began swimming in the ocean. Cephalopods were once one of the dominant life forms in the world's oceans. These are found in all of the world's oceans, from the warm water of the tropics to the near freezing water at the poles. They are found from the wave swept intertidal region to the dark, cold abyss. All species are marine and with a few exceptions, they do not tolerate brackish water. Octopus inhabits many diverse regions of the ocean; they occur in shallow coastal waters in the intertidal and subtidal areas among rocks, stones or coral reefs hiding themselves among crevices. There are about 289 different octopus species known to exist, which is over one-third the total numbers of cephalopod species. About 38 species of octopus belonging to the family Octopodidae, Tremoctopodidae and Argonautidae have been recorded from Indian seas (west and east coasts) including Andaman and Lakshadweep water (CMFRI, 2004).

*Octopus membranaceus* species is distributed around the world in different countries. Some of the countries which catching *O. membranaceus* are Andaman Islands (India), Australia, Bangladesh, Brunei Darussalam, Cambodia, China, Christmas Island (Australia), East Timor, France Polynesia, Guam (USA), Hong Kong (China), India, Indonesia, Iran, Japan (Pacific ocean coast), Japan (Sea of Japan), Korea (North and South), Macau (China), Malaysia (East Peninsula), Malaysia (Sabah), Malaysia (Sarawak), Malaysia (West Peninsula), Maldives, Micronesia, Myanmar, Ogasawara Islands (Japan), Oman, Pakistan, Palau, Papua New Guinea, Philippines, Pitcairn (UK), Ryukyu Islands (Japan), Saudi Arabia (Persian Gulf), Saudi Arabia (Red Sea), Singapore, Solomon Islands, Somalia, Sri Lanka, Taiwan, Thailand, United Arab Emirates, USA (North Marianas), Viet Nam and Yemen.

### 2.3. Present status of octopus fishery / Industry, consumption and benefits

Among cephalopod resources, Octopods are the least exploited in India. In earlier years there has been fishing of Octopods in some areas along the mainland coast.
Only little information is available on the fishery of octopus. However, reports indicate that the fishery exists in the Andaman-Nicobar and Lakshadweep areas, Gulf of Kutch, Gulf of Mannar, Pak bay etc. Fishery of *Octopus membranaceus* have been recorded at Mangalore, Malpe and Cochin coasts also. In India domestic consumption is very less, but the catch is exported to different countries viz., Japan, USA, Austria, Belgium, France, Germany, Greece, Italy, Netherlands, Portugal, Spain, UK, China, Taiwan, Thailand, Vietnam, Israel etc., in different form like frozen octopus, frozen baby octopus, IQF octopus tentacles, IQF baby octopus, frozen octopus tentacles, frozen octopus rings, AFD octopus, frozen octopus (whole cleaned) etc. Currently, about 68 seafoods processing industries are engaged in processing and export of octopus in India.

Japan is the main market for octopus. Increase in prices of octopus was noticed during the course of 2004, exceeding US$ 12.00/kg for the larger-sized specimens and was one of the most expensive seafood species traded. Spain was the second most important octopus market, with a tradition of octopus consumption in the main fishing regions. Morocco had lost its dominant position of the Japanese market, which the country held for decades. On the other hand, China is becoming the main player on the Japanese market, selling at very convenient prices. Compared to Spain, Italy had always to rely on imports for its octopus supply. The preference goes to small octopus and imports depend on availability and price levels.

Kreuzer (1984) has extensively reviewed cephalopods as raw material for processing industry, systematics and distribution of commercially important species, as well as their biological structure, chemical composition and nutritive value. Handling and processing methods of cephalopods at sea and ashore, products and product development, with emphasis on those available in Japan, methods used for quality assessment, as well as quality requirements in the trade, problems of the consumer acceptance of cephalopods have been discussed. Arocha (1989) have reviewed the cephalopod resources of Venezuela and noticed *Octopus vulgaris* was abundant in the catches from June until October with a peak in August-September. *Octopus vulgaris* is currently ranked third in landings and generates the highest revenue than all species caught in Portuguese fisheries. Octopus is mainly caught by artesian gear types, particularly octopus pots and traps (FAO. 1997). Roh (1991) has given an account on cephalopod products exported from Korea which include seasoned squid, frozen cuttlefish, frozen octopus, frozen processed squid, fresh octopus and dried squid. The process of liberalization for cephalopods and changes in the import policy and their influence on the fishery industry is outlined.
In most species of octopus, younger and smaller one is more tastier and tenderer than older, larger ones. The eight tentacles and body of the octopus are edible, but the eyes, mouth area, and viscera are not. Octopus can be prepared raw, boiled, pickled, grilled, or sauteed. Cooked octopus (84.9 g) in moist heat provides 139 calories, 25.3 g of protein, 3.7g of carbohydrate, 1.7 g of total fat. Octopus is an excellent source of iron (8.1mg), selenium (76 mcg), and vitamin B\textsubscript{12} (30.6mcg), Good source of zinc (2.8 mg), When cooked (moist heat), octopus provides 0.314 g of omega-3 fatty acids, derived from EPA (0.152 g) and DHA (0.162 g), per 100 g of octopus.

2.4. Chromatophore discoloration

Chromatophore is a container for the colour particle-pigment. It consists of a cell with expandable membrane as a wall. Several muscle fibres are radially attached to the membrane and each fibre is connected with a nerve which links the muscle fibre, via the brain, with the eyes. The eyes signal to the brain the required colour and the brain monitors with high speed via the muscle fibres, the colour change. After death, the muscles attached to the chromatophores are no longer controlled by the nervous system. The chromatophores remain expanded and the muscles relax slowly. Thus, within some hours of death, the skin colour changes from dark to light called “discoloration”. Black discoloration in cold store or during thawing is caused by pigment flowing out of broken chromatophores. A red discoloration appears when the released pigment makes contact with basic substances; for example, ammonia or other similar substances produced during protein degradation. Deviation from the natural colour may adversely influence the commercial value of the product. In Spain and Japan, deviations from the colour of the fresh skin are regarded as the loss of freshness of the raw material. These colour change can only be stopped by freezing (Kreuzer, 1984).

In general, surface color is important for whole squid and significantly less important for a product which is destined for skinning and further processing (Learson, 2000). Studies on the effects of chilling and hypoxia treatments on skin color, measurements of chromatophores expansion of Japanese common squid Todorodes pacificus and spear squid Loligo bleekeri showed that skin color can be maintained well after death by keeping the bodies well-oxygenated and avoiding direct contact with ice (Okada et al., 2004).
2.5. Presence of chemical contaminants

Cephalopods are predominant in the tropic system and they are eaten by many oceanic animals, such as marine mammals and sea birds. Some studies on elemental bio-accumulation show that cephalopods accumulate high levels of trace elements, particularly cadmium (Cd) and copper (Cu) in different parts of the body viz., muscle tissue, liver, digestive gland etc. (Martin and Flegal 1975; Miramand and Guary 1980; Smith et al., 1984; Miramand and Bentley 1992). Thus cephalopods represent important species for studying the transfer of heavy metals into marine food webs. Chen et al., (1984) have found highest content of Cu in crustaceans, cephalopods, gastropods and oysters. Oehlenschlaeger (1989) frequently observed high concentrations of toxic heavy metals, especially cadmium and lead in squids (Loligo sp.) and squid products from India and Thailand. Hadj et al., (1986) have observed the total mercury content of fish, bivalves, shrimp and cuttlefish, which are very low and varying from 29±14 ppb to 198±18 ppb when compared to the tolerated concentrations fixed by FAO for (5000-1000 ppb) mercury and is not dangerous for human health. Barska et al., (1988) have studied the most toxic lead, cadmium, zinc, copper, mercury concentrations in two species of squid: Loligo patagonica and Illex argentinus. Schuhmacher et al., (1994) have analysed a total of 592 samples of 21 species of fish, cephalopods, crustaceans and molluscs for mercury concentrations of which fish and crustaceans being the groups which accumulated the highest levels of this element. The individual dietary intake of mercury from fish and seafood consumption by the population of Tarragona province was estimated to be 16 μg/day. This intake of mercury would not signify a health hazard for consumers of fish and seafood.

Ghazaly (1988) has recorded high metal concentrations in gills although elevated concentrations were noted in edible soft tissues of Donax trunculus, Venus verrucosa, Sepia officinalis and Octopus vulgaris. Oehlenschlaeger (1990) has observed low levels of cadmium and lead contents in squid and cuttlefish mantle and suggested to cut the intestines from the mantle immediately after catch to avoid transportation of heavy metals from the intestines to the mantle during freezing. Miramand and Bentley (1992) have studied the concentrations of Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn and their distribution in the tissues like digestive gland, branchial hearts, gills, digestive tract, kidney, genital tract, muscle, skin, shell of Eledone cirrhosa and Sepia officinalis. The distribution of heavy metals in tissues of both species were similar: the digestive gland, branchial hearts and kidney were the major sites of concentration for all the metals; the digestive gland accumulated more than 80% silver, cadmium, cobalt, copper, iron, lead and zinc, the branchial hearts accumulated high concentrations of copper, nickel and vanadium, and the kidney high concentrations of manganese, nickel and lead. High
concentrations of cadmium (30.7 ± 47.1 and 27.3 ± 54.4 ng/g dry weight respectively) and low concentrations of copper and zinc were found in squid (*Graneledone* sp.) and octopus (*Benthoctopus thielei*) from Kerguelen Islands, southern Indian ocean (Bustamante et al., 1998). Sapunar *et al.*, (1989) have found significantly higher concentrations of cadmium content in cephalopods from industrially polluted Kastela and Rijeka bays and a control area in the Adriatic Sea.

Storelli and Marcotrigiano (1999) have noticed higher concentrations of cadmium and total mercury in spider octopus (*Octopus salutii*) than in broadtail squid (*Illex coindeti*) caught in the south Adriatic Sea. Higher concentrations were observed in hepatopancreas than flesh. No flesh sample showed cadmium and total mercury concentrations exceeding the peak permitted values of 2 mg/kg wet weight and 0.5 mg/kg wet weight respectively. Nessim and Riad (2003) have recorded heavy metal concentrations in various tissues like hepatopancreas, branchial hearts, salivary gland, gills, genital tract, mantle, arms and skin of *Octopus vulgaris*. Heavy metal concentrations in most tissues displayed significant differences among sites, sizes and sex. Hepatopancreas and to a lesser extent branchial hearts are better indicators of chronic Cu, Fe, Zn and Cd contamination than edible tissues. Similar studies were also conducted by Raimundo *et al.*, (2004) in different species of octopus the metal concentrations in tissues did not vary significantly with size/weight, sex or sexual stage. The abundance of metals in each analysed tissue was: Zn>Cu>Cd>Pb>Hg. Concentrations in the digestive gland reached one (Pb, Hg) and two (Zn, Cu, Cd) orders of magnitude higher than those found in arm and mantle, which indicates that the digestive gland contains the major storage sites for these elements, acting as a detoxification organ.

2.6. Post harvest handling

Because of their physiology, octopus requires special handling during harvesting, refrigeration and processing to retain the highest quality. Being a "soft-bodied" species, octopus are prone to physical and mechanical damage such as crushing, torn mantles, and loose skin - all of which result in poor quality and lower market value (Learson, 2000). Icing of octopus in boxes is normally practiced and sent to the processing factories where it is processed and exported to different countries in various forms.

High level of proteolytic activity exists in the muscle due to presence of indigenous proteolytic enzymes (Stanley and Hultin, 1984). The proteolytic activity is as high as 6 times greater as in fish. Spoilage by proteolytic enzymes favours rapid microbial growth. Thus special means of preservation is very much required to preserve the freshness of fresh octopus, so quick chilling or freezing of fresh octopus and storage
at lower temperature are important means to arrest the microbial activity and to reduce undesirable changes in quality.

2.6.1. Post mortem changes

Most crustaceans are capable of respiring outside the aquatic environment by absorption of atmospheric oxygen for limited period, under anaerobic conditions; ATP may be synthesized by two other important pathways from creatine phosphate or from arginine phosphate. The former source of energy is restricted to vertebrate muscle (teleost fish) while the latter is characteristic of some invertebrates such as the cephalopods (squid and octopus). In either case, ATP production ceases when the creatine or arginine phosphates are depleted. It is interesting to note that octopine is the end-product from the anaerobic metabolism of cephalopods and is not acidic (unlike lactate), thus any changes in post mortem pH in such animals are not related to the production of lactic acid from glycogen.

2.7. Microbial profile

After catch, the fish and by-catch are dumped on deck which may be heavily contaminated. Bacterial counts of around \(10^4-10^8\) have been reported to build up on deck surfaces during a fishing trip (Iyer et al., 1971; Castell, 1973) washing of fish if carefully done, can reduce the microbial load by 10-90% (Georgala, 1957). The effect of gutting during storage has been studied by several workers. According to Shewan (1961) it takes several days for bacteria in the viscera of uncut fish to invade the muscle and therefore the main advantage of gutting is to prevent autolytic rather than bacterial spoilage. Stansby and Lemon (1941) found that gutting of fresh mackerel could increase rather than decrease bacterial numbers. Ravesi et al., (1985) demonstrated that spiny dog fish stored whole, had lower counts than gutted fish which in turn had lower counts than those headed and gutted. They recommended gutting in fishing trips longer than 2 days since it resulted in an increased shelf life of fillets.

According to FAO (1973) recommendation, fish should not be exposed to direct sun light or to drying effect of wind but should be carefully cleaned and cooled down to the temperature of melting ice (0 °C) as quickly as possible. Holston and Slavin (1965) pointed out some of the disadvantages of icing which include tendency to injure and bruise the flesh, leaching of flavour compounds, nutritionally desirable minerals and water soluble proteins. To overcome this problem, systems using refrigerated sea water (RSW) and chilled sea water (CSW) have been suggested. Nagaraj (1994) have observed total plate count of \(5.20\times10^5\) cfu/g in freshly caught squid (L. duvaucelli) whereas Nagesha (2002) has recorded \(6.80\times10^4\) cfu/g in squid. Higher counts of mesophiles \(4.36\times10^5\) cfu/g) and psychrophiles \(5.37\times10^5\) cfu/g) were also recorded by Sastry (1981) in cuttlefishes. Sanjeeva et al., (1986) studied on the coagulase-positive
Staphylococcus in 66 samples of frozen crab, shrimp, mussels and cuttlefish meat collected in India of which 72.2% of samples were contaminated.

Seafoods are one of the important vehicles of transmission of food-borne diseases. The important pathogens associated with seafood and often implicated in gastrointestinal and extra intestinal problems are E. coli, Salmonella spp., Vibrio cholerae, V.parahaemolyticus and V. vulnificus. While the members of the family Vibrionaceae are the natural inhabitants of the aquatic system and are likely to be present in seafoods, presence of pathogens such as E. coli and Salmonella is due to secondary contamination of the seafoods.

2.7.1. Vibrio cholerae

V. cholerae are curved or comma-shaped rods, Gram-negative, aerobic, non-spore forming and are actively motile. Different serotypes of V. cholerae are Ogawa, Inaba and Hikojima. Vibrio cholerae (01) is the causative agent of the disease called "cholera". The only natural habitat of this organism is man. The primary vehicle of the spread of V. cholerae is water. This in turn may contaminate fish and other food items. The same applies to the spread of gastroenteritis due to non-01 Vibrios. They are destroyed by heat. At 56 °C they are killed in 30 minutes. V. cholerae can survive in stagnant water with alkaline pH for long periods. It can enter a viable but non culturable phase in the aquatic environment (Joseph et al., 1982; Rozak and Colwell, 1987). The incubation period is 1-5 days, symptoms are sudden onset of nausea, vomiting and profuse diarrhea with abdominal cramps. The stool resembles "rice water". There is a rapid loss of fluids and electrolytes, which leads to dehydration and circulatory collapse. Minimal infective dose is $10^3$ cells. Several cholera outbreaks related to seafood have been reported. Mostly shellfishes and crustaceans are involved in cholera out break than fin fishes. According to quality standards V. cholerae should be totally absent in seafoods. According to James (2003) the incidence of Vibrios in frozen sea foods is much less compared to fresh or refrigerated sea foods. Bacterial death by freezing can be in two phases, sudden mortality immediately on freezing and gradual death from prolonged storage at frozen storage temperature. The psychrotrophic strains of vibrios may have better survival potential than other mesophilic strains in the frozen products and could probably enhance the risk of Vibrios in frozen foods. V. alginolyticus and V. mimicus were also found in seafoods. Some of the psychrotrophic strains have good survival at low temperature and could probably enhance the risk of Vibrios in frozen foods.

Non 01 serotypes of V. cholerae have been reported from seafoods and aquatic environment (Blake et al., 1980; Colwell, 1984; West, 1989; Karunasagar et al., 1990.). Mathew et al., (1988) recorded the presence of non 01 V. cholerae in seafood samples
Counts of non 01 *V. cholerae* as high as $10^4$ per 100 ml have been recorded in waters (West and Lee, 1982; Roberts *et al.*, 1984) and it is suspected that bioconcentration of this organism might occur in filter feeding molluscs and zooplankton (West, 1989). A small percentage of non 01 *V. cholerae* has been reported to possess genes for production of cholerae toxins (Kaper *et al.*, 1986) and cause clinical symptoms indistinguishable from cholerae (Arita *et al.*, 1986). A TDH related hemolysin (You *et al.*, 1986) and toxin related to shiga toxin (O'Brien *et al.*, 1984) have also been demonstrated from some strains. Interestingly, Shimada and Sakazaki (1988) isolated a serogroup of non 01 *V. cholerae* possessing Inaba antigen of *V. cholerae* o1 from shrimp. However, these *Vibrios* designated *V. cholerae* did not produce cholerae toxin and did not agglutinate with monoclonal antibody for factor A of *V. cholerae* 01. Saravanan (2003) have recorded 33.33% incidence of *V. cholerae* in seafood samples from Mangalore landing centre.

### 2.7.2. *Vibrio paraheamolyticus*

Outbreaks of *V. paraheamolyticus* gastroenteritis have received considerable attention in Japan, where it is one of the most commonly occurring food-poisoning syndromes. The pathogenicity is associated with a thermostable direct hemolysin (TDH) and strains producing this hemolysin are referred as Kangava positive. In recent years, this organism has been responsible for several food borne outbreaks in other parts of the world (Joseph *et al.*, 1982). Infective does is $>10^5$ (Kanagawa positive strains). Symptoms are abdominal pain, vomiting, diarrhoea and fever. Incubation period is 2-48 hours. Presence of *Vibrios* have been observed in various samples such as sea and brackish water, sediments and seafood organisms such as fish, shellfish, as well as from culture ponds (Chatterjee and Sen, 1974; Karunasagar *et al.*, 1984, 1986). *V. paraheamolyticus* is most commonly an inhabitant of estuaries and is infrequently found in freshwater or full strength seawater. The incidence of *V. paraheamolyticus* in seawater decreased with depth. Baross and Liston (1970). Schwartz and Colwell (1974) reported that the organism could not grow in any hydrostatic pressure seen in the deep oceans.

*V. paraheamolyticus* is gram-negative, straight or curved motile rod shaped bacterium. It will grow within a temperature range typical of mesophiles, with a minimum growth between 9-10 °C, a maximum growth temperature of approximately 44 °C and an optimum between 35 and 37 °C (Beuchat, 1973; Johnson and Liston, 1973; Jackson, 1974). The pH range of growth of the organism is from pH 5 to 11 with an optimum between pH 7.5 to 8.0 (Beuchat, 1973; Lee, 1972). *V. paraheamolyticus* is a moderately halophile. Growth occurs at NaCl concentrations of 0.12 % to approximately 1.2% with an optimum of 0.5% NaCl (Morishita and Takada., 1976; Gray and Muir., 1977). The organisms can be readily killed by proper cooking of the food. Both raw and cooked
seafoods, for example crab, lobster, shrimp and prawns are Vehicles of infection. Cooked foods may be contaminated by the raw products in kitchen. It is a facultative anaerobe, possessing both respiratory and fermentative metabolism. It produces catalase and cytochrome oxidase. It is sensitive to the vibriostatic agent 0/129 (2, 4 diamino-6, 7-disopropyl pteridine) and in general sensitive to chloramphenicol, gentamycin, kanamycin, colistin and penicillin. (Bonang et al., 1974; Joseph et al., 1978). ICMSF has recommended an acceptability of $10^2$ g$^{-1}$ in frozen shrimp and lobster tails, frozen cooked shrimp and lobster tails, frozen raw and breaded shrimp and cooked picked crab meat. Karunasagar et al., (1984) showed that *V. parahaemolyticus* could be isolated from frozen tropical shellfish.

2.7.3. **Salmonella spp.**

*Salmonella* spp. is one of the most important causative agents of the food borne diseases throughout the world (Lacey, 1993). *Salmonella* are Gram-negative, rod-shaped bacterium, mostly motile and do not form spores. Primary habitat of *Salmonella* is the gut of warm blooded animals, birds, insects, lizards, rodents etc. They reach food directly or indirectly from animal etc. or water polluted by sewage, in the kitchen they may be transferred from raw or cooked foods by hands, surfaces, utensils and other equipments. Illness is more likely to occur when the organisms are ingested in large numbers ($10^4$ to $10^7$ cells), a chance contamination of food by a small number of cells may not be harmful, but if they are kept at room temperatures for some hours they will multiply in the food to produce symptoms when consumed. It is well established that newborns, infants, the elderly and immuno compromised individuals are more susceptible to *Salmonella* infection than healthy adults.

The onset of illness occurs usually within six to thirty six hours of eating the contaminated food. The symptoms are characterized by fever, headache and general aching of the limbs, as well as by diarrhoea predominantly and vomiting. The duration of illness is from one to seven days or longer. Complete recovery is possible only in about 20 days. At this stage, generally organisms disappear from the intestinal tract of most of the patients, upto 5% of the victims may become carriers of the organism for about 3 to 4 months. In fact "Carriers" are known to be the main source of *Salmonella* contamination in many types of food materials.

Food poisoning caused by *Salmonella* is known as "**Salmonellosis**". Fresh fish collected from the open sea is free from this organism. However, fish from polluted coastal waters are usually contaminated with *Salmonella*. There are reports on the incidence of *Salmonella* in freshwater fishes collected from different parts of the world. Salmonellosis due to consumption of fish cakes, raw oyster, canned salmon, smoked white fish and whale blubber has also been reported. Quality standards specify that the
organism should be totally absent in seafoods. More than 2000 serotypes of *Salmonella* are known to exist. The presence of any serotypes of *Salmonella* in a food material has to be regarded as a potential hazard. Sanathkumar (2001) has isolated *Salmonella* spp. in fish and shellfish samples collected from different places around Mangalore.

### 2.7.4. Handling and processing of octopus

In Spain, Portugal and Italy, after washing with potable water, octopus is held in such a way that the inner part is exposed to the surface. With the right hand, the viscera are torn out with a downward movement. The operation is continued until all the viscera are extracted. Finally the viscera are cut away at the throat. At the end of the operation, the beak is removed, but the eyes are usually left. The body is thoroughly cleaned of all the remaining viscera, then the octopus is thoroughly washed (Schwartz, 1973). This is used for further processing. For canning, tentacles are cut off from the head and treated with 1% salt and steamed in a cooker at 104-105 °C for 55 min. for larger tentacles and about 45 min. for medium sized ones. After steaming, the skin is removed from the tentacles by tearing off by hand, starting from the thickest part and proceeding downward to the tip of the tentacles. This operation is carried out while the tentacles are still hot, otherwise the meat is liable to damage and skinning will take almost three times longer. Head/mantle is used without skinning but it is cooked in 1% salt solution before use. The skin is considered to give a characteristic aroma to the product (Kreuzer, 1984). In Spain, octopus frozen at sea is mainly exported to Japan. Octopus is prepared on shore; the tentacles are tenderized by beating and then packed into cardboard boxes with the tentacles neatly arranged on top of the octopus. These boxes are frozen in an air blast freezer, at a temperature of -35 °C for 5-7 hours to a core temperature of at least -18°C (Schwartz, 1973).

The dressing yield varies according to species, size, sexual maturity, feeding stage, processing methods etc. Mantle and tentacles are separated and used for making small fillets / pieces. Generally the dressed yield of cephalopods varies from 45-75% of the total weight. Different scientists have observed varying % of dressed yield of cephalopods. Suryanarayan *et al.*, (1973) have reported an edible content of cephalopods ranging from 68-70%. Joseph and Perigreen (1988) have observed 35% yield of fillets from cuttlefish. Nagaraj (1994) has observed 45.08% of yield from mantle portion of squid and 50-56% yield was also recorded by Nagesha (2002) in squid.

### 2.7.5. Proximate composition

Cephalopods are group of species with an exceptionally high percentage of edible content, which is primarily protein and accounts for between 60 and 80% of the total weight, depending on the species, size of the specimen and sexual maturity (Sikorski and Kolodziejska, 1986). Proximate composition typically comprises water,
protein, fat, and ash, expressed as percentage of the weight. Proximate composition of the different fish species will show variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. Pandit and Magar (1972) found 79.73 percent moisture, 0.81%, 16.52% and 1.49% fat, protein, and ash respectively in *Loligo vulgaris*. Suryanarayan *et al*., (1973), Joseph *et al*., (1977) and Sarvaiya (1977) have analysed the proximate composition of different species of squid. Shchenikova *et al*., (1987) observed lipids of *Todarodes pacificus*, *Berryteuthis magester*, *Ommastrephes bartrami* and *Sepia officinalis* were ranged from 0.5-2.6%. Saturated fatty acids such as $C_{16:0}$, $C_{14:0}$ and $C_{12:0}$ were found to be predominant. According to Dunstan *et al*., (1988) flesh of cephalopods contained high levels of eicosapentaenoic acid than the fish. Joseph and Perigreen (1988) have noticed an average moisture content of 76.85% and fat 0.82% in frozen stored cuttlefish (*Sepia aculeata*) fillets for 16 months. Dumont *et al*., (1992) have observed preponderance of the polyunsaturated fatty acids, especially of the (n-3) family in *Sepia officinalis*.

Chu *et al*., (1992) have observed higher moisture content in mantle and lower myofibrillar proteins and NPN in octopus than other squid species. The differences in proximate composition among these squid mantles were non significant. Nagaraj (1994) found 80.68% moisture, 17.65% protein, 1.24% fat and 1.36% ash in squid (*Loligo duvauceli*). Moustafa *et al*., (1996) have noticed small changes in the proximate composition of the hot smoked and frozen stored squid (*Loligo vulgaris*) and cuttlefish (*Sepia officinalis*) mantles and tentacles for 6 months and the products were found good for consumption throughout the storage period. The cuttlefish lipids were characterized by the highest level of mono-unsaturated fatty acids. Ruiz-Capillas *et al*., (2003) have noticed proximate and amino acid composition of valador, pota and octopus. In case of octopus, moisture contents were 81.19 and 80.21% in immature, 80.04 and 78.54% in mature mantle and arms respectively; lipid contents were 1.24 and 1.14% in immature, 1.13 and 0.98% in mature mantle and arms respectively; protein content were 15.67 and 16.64% in immature, 15.85 and 17.89% in mature mantle and arms respectively. The analysis of amino acid content of mantle and tentacles of octopus indicated that highest proportion of amino acids are glutamic acid, aspartic acid, lysine, leucine, and arginine, which together amount to slightly more than 50% of the total amino acids.

**2.7.6. Chilling of sea foods with ice**

Fish and shellfish are among the most perishable of foods. Spoilage begins immediately after death, can be retarded by reducing the temperature of the fish by either chilling or freezing as soon as possible after capture. A simple and effective method is to use ice. More rapid cooling can be achieved by using slush ice or binary ice initially and allowed to drain so that the fish to ice ratio is about 1:1, fish will cool from 10
°C to 0 °C in about 1½ hours. Alternatively, chilled water stowage either in tanks of chilled sea water (CSW) using ice for chilling or in mechanically refrigerated sea water (RSW) can be used particularly for marine pelagic species. The temperature of sea water can be lowered to about -1 °C without freezing the fish and the catch can be cooled more rapidly in chilled or refrigerated sea water than stowage in ice. Under optimal conditions, the refrigeration capacity of an RSW system should be sufficient to cool the fish to 0 °C within a few hours. At temperatures less than -1 °C, the fish begins to freeze and reducing the temperature to the range -1.5 °C to -3 °C is known as superchilling or partial freezing. Cooling fish or fish products to a temperature just above and as close to 0 °C as possible by forced circulation of cold air over the fish is known as air blast chilling.

Ice is available in different shapes; the most commonly used in fish chilling are flake, plate, tube and block. Block ice is ground before being utilized to chill fish. Under tropical conditions ice starts to melt very quickly. Part of the melted water drains away but part is retained on the ice surface. The larger the ice surface per unit of weight, the larger is the amount of water retained on the ice surface. Generally flake ice will allow for an easier, more uniform and gentle distribution of ice around fish and in the box or container and will produce very little or no mechanical damage to fish and will chill fish rather more quickly than the other types of ice. On the other hand, flake ice will tend to occupy more volume of the box or container for the same cooling capacity and if wet, its cooling capacity will be reduced more than the other types of ice (since it has a higher area per unit of weight). Large and sharp pieces of crushed ice can damage fish physically. However, crushed ice usually contains fines that melt quickly on the fish surface and large pieces of ice that tend to last longer and compensate for thermal losses. Block ice requires less stowage volume for transport, melts slowly, and contains less water at the time of crushing than flake or plate ice. Probably tube ice and crushed ice are more suitable for use in CSW systems if ice is wet, since they will contain less water on their surfaces. Cooling rates depend mainly on the surface per unit of weight of fish exposed to ice or chilled ice / water slurry.

Generally, both enzymatic and microbiological activities are greatly influenced by temperature. However, in the temperature range from 0 to 25 °C, microbiological activity is relatively more important and temperature changes have greater impact on microbiological growth than on enzymatic activity. Many bacteria are unable to grow at temperatures below 10 °C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0 °C. At 0 °C the growth rate is less than one-tenth of the rate at the optimum growth temperature. Microbial activity is responsible for spoilage of most fresh fish products. The shelf life of
fish products, therefore, is markedly extended when products are stored at low temperatures. While broad differences are observed in shelf lives of the various seafood products (Cann et al., 1984; Cann et al., 1985).

2.7.7. Icing of cephalopods

Icing of cephalopods is normally practiced on board the fishing vessels to extend the shelf life. At 0 °C, the decrease in ATP content of squid mantle muscle was noticed and well correlated with a loss of translucency or increase in the turbidity and development of dark color on the surface skin of mantle (Kinoshita et al., 2003). Independent of the sexual maturation stage of squids, as compared with whole squid mantles, isolated mantles had a lower content of AMP and IMP and a higher content of Hx in 6 days of ice storage (Sagedhal et al., 1997). Luong et al., (1992) have assessed the fish freshness of cod, salmon and trout stored in ice (0-4 °C) and at 20 °C using capillary electrophoresis and an immobilized enzyme procedures to monitor degradation of inosine-5'-monophosphate (IMP) to inosine (HxR) and hypoxanthine (Hx). The two procedures agreed and for all species, H ratio and K values increased with storage time. Application of high pressure was found effective in reducing the autolysis of myofibrillar proteins and the microbial load in the octopus muscle (Hurtado et al., 2002). The proteolytic activity of octopus arm muscle exhibited optimum activity at 40 °C and 60 °C, at optimum pH 2.5 and 4.0, respectively. Hurtado et al., (2001) have noticed 43 d longer shelf life with reduced autolytic activity and WHC in pressurized octopus muscle when stored at chilled condition. Studies on different methods of icing showed that the packaging of the squids resulted in more intense changes in the characteristic colour of the muscle and did not promote a significant reduction in microbial growth. The non-contact ice storage method presented no advantages when compared with the contact ice storage method with respect to the quality preservation of L. plei (Lapa-Guimeranes, 2002). Similar type of results were obtained by Prafulla et al., (2000) in squid (Loligo duvauceli) and cuttlefish (Sepia pharaonis) stored under direct icing, indirect icing and in a mixture of ice and 3% salt conditions for 12 days, the indirect icing did not increase the shelf lives while chilling in a mixture of salt and ice gave a product of better quality. According to Joseph and Sherief (2003), treatment with the salt and citric acid was found to improve the appearance and overall quality of cuttlefish fillets stored in ice. Some studies pertaining to the ice-storage characteristics of squid and cuttlefish have been made (Joseph and Perigreen, 1988; Bykovski et al., 1990; Lakshmanan et al., 1998; Joseph and Sherief, 2003), but studies on chilled storage of octopus are very scanty.

2.7.8. Quality changes during ice storage

Spoilage caused by autolysis of the muscle is intensive in cephalopods because of the high level of proteolytic activity in the muscle produced by their active metabolism
(Stanley and Hultin, 1984), and this favours rapid microbial growth. As regards to this factor, the spoilage of cephalopods in chilled storage is governed chiefly by gram negative bacteria (Paarup and Moral, 1996). Whereas in vacuum packed samples, there are qualitative and quantitative changes in the growth of the microbial flora as aerobic organisms are inhibited (Barhs, 1985) and spoilage is largely dominated by gram positive bacteria (Stammen et al., 1990). Among Gram-positive bacteria lactic acid bacteria are favored, to the extent that they can become the dominant flora (Lerol et al., 1996).

Growth of this kind of bacteria could improve bio preservation of the product (Helander et al., 1997). Application of high pressure up to 400 MPa was effective in reducing proteolytic activity and microbial load and prolonging the shelf life of octopus in chilled storage (Hurtado, 2001), and changed microorganisms to Gram-positive bacteria as the dominant flora. The extension of shelf life is mainly due to substantial reduction of total microbial load (Hurtado et al., 2002). Thus, quick chilling or freezing of fresh octopus and storage at lower temperatures are important to arrest the microbial activity, to reduce proteolytic activity and undesirable changes in quality. Suvanich et al., (2000) have observed increase in TVBN of cat fish washed mince during refrigerated storage.

Sas astry (1981) has observed gradual increase in moisture, VBN, tri-methyl amine nitrogen, mesophiles and psychrophiles and decrease in water soluble nitrogen, salt soluble nitrogen, non protein nitrogen and total nitrogen during storage of cuttlefish in ice. Similar trend was also observed by Dhananjaya (2000) in green mussel stored in ice and Sunilkumar (2002) in smoked clam stored under refrigerated temperature. Hurtado et al., (2001) have observed total viable bacterial count of 4 log unit in octopus which increased to 6 log units at the end of 20 days of storage at chilled condition.

Microbiology of iced fish has been studied by a number of workers. Hobbs (1983) suggested that most of the bacteria which grow in iced fish have optimum growth at 18 °C -20°C. Gram-positive bacteria such as Coryneforms and Micrococi grow well at this temperature. The total number of bacteria growing at 0 °C -20°C increase systematically and gram negative bacteria such as Pseudomonas, Moraxella, Alteromonas and Acinetobacter comprise most of the flora and at the end of shelf life Pseudomonas assume a dominant position. (Shewan, 1949 and 1977; Hobbs, 1983). Liston (1980) has suggested that some Pseudomonas have very short generation times at 0 to 5 °C and are capable of utilizing NPN compounds much more rapidly as compared to other psychrotrophic bacteria. Bacteria have been shown to produce tri-methyl amine nitrogen from TMAO, H2S, dimethyl sulphide and methyl mercaptan from sulphur containing amino acids, various amines and ammonia from amino acids. The importance of TMA in fish spoilage is stressed by Ester et al., (1982) that many spoilage bacteria are able to utilize TMAO as terminal hydrogen acceptor enabling these non fermentative bacteria to grow
rapidly under micro-aerophilic or anaerobic conditions resulting in fish tissues undergoing spoilage. TMA formation in squids stored at chilled temperatures was investigated by Prafulla et al., (2000), Nagaraj (1994) and Sastry (1981), Joseph and Sherief (2003) in cuttlefishes. Hurtado et al., (2001), have noticed higher (25 mg / 100 g octopus muscle) TMAN on 19th day of storage at chilled condition. Disney et al., (1984) have pointed out that fish from tropical waters might have a longer storage life in ice compared to fish from cold waters. This could be because of psychrophiles which are involved in spoilage form an insignificant part of the flora of tropical and subtropical fish.

2.8. Freezing

Freezing is the process of reducing the temperature of fish in a freezer to a point at which more than 90% of the water in it has solidified. The best method of preserving of seafood is freezing and storing at low temperatures. If properly frozen, seafood retains quality and flavour. Quickness of freezing determines the quality after thawing (Pigott and Tucker, 1990). The recommended temperature to which fish should be reduced during freezing is -30 °C, at which temperature the fish should be stored. The freezing process itself has no effect on the taste or nutritional value of the food and ideally after thawing there should be no distinguishable differences between the frozen and fresh fish. Freezing is advantageous during periods of glut and scarcity. The fish may be frozen when plentiful and distributed throughout the periods of glut and scarcity. Freezing also makes it possible to move large quantities of seafoods over the long distance.

The seafood processing industry in India at present is largely export oriented and dominated by the freezing sector having major share in earning a total export value of Rs.6,646.49 crores in 2004-05. The frozen shrimp constitutes the single major item in terms of value; cephalopods are ranking next and other major items to fin fish and shellfishes for export (MPEDA, 1998). Extensive work on the influence of freezing on quality of seafoods indicates that the rate of freezing is critical. Love (1958) has carried out extensive work on freezing of animal tissue and its effects on eating quality. Further he noticed that fast freezing rate do not allow moisture migration into the extra cellular spacing with the result small ice crystals are formed. On the other hand, slower freezing rate induces high salt concentration in the extra cellular fluid by drawing out moisture from inside the cells by osmosis. Leljimank (1969) has given an account on the influence of freezing on cohesiveness of the muscle structure. FAO (1977) recommended the freezing rates in various freezing methods which can be defined in terms of the thickness of flesh frozen per unit time. Many studies indicate that freezing rate may influence quality deterioration during subsequent storage. Lee (1982) observed that freezing in liquid nitrogen resulted in less drip and muscle toughening is less than conventional freezing after storage at -20 °C for 2 months. Storage temperature had a much larger
influences on deterioration process than freezing rate (Reid et al., 1986). The freezing point of fish muscle depends on the concentration of different solutes in the tissue fluids: for cod and haddock, it is in the range of -0.8 to -1 °C, for halibut -1 to -1.2 °C, and for herring about -1.4 °C.

2.8.1. Quality changes during frozen storage

Once most of the water inside fish flesh is frozen to around -25 °C the bacterial action becomes dormant and most enzymic activity ceases; protein denaturation, oxidation of fats and colour changes in the flesh are reduced. Oily fish have relatively short frozen storage life than lean fish. Dehydration may occur if the products are not properly glazed, packaged or stored. Deep dehydration adversely affects the appearance and surface texture of the product and is commonly known as "freezer burn". Upon thawing, there is a loss of fluid from the flesh of any fish product, which is explained by the denaturation of protein during the freezing and frozen storage, which causes the protein to reduce its water-binding capacity. Ice crystal formation and lipid oxidation products are the major factors that cause protein denaturation in lean frozen fish, protein denaturation and texture changes are minimized by cryoprotectants, as well as antioxidants or a mixture of antioxidants and cryoprotectants. Lee et al., (1999) have noticed more rapid denaturation of actomyosin from the obliquely striated mantle muscle of squids (Todarodes pacificus) at -11 °C than at -5 °C. To reduce freeze-induced protein denaturation, phosphate, sugar, and sorbitol have been used in surimi as cryoprotectant.

Denaturation is the phenomenon of an alteration in the tertiary structure of a protein. Covalent bonds such as disulphide bonds might be broken during denaturation, but there is no breaking the polypeptide chain of the molecule. Denaturation almost invariably results in loss of biological activity of the protein, such as enzymic activity, solubility in water or dilutes salt solutions etc. Susceptibility to denaturation differs among proteins, and can be brought about by a variety of treatments, chemical and physical. The processes most frequently encountered in fish technology are heating - cooking, pasteurizing, retorting, freezing and treatment with acids. The main muscle proteins in fish, the actomyosin complex, become more difficult to solubilise in dilute salt solution as storage proceeds, and this process is often referred to as ‘denaturation’ in descriptions of changes occurring during frozen storage of fish and fishery products. However, it is not clear that denaturation of the proteins has occurred; other mechanisms such as cross-linking of actin and myosin molecules, with themselves or other proteins, or with structural elements of the muscle fibre could lead to loss of solubility with unfolding of tertiary structure.

The amount of protein denaturation depends on the concentration of enzymes and temperature (Garthwaite, 1992). Drip loss, or the release at water during thawing,
implies nutrient loss. Little drip loss occurs when the products are frozen quickly and stored properly, if not, excessive drip loss can occur and render the products unfit for consumption (Graham, 1982). It has been reported that the usage of polyphosphate dips increases water holding capacity of flesh and reduces drip and deterioration of the quality (Pigott and Tucker, 1990).

Gomez-Guillen et al., (2003) have observed significant drop in protein functionality that negatively affected the thermal gelation profile of squid (Loligo vulgaris) mantles that underwent chilled storage. The rate of proteolysis remained very high throughout frozen storage; however functional properties and thermal behavior remained very stable. Suwanich et al., (2000) have observed increase in TVBN, decrease in SSP and not much change in moisture content and pH of channel cat fish washed mince treated with cryoprotectant and it was good in 3 months of frozen storage. Joseph and Perigreen (1988) have observed decrease in non protein nitrogen and AAN in frozen stored cuttlefish (Sepia aculeata) fillets. They have also observed that the colour of fillets initially, were white and showed signs of desiccation by 4 months storage which increased on further storage and the fillets finally became dull white with yellow discoulouration. The firm and chewy texture of the cooked fillets changed to rubbery even though the product was slightly sweet at the end of the storage period of 16 months.

Sastry (1981) also observed decrease in moisture, total nitrogen, salt soluble nitrogen, non protein nitrogen, and increase in VBN, TMA, PV, and free fatty acids values of phosphate, NaCl treated and control cuttlefish during frozen storage. The changes were more prominent in control samples than the treated samples. Similar results were also obtained by Nagaraj (1994) in cryoprotectants treated and frozen stored squid stored for 105 days and Nagesha (2002) in ready to eat squid products frozen stored for 21 weeks. Moral et al., (2002) studied the protein solubility of octopus for 12 months of frozen storage in 5% NaCl and found that it remained high over 60%. The solubility of mantle muscle was more in younger specimens than mature ones. The mantle muscles of immature specimens of octopus were suitable for freezing. Ruiz-Capillas et al., (2003) have observed greater levels of apparent viscosity and emulsifying capacity of octopus during frozen storage. Mantle muscles showed greater stability than the arms, mantle muscles of younger octopus showed more stability than matured specimens.

2.8.2. Prevention of protein deterioration using cryoprotectants / chemicals

Cryoprotectant is a substance permitted to be added to products of fish muscle before freezing to prevent reactions in the products during frozen storage that result in impaired functional properties of the muscle proteins. The cryoprotectants most frequently used in fish processing are sugars and polyalcohols, (sometimes abbreviated
to polyols), i.e., compounds having many hydroxy groups in the molecule such as sucrose and sorbitol (Suzuki, 1981). About 8% of cryoprotectants, singly or in combination, are routinely added to surimi before making into blocks for freezing (Park et al., 1987; Park, 1994). Their action seems to be a combination of water holding to prevent migration of water (Matsumoto, 1980). Noguchi (1974) screened about 150 compounds for cryoprotective effects on carp actomyosin in-vitro model systems. About 30 compounds are found to be markedly effective. The effective compounds were amino acids, di-carboxylic acids, poly alcohols and polyphosphates etc. He concluded that cryoprotectants will have the following structure:  (1) a molecule with one essential group either -OH, -COOH or -OPO$_3$H$_2$; (2) a functional group with suitable space and properly oriented (3) molecule with comparatively small size. Such cryoprotectants act by association with protein via, ionic or hydrogen bonding thus increasing electrostatic repulsion.

2.8.2.1. Sodium tri-polyphosphate (STPP)

Sodium Tripolyphosphate is a white granules or powder which is odorless. It quickly dissolves in chilled water, significantly increases yield and water binding during processing and freezing. STPP prevents drip and moisture loss during frozen storage, stabilizes color, taste and appearance. It Increases tenderness and flavor during storage, contributes to significant binding and homogeneity giving a good spread of fat and water. It reduces nutritional loss and microbial growth during frozen storage. Polyphosphate treatment of fish before freezing often reduces the amount of thaw drip. Good quality fish with properly frozen and cold stored, normally develops little thaw drip; therefore, application of polyphosphate to such material is only of limited value. The poor quality fish may drip much more after freezing and thawing stages and treatment will reduce the loss to some extent.

Polyphosphates are legally permitted additives that are widely used to facilitate processing or to improve the quality of many foods (Brotsky, 1980), particularly that of meat and fish products. The main function of polyphosphates lies in increasing the water-retaining capacity of protein in fish. The phosphates used in foods may be simple phosphates, containing one phosphate unit; pyrophosphates, containing two phosphate units; tripolyphosphates, containing three units; or polyphosphates, containing more than three phosphate units (Aitken, 1975). Monophosphates and polyphosphates are used alone or in combination with salt in meat and fish processing (Antoine et al., 2000). Sugars synergistically enhance the effect of phosphates particularly under alkaline conditions (Sorenson, 1980).

In Japan sugar, sorbitol or dextrose are used to protect the protein during freezing and storage (Noguchi, 1974). Addition of 3% sucrose and 0.2% polyphosphates
to the mince of Lizard fish was found useful to reduce denaturation of proteins during freezing (Yasui et al., 1987) and further storage. Different scientists Dawood et al., (1983), Krivchenia and Fennema (1988a), Sastry (1981) and Park et al., (1987) have employed sodium tripolyphosphate as a cryoprotectant in single or in combination with other chemicals to reduce the freeze denaturation of protein in fish or fish mince during freezing and frozen storage. Joseph et al., (1977) and Selvaraj et al., (1992) reported that favorable results were obtained when squid was treated with polyphosphate. Nielsen and Pigoit (1994) noticed gel strength of commercial surimi was increased by addition of phosphate blends. Hulya et al., (2003) have noticed that neither phosphate usage nor glazing treatment was effective to prevent drip loss in the frozen rainbow trout. However, the combination of glazing and packaging did prevent drip loss and protect the moisture content of the inner and surface layers of the product.

Suzuki (1981) reported that the addition of 10% sucrose alone to surimi prevented denaturation of minced meat, but the effect was much stronger when used with polyphosphate. Care must be taken because it might make the taste too sweet or turn the finished product a brownish color. Matsumoto et al., (1985) reported that at around pH 7.5 the protective effect of sugar was enhanced on the denaturation of myofibrillar protein during frozen storage. Phosphate raises the muscle pH and chelates metal ions in muscle. Phosphate functionality often depends on its biochemical properties. However, effects of various blends of phosphates on the biochemical properties of fish protein have seldom been investigated.

Commercially polyphosphates are used at 10% concentration (Graham, 1982). However, when used with salt concentration is reduced to 3-8% phosphate + salt (Sutton, 1969). Phosphates stabilize proteins against denaturation, increase water binding capacity, improve emulsification and buffering capacity (acid base relationships), contribute nutrients, chelate metal ions and function as antioxidants to a variety of foods. Protein reactions and water binding are perhaps the most important reasons for the usage of phosphates in seafoods. Phosphates are effective on fish and shellfish in preventing drip loss when frozen products are thawed and in enhancing tenderness by restricting protein denaturation during freezing and frozen storage (Reddy and Finne, 1986). Hydrolysis of polyphosphates either enzymatic or non enzymatically occurs during fresh and frozen storage with orthophosphate, which cannot be differentiated from the naturally occurring phosphate (Storno et al., 1987).

2.8.2.2. Sodium citrate (SC)

Sodium citrate is used as sweetener in chewing gum and in low calorie foods. Sodium citrate inhibits gram-positive bacteria like Enterococci. Sammel and Claus (2003) reported that sodium citrate consistently reduced natural or induced pink color in ground
turkey rolls but had no effect on pink color of intact turkey breasts, no reduction in pH and cooking yields. Weilmeier and Regenstein (2004) have noticed reduction in formation of thiobarbituric acid-reactive substances in brook trout muscle treated with phosphates and other antioxidants. When copper was added to accelerate oxidation, only EDTA completely prevented TBARS formation. Sodium citrate was more concentration-dependent and slightly less efficient in prevention of TBARS formation than STPP. Hoke et al., (2000) have observed Sodium citrate, sodium erythorbate, sodium citrate plus sodium erythorbate, sodium citrate plus sodium erythorbate, and polyphosphates used in washing of channel cat fish mince reduced the formation of Thiobarbituric reactive substances and FFA changes during frozen storage. Addition of antioxidants did not significantly improve the overall quality and shelf-life of the frozen mince.

2.8.2.3. Ascorbic acid (Vitamin C)

Vit-C is a naturally occurring vitamin found in plant products, but found in trace amounts in fish muscle. Ascorbic acid is widely known as an antiscorbutic factor. Apart from its therapeutic property on account of its easily oxidisable nature, it is a strong reducing agent. This property has brought its importance in food industry to use as antioxidant. In fish it has also been used to retard the development of rancidity. Ascorbic acid and its salts are virtually insoluble in fat and oils. Being water soluble they are used as antioxidant. Deng et al., (1978) tested the effectiveness of ascorbic acid as antioxidants on ground mullet fillets. Richard and Stephen (1986) used ascorbic acid to prevent rancidity in frozen cooked mussel Mytilus edulis. Ascorbic acid and sodium metabisulphite are used to control black-spot formation in shrimps (Annon, 2001). Ascorbic acid is also used to control the drip in frozen fishery products. Selvaraj et al., (1991) have found an improvement in quality of frozen squid (L. duvauceli) pretreated with 0.5% ascorbic acid.

2.8.2.4. Sodium chloride

It is used in food for various purposes like preservative, to give taste to the product, as pretreatment in smoking process. The preservative effect is due to its bacteriostatic and bactericidal effect at various concentrations. Brine at low concentrations is used to get firmness and shiny appearance at the surface of the fish.

2.8.2.5. Hydrogen peroxide

Hydrogen peroxide is not a cryoprotective agent. It is odorless and colorless, but not tasteless liquid. When stored under the proper conditions, it is a very stable compound. When kept in the absence of light and contaminants, it dismutates (breaks down) very slowly at the rate of about 10% a year. This can be slowed even further by storing the liquid in the freezer. It boils at 152 °C and freezes at -2 °C. Hydrogen peroxide
is a very strong oxidizer and if not diluted, it can be extremely dangerous or even fatal. Most harmful bacteria (and cancer cells) are anaerobic and cannot survive in the presence of oxygen or $\text{H}_2\text{O}_2$. Hydrogen peroxide (300 or 600 ppm) treatment followed by refrigerated storage may provide an alternative to thermal pasteurization to meet the 5-log reduction standard in cider and orange juice (Williams et al., 2005). 1% Hydrogen peroxide wash at 20 or 40 °C for 15 or 30 min. used to decontaminate apples and Cantaloupe melons showed useful in achieving reduction of 3 log counts of $E. \text{coli}$. (Sapers and Sites, 2003). Kim et al., (1996) reported that treatment with hydrogen peroxide was found to be useful in reducing the TBARS, slow down the odour spoilage and improving color of Channel Catfish. Fresh mushrooms treated with 5% $\text{H}_2\text{O}_2$, followed by application of sodium erythorbate-based browning inhibitor was found useful in colour improvement and reduction of the enzymatic browning during storage at 4 °C and at 10 °C (Sapers et al., 2001). However, this is used to improve the flesh colour and appearance of fish and fishery products.

2.8.3. Effect of cryoprotectants on various biochemical parameters

Treatment of fish with cryoprotective agents will certainly have an effect on biochemical parameters. Changes in pH of the treated muscle were noticed by several workers. Cormier and Leger (1987) reported that polyphosphate treated fillets had higher pH, on the other hand a decrease in pH in mackerel and Hoki ($\text{Macruronus novazelandiae}$) during frozen storage has been reported by Abdullah and Yu (1985) and Mac Donald et al., (1992). Selvaraj et al., (1992) have observed the effect of STPP on frozen squid mantles and they found decrease in pH throughout the storage. Chun et al., (1991) reported that pH of the sample showed substantially higher value in the phosphate treated muscle than the control, and decreased after 3 months storage in all the white fish and burbot samples. Sastry (1981) and Joseph et al., (1977) have observed the effect of polyphosphate on $\text{Sepia aculeata}$ and squid respectively and found decrease in the moisture of treated samples. Frozen stored squid treated with STPP showed decrease in moisture content throughout storage (Selvaraj, 1986).

Joseph et al., (1977) have observed decrease in total nitrogen in all the treated squid samples. With increase in duration of frozen storage there was a sharp decrease in the salt soluble nitrogen. Sastry (1981) noticed small decrease in total nitrogen in polyphosphate treated sample as compared to control, and among the treatments the protein extractability was found slightly better in polyphosphate treated sample than the NaCl treated sample. Park and Lanier (1987) reported that the freeze-induced aggregation was reduced effectively by combination of phosphates and carbohydrates, and less effectively by phosphates or carbohydrates. Selvaraj (1986) noticed reduction in crude protein and SSN content of frozen stored squid mantles treated with STPP, but
reduction in treated sample was less compared to control. Dora (1992) observed a steady decrease in total protein and decrease in WSP content and gradual fall in SSP in both croaker and pink perch mince treated with STPP and mixture of sucrose and sorbitol. According to Lanier and Mc Donald (1992) the myofibrillar proteins of most fish are known to be liable for denaturation, for this reason the inclusion of a cryoprotective component to surimi prior to freezing is necessary to ensure long term stability of the proteins in frozen storage.

There was no significant difference in the fat content of control as well as polyphosphate treated samples of cuttlefish (Sastry, 1981) during frozen storage. Increase in PV and FFA was more in control than either polyphosphate or NaCl treated cuttlefish. STPP treated and frozen stored squid showed increase in PV throughout storage (Selvaraj et al., 1992). Dora (1992) noticed a slight fluctuation in lipid content and increase in PV and FFA of pink perch and croaker treated with STPP and mixture of sucrose and sorbitol and stored at -20 °C.

Sastry (1981) reported decrease in NPN and increase in TVB-N of control as well as polyphosphate and NaCl treated cuttlefish during frozen storage. Similar results were also recorded by Joseph et al., (1977) in squid meat. Selvaraj et al., (1992) observed increase in TVB-N and decrease in NPN of squid treated with STPP during frozen storage. Dora (1992) observed the NPN content of pink perch and croaker mince showed a decreasing trend in both STP and the mixture of sucrose and sorbitol treatments during frozen storage, whereas VBN showed increasing trend in both species, STP treatment was found to be advantageous over mixture of sucrose and sorbitol. Considerably low volume of drip in treated sample of squid was noticed by Joseph et al., (1977). Among the treated samples rate of increase of drip during storage was more in salt treated sample than the polyphosphate treated sample. Sastry (1981) has observed less drip in polyphosphate treated sample of cuttlefish, whereas Devriendt (1979) is of the opinion that polyphosphate had no practical value in the reduction of drip loss in fresh cod fillets. Selvaraj et al., (1992) have observed decreased thaw drip and cooked drip throughout the frozen storage in STPP treated and frozen squid than the control. Croaker and pink perch minced meat treated with sodium tri-polyphosphate and the mixture of sucrose and sorbitol showed increased drip loss and cook loss and gradual increase in thaw drip during frozen storage, but sodium tri-polyphosphate treated sample showed comparatively less cook loss and thaw drip (Dora,1992).

Sastry (1981) has observed rapid decrease in mesophiles and psychrotrophic counts in NaCl treated cuttlefish and only mesophiles in case of control and polyphosphate treated samples during first month of frozen storage at -20 °C. Low counts of psychrotrophs were observed in case of control and polyphosphate treated
sample during storage. Devriendt (1979) has noticed no difference in keeping quality and bacteriological safety of the fillets dipped in a solution of polyphosphates and control.

Lee et al., (1988) pointed out the bacteriostatic effect of condensed phosphate on the growth of bacteria in various meat products. A bactericidal property of tripolyphosphate was also noticed by Basu (1990). Selvaraj (1986) has observed reduction in TPC in first three months of frozen storage in STPP treated squid samples and at later stages there was increasing trend throughout storage period. Dora (1992) has accounted that both pink perch and croaker minced meat treated with STPP and the mixture of sucrose and sorbitol showed decrease in TPC compared to control at the end of frozen storage.

2.8.4. Lipid changes during frozen storage and its control

The polyunsaturated fatty acids found in fish lipids are very susceptible to oxidation. Lipids undergo oxidation during storage; the initial step of oxidation of lipid is for oxygen to attach to unsaturated bonds in the molecule to form a hydroperoxide, which later breaks down to other products (Melton, 1983; Bligh et al., 1988). The peroxide value measures this initial formation of hydroperoxides. Its value increases then decreases as oxidation proceeds, as the hydroperoxides are first formed then decomposed. Once fat gets oxidised, it continuously combines with other compounds such as denatured protein, other proteins also with free -NH\textsubscript{2} group resulting in an irreversible change to flesh quality and giving it a fish-oil taste (harsh, somewhat bitter flavour and odour of oxidised fats) and yellowish colour. Oxidation of fats results in a variety of chemicals of different types, many of which have strong odours and flavours. These give rise to the rancid sensation. Because rancid flavours are due to mixtures of chemicals, the exact character of rancidity can differ a little among sources of the fat and the extent of oxidation. The complete elimination of rancidity development in fresh or frozen fish is not yet possible. Although rancidity in fish cannot be completely eliminated there are a number of reported steps that can be taken to minimize the rate of rancidity development. The important steps to control rancidity are;

1) **Storage temperature:** The storage temperature as low as -30 °C to -40 °C is needed in order to achieve a significant increase in storage life for most fish species (Ke et al., 1977). It is worth remembering that the rate of rancidity is higher at about -5 °C than it is at 0 °C. This is because the formation of ice crystal causes the reactant remaining in solution to become more concentrated and thus stimulate the reactions to go faster (Allen and Hamilton, 1994). Storage at lower temperature helps to extend the shelf-life.

2) **Oxygen Control:** Oxygen is required for rancidity to proceed. A number of control measures are taken to limit the level of oxygen. Glazing of fishery products will help to
avoid contact of oxygen with the muscle, Oxygen level is significantly reduced if the fish and shellfish is vacuum packed in gas impermeable packaging (Santos and Regenstein, 1990). The work of Jeong et al.,(1990) clearly indicate that the lipid deterioration of oyster were inhibited effectively by storing at -35 °C as well as by storing in enclosed deoxygenizer at -20 °C.

3) Antioxidants: They function as free radical acceptors, thus terminating the oxidation process at the initial stage. Chain breaking antioxidants used in the food industry are phenols such as Butylate hydroxyanisole, Butylate hydroxytoluence and t-butylate hydroquinone and propyl gallate. Such compounds lose their efficiency at high temperature. The t-butylate hydroquinone is the newest and very effective antioxidant. It is stable at high temperature, with fairly good carry-through properties and is effective in extending the induction period. Ke et al., (1977) found that T-BHQ is more effective than BHA and BHT in inhibiting the oxidation of skin lipid in mackerel. Chelating agents help to establish, maintain and enhance the integrity of many food products, including seafoods. Chelating agents react with metals in the food to form complexes. Citric acid, ethylene diaminotetraacetic acid, tartaric acid, polyphosphoric acids are some of the important chelating agents commonly used in seafood industry.

Thanonkaew et al., (2005) have observed lipid oxidation and yellow / brown discoloration of squid muscle during frozen storage. Non-enzymatic lipid oxidation increased with increasing temperature (4 °C to 37 °C), iron (0 to 100 μM), and ascorbic acid (0 to 200 μM) concentrations. As lipid oxidation in the microsomes or isolated microsomal lipids increased, color changes were observed. The ability of iron and ascorbate to promote both lipid oxidation and pigment formation in the microsomal fraction suggested that this pathway could be responsible for quality deterioration of squid muscle during storage. Weilmeier and Regenstein (2004) have stated that phosphates are good chelators, provided cooking has eliminated phosphatase hydrolysis in brook trout muscle.

2.8.5. Free Fatty Acids interaction

Free fatty acids are derived from enzymatic or non enzymatic hydrolysis of lipids, particularly the phospholipids, which are located primarily in the cell membrane. Both lipase and phospholipase enzymes have significant activity in producing FFA in various fish and shellfish species stored at -12 °C or -14 °C (Olley et al., 1962). Dyer and Dingle (1961) have reported that the accumulation of FFA was found to increase with prolonged storage time and at elevated frozen storage temperature. FFA is believed to attack primarily the myofibrillar proteins. Actomyosin was considered by many researchers to be the prime target and to be largely unextractable in the presence of FFA. Anderson and Ravesi (1970) have reported that a decrease in protein extractability occurred more
rapidly in the first 8-10 weeks than in subsequent storage period. Sikorski et al., (1976) have concluded that the FFA attaches themselves hydrophobically or hydrophilically to the appropriate site on protein surfaces, consequently creating more hydrophobic regions.

Trimethylamine oxide is a compound naturally present in many marine animals. The bivalves contain very small amount of TMAO. Besides fish, a few members of the invertebrates, (squid, bivalves and gastropodes) show some capacity to form dimethylamine and formaldehyde (Harada, 1975).

2.8.6. Methods of measuring protein denaturation

There are many methods of measuring the fish and shellfish protein denaturation. The methods are solubility of myofibrillar proteins, emulsifying capacity, viscosity, gel forming properties, ATPase activity etc. Some of the earliest report on relation of frozen storage and protein denaturation was made by Finn (1932). He demonstrated that protein in the pressed juice from fish meat coagulated and settled during frozen storage. Reay and Kuchel (1937) revealed that extractabilities of protein in 7% Lethium chloride decreased as a result of frozen storage of haddock at -3 °C and they also demonstrated that the myosin fraction was diminished as a result of frozen storage.

Dyer et al., (1950a) and Dyer and Morton (1956) studied the relationship between the solubility of myofibrillar protein from frozen cod and halibut and their sensory valuation. No change in solubility of sarcoplasmic protein was observed but, the amount of actomyosin extracted decreased with increase in storage periods which also correlated with low sensory changes. The loss of extractability of proteins during frozen storage is mainly related to changes in the properties of myosin, actin and actomyosin system. Pre-freezing conditions, freezing rate and storage temperature have been known to influence the solubility of myofibrillar protein.

Iguchi et al., (1981) have reported that when the mantle muscle of squid was frozen stored at -20 °C and extracted with 0.6 M KCl, the amount of soluble actomyosin extractable from the frozen meat and the ATPase activity of the actomyosin were decreased only slightly even after a long freezing period. The rate of insolublization of protein in fish muscle is also dependent on the temperature of frozen storage. Krzynowek and Wiggin (1979) have shown that frozen storage does not affect the extractable protein which remains constant throughout the period in case of blue mussel (Mytilus edulis) whereas a gradual decrease in the sarcoplasmic and myofibrillar proteins during frozen storage has been observed in fish (Dhananjaya and Hiremath, 1988), mussel (Chinnamma et al., 1970) and in clam (Mishra, 1988).

The changes that occur in muscle proteins during frozen storage can be evaluated by studying their functional properties. Moreover, knowledge of their
functionality provides an indication of the suitability of these proteins for certain technological processes. Consequently, evaluation of protein functionality in frozen storage is an index for determining the quality of the muscle. Protein solubility is therefore widely used as an indicator of structural changes in protein. Apparent viscosity provides information on physicochemical interactions of proteins leading to structural changes. Apparent viscosity of muscle solutions is thus used as a quality index of muscle. From the point of view of quality, the studies of these functional properties are obviously interesting for industry. Moreover, the techniques are quick and do not require very complicated equipment, which makes them extremely versatile.

2.8.7. Non-protein nitrogenous matter

Non-protein nitrogenous compounds in fish, crustaceans and molluscs are very important (Simidu, 1962) and the relationship of non-protein nitrogenous matter to taste, its variations in different species of fishes, its seasonal changes etc., have been very well studied. These compounds though present in small quantity, play a key role in the metabolic processes of aquatic animals and their spoilage, besides contributing to the flavour (Simidu, 1962; Suseela, 1999). The NPN content in fish and shellfish varies widely ranging from 9-18% of total nitrogen. Among Indian seafoods crustaceans and molluscs have higher NPN content than teleost fish (Velankar and Govindan, 1958).

A gradual decrease in the NPN content has been noticed by Chinnamma et al., (1970) in clam meat whereas a slight increasing trend has been noticed by Basu and Gupta (1984) during ice storage. Sastry and Srikanth (1986) have reported a decrease in NPN content from 19.11 to 9.11% of total protein during the 14 days storage of the cuttlefish in ice. Chinnamma et al., (1970) have reported that the higher rates of spoilage of shellfishes may be due to higher levels of free alpha-amino nitrogen content which contributes about 50% of total NPN factors. Chinnamma (1974) has observed a gradual decrease in free AAN content in mussel and clam meat stored in ice as well as during frozen storage. The test for total volatile base nitrogen has had a long history (Stansby et al., 1957; Liston et al., 1961). For determination of early stages of spoilage, TVBN determination has been revealed to be the best method to identify molluscan freshness (Bianchis et al., 1979). Chu et al., (1992) have found higher moisture, lower myofibrillar proteins and non protein nitrogen in octopus than other squid species.

2.8.8. Drip

Drip is the liquid exudes from fish flesh, especially when frozen fish is thawed. It is also called thaw drip and is usually expressed as a percentage of the original frozen weight. Heena and Karsti (1965) have defined drip as the exudates of tissue fluids that flow free from fish muscle during thawing of frozen fish. Volume of drip is still used to indicate general rough changes in quality (Miyauchi, 1963). To a rough degree, it reflects
the extent of proteins denaturation resulting from surface dehydration, ice crystal formation and cell rupture (Tsuchiya and Uchimi, 1959). Analysis of drip content is used as an indicator of cell damage, either by rupture or alteration of permeability properties (Love, 1958), although the solid content is not reliable, the DNA content has been used (Love and Heraldsson, 1958). The protein composition of drip is similar to that of mechanically disrupted cells (Seagran, 1959). Mishra (1988) has reported that the drip loss in clam meat increases as the storage period increases. Similar observation was also made by Chinnamma (1974) in frozen clam and mussel meat and in squids by Sastry (1981), Nagaraj (1994) and Nagesha (2002).

2.9. Value added products

The term value-added refers to value that is added to a product from the time it enters the processing plant to the time it leaves (Joscepeit and Franssu, 1992). Most people in the western world now have a higher disposable income but have less time available for meal preparation from raw material. The demand for value-added food products is slowly increasing in our country. Today various types of value added products are available in the market. The scope for value added products from shrimp, bivalves, squids, minced meat etc, are immense both for international and domestic markets. The gradual increase in demand for easy to cook or ready to serve food products among urban Indian population is primarily due to increase in the purchasing power. The opportunities for innovative seafood products are great in India (Dhananjaya, 2000).

In recent years, the processing of cephalopods has shifted to more value-added products rather than the traditional product forms. With mechanisation in the processing operations, high quality products have emerged, packed in superior quality packaging materials and introduction of innovative techniques (Dey, 2000). The dietary habits of the people are changing fast and India is gearing itself to produce value added products in convenience packs by adopting latest post harvest techniques in seafood handling and processing.

A lot of Research and Development work is required within the country to develop new products for export. Technology is imported in several areas for processing new products to make use of India’s unexploited and under exploited fishery resources (MPEDA, 2005). Value addition gives the consumer products a step closer to his meal. Moreover, he gets his choice at the exact weight measurements. Realizing the importance of value addition in exports, MPEDA has been concentrating on the development of diversified / value added sea food products. It introduced new technology and encouraged sea food processors to adopt consumer packaging. The efforts have proved to be fruitful as the country has expanded its overseas markets and
achieved higher market value realization. Value added products have already penetrated international markets elicit export incentive from development agencies like the MPEDA.

Eys (1987) has surveyed pilot projects conducted in India, Indonesia, and Thailand regarding shrimp, tuna, cephalopods, their trade potential, feasibility of the production and export of various high quality value added products. There are good prospects of increasing exports of frozen squid and cuttlefish in India (Nair, 1988).

2.9.1. Product development

The most important factor responsible for the success of new product is primarily the consumer satisfaction and long term repeat purchase by the consumer. The new product development is without doubt a risky business and is a complex activity requiring a systematic and thorough approach. Among the wide variety of value added products, the breaded seafoods are well established. In the advanced countries, they are commonly known as coated/enrobed seafoods. The consumer market for fresh and frozen coated seafoods has been expanding rapidly in recent years. The ability to formulate and apply batter-based coatings and breading to a wide variety of seafoods has evolved from an art into a science. The technology appears to be a fertile ground for new research. Coating is the most common way of value addition (Hunter, 1991), to put it in a simple term, coating or enrobing is a means of surrounding a high value substrate such as seafoods with low value coat such as batter and bread crumbs. Coated vegetables such as onion, potatoes, chillies and flesh foods like fish, chicken are familiar in Indian culinary preparations. The battering and breading process will change the raw seafood into a different product, the appearance and taste of which will depend primarily on the type of coating systems which comprises a number of layers of coating material. Each layer is important, not only with regard to appearance, taste, weight and size, but also in its functional characteristics. Coatings are best applied with mechanical equipments, since application of batter and bread is difficult in manual operations resulting in variation in percentage of coating. Batter and bread crumbs selection is critical. The texture, taste and appearance of the product can be varied by using different types of batter and breading materials.

In advanced countries, commercial batter and bread materials are available which can withstand the rigorous of the various processing steps like frying, freezing, packaging, transportation, frozen storage and final preparation and give consistent product quality with regards to flavour, colour, texture and appearance. Optimum frying time, temperature, oil quality and use of standard frying equipment make the product more attractive. Specially designed battering, breading and frying equipments are available for continuous and efficient production of coated products which can be easily dismantled for cleaning purposes.
Most of the new product development works are being carried out by the research institutes or by fish processing industry itself. The reason for new product development may be varied like better utilization of available resource, consumer need and convenience. Sullivan (1969) has given in-depth account on the conceptual approach of new product development; product innovation, the consumer demand and marketing. The importance of product diversification in seafood trade has been highlighted by many researchers (Saralaya, 1979; Gopakumar, 1978; Shenoy, 1984; Girija and Ravinath, 1986). There are reports on various fish products such as fish curry, fingers, sticks and cutlet (Revankar and Baliga, 1982), battered and breaded prawn (Kasinath, 1994), green mussel (Dhananjaya, 2000), and value added products from squids (Nagesha, 2002). Recent innovation in product developments is to produce value added products from shrimp, squid, cuttlefish etc. Value-added products include battered and breaded, IQF, mince based analogues etc. The recent developments in battering, breading and frying equipments have contributed immensely to the growth of coated products, such products have got great market potential (Johnson, 1982).

Kreuzer (1984) has extensively reviewed handling and processing methods of cephalopods at sea and ashore, products and product development, with emphasis on those available in Japan. Export of items like battered and breaded shrimp, double skinned cuttlefish, fish burgers, sea food mix, squid fillets etc. have made their presence felt and it is expected to increase in leaps and bounds by the turn of the century.

A review on value-added frozen fishery products, advantages of value-added products, steps for production of battered and breaded seafood and their marketing is given by Dhananjaya and Naik (1996). Steps for production of battered and breaded shrimp products, squid rings, stuffed squid and cuttlefish fillets were explained by Naik (1996). Gopakumar (1997) has reviewed the utilization of fish in India, different products prepared for export which include squid, shrimp, prawn etc. He also briefed about the production of value-added products which includes battered and breaded products, IQF products, surimi etc. Nagesha (2002) has studied the frozen storage stability of ready to eat squid products. Dhananjaya (2000) has prepared different value added products from green mussel (Perna viridis) and observed their quality changes during frozen storage. Sunilkumar (2002) has developed value added product from clam and studied its stability during frozen storage.

2.9.2. Smoking

Smoking is one of the historically important methods of preparing food because of its preservative qualities. Before the age of refrigeration, that was extremely important. It is one of the oldest forms of food preservation acceptable to a large proportion of the world’s population (Borgstrom, 1965, Aitken, 1982 and Storey, 1982). Fish is smoked as
it dries over a smoldering fire. Wood smoke adds flavor and color; the brining process helps to preserve the fish. In developed countries this process is primarily designed to produce a product of desirable appearance, odour and flavour, but in some parts of the world smoking is used as a preservative process. Preservation is achieved by drying. Prior to smoking fish products are given brine treatment to have desired texture and flavour. The brining will bring about an initial change in the microflora. Generally, smoking will cause a shift in the microflora from gram-negative to gram-positive. Coryneforms bacteria, Micrococc and Bacilli are frequently encountered in the hot smoked products. In cold smoked products, a typical Pseudomonas spoilage flora develops during subsequent storage. In the case of hot-smoked products, internal temperature of the fish during smoking is important. It has been observed that chub smoked at 140°F for 30 minutes had a microflora dominated by Cocci (61%) and non-sporing rods (34%). When smoked at 160°F, the product showed 81% spore forming rods with cocci and non-sporing forming rods composing only 9% and 10% respectively of the microflora (Liston, 1980).

The danger of botulism due to growth of Clostridium botulinum type E in smoked fish is now very widely recognised. Botulism from smoked products is traceable to improper holding practices. Unless the products are dried to a water activity (a_w) below 0.93 during smoking or by heavy salting, they should be considered potentially capable of supporting the growth of C. botulinum. Those products should be kept under refrigeration or consumed shortly after preparation to overcome this problem. Dried and smoked products are normally spoiled by moulds. Philips and Wallbridge (1977) isolated Aspergillus (six species), Wallemia (Spirendonema) Penicillium (five species), Acremonium and Rhizopus in smoked-dried products from tropical region. Chandrashekar (1986) has reported that smoking of Indian sardine for 3½ hours at 45 °C followed by 1 hour at 70 °C is lethal to the bacteria and fungi which are present in the fish.

2.9.3. Brining / salting

Salting is one of the preservation methods of fish, but in smoking process it is an important and essential step mainly used to give the taste and odour to the product. Different methods of salting are practiced all around the world. Wheaton and Lawson (1985) have extensively reviewed the various methods of salting for fish. The fish may be dry salted or brined until it has absorbed the desired amount of salt. The penetration of salt through different parts of fish was studied by Narayanaswamy et al., (1980). Brining improves the efficiency of handling due to cleansing and improvement of texture.

Brine of different salt concentrations was employed as a preliminary step to smoking by different workers. Muraleedharan (1979) used 5% brine for 5 minutes when
mussels were smoke cured. Anthony (1993) used saturated brine for seer fillets for a short period of 6 minutes. Brining in 10% brine for 5 min. was employed for brining before smoking of green mussels (Dhananjaya, 2000). This uptake of salt due to salting merges with the deposition of smoke components resulting in moisture loss to give combined effect in the product from spoilage process. Typically, the salt content of smoked fish is 10 to 15% and the fish loses 20 to 30% by weight during the smoking process. While brining, as the brine concentration Increases, increase in weight loss in rainbow trout fillets was noticed (Jittinandana et al., 2002).

2.9.4. Methods of smoking

There are two principal smoking processes, hot smoking and cold smoking based on temperature employed for smoking process (Cutting, 1965). In cold smoking, the smoke temperature does not exceed 30 °C and the fish remains uncooked. In hot smoking, the smoke temperature is raised to at least 70 °C at some stage during smoking, so that the fish are partially cooked during the process, and the product can be eaten without further cooking. In some products the temperature may be raised in stages to as high as 95 °C. Petersen (1982) identified three different stages during hot smoking process based on the temperature, they are (a) Preliminary drying period at a temperature of 30 °C during which the skin is toughened against breakage (b) smoking and partial cooking period (50 °C) and (c) final cooking period (80 °C).

Traditionally, fish was subject to heavy smoking (a heavy cure) in which there was prolonged exposure to smoke to preserve it, often in combination with salting and prolonged drying. Cold smoking for a prolonged period until the fish is hard, mainly as a result of drying is known as hard smoking (a hard cure). Traditional smoked products are still produced and remain important in some countries, but they tend to be too salty, too dry and have too strong smoky flavour for the mass market in developed countries. In modern smoked products for these markets, salt and smoke are used primarily to flavour the products, and mild and light smoking processes are used. These milder processes contribute only a limited preservative effect resulting in only a small extension of shelf-life compared with that of the raw fish and in some cases should be regarded as having much the same shelf life as unsmoked fish. If such a product is intended to be eaten without further cooking, the salt concentration in the water phase of the product and proper refrigeration of the product below 5 °C along the whole chill chain from producer to consumer are critical factors for product safety.

David et al., (1990) have studied the quality characteristics of traditionally prepared masmin in a mechanical kiln. The quality of the mechanical kiln prepared product was superior. The rate of smoke deposition on fish surfaces depends on smoke density, air circulation, humidity, temperature, and nature of the surface (texture and oil
content). A brief drying period to form a coating of protein (pellicle) on the surface of the fish will prevent the accumulation of surface moisture and assist in the even deposition of smoke.

2.9.5. Smoking kiln

The smoked fish is produced in a traditional or mechanical smoking kiln. In the traditional kiln, smoking operation depends on natural convection and in mechanical kiln with forced convection and temperature control can be designed as a batch or continuous operation. The smoke is usually generated by burning wood, wood chips, (wood shavings), or sawdust (or a combination of these) obtained from hardwood or softwood or some combination of these. The smoke density or thickness is used as an indication of the concentration of the invisible smoke vapours in a kiln. In a traditional kiln, the movement of smoke and warm air depends entirely on natural draught, usually by means of a chimney, as in the chimney kiln, where the smoke rises by convection through fish or fillets hung above a fire of smouldering sawdust. The results are very variable and there is very little element of control. It is also known as a tall kiln or vertical smokehouse. Various designs have been built such as the London kiln or smaller north kiln, both used mainly for cold smoking, or those designed mainly for hot smoking.

In a mechanical kiln, the smoke is moved over the fish vertically or horizontally by means of a fan. The temperature of the smoke can be controlled much more closely because the smoke is generated outside the kiln in a separate smoke producer, designs of which vary. The smoke can be distributed much more evenly over the fish so that the fish are all smoked and dried consistently at the same rate. The Torry kiln, originally developed in the 1930s at Torry Research Station, is a batch kiln that can be built to a range of capacities from 100 kg to 1200 kg / 4 hours. The temperature, humidity and speed of a constant horizontal stream of smoke can be controlled.

The smoke producer (fire box) is the apparatus in which the wood is burnt to produce the smoke. It can range from a simple hearth with dampers controlling the supply of air, to an automatic smoke producer (smoke generator) in which the wood is burned continuously and the combustion temperature is controlled. Typically, sawdust is fed continuously from a hopper onto an electrically heated plate or fire bed, but smoke can also be produced by blowing hot air or super-heated steam through a bed of sawdust, or combustion can be achieved by application of friction to a block of wood. The smoke density in the kiln can be measured using a smoke density meter or smoke meter usually designed to measure the degree of light scattered by the smoke. In modern kilns the production and intensity of the smoke, and the temperature and humidity are computer controlled, the smoke is recirculated, weight loss can be monitored, emissions are controlled, and the consumption of sawdust or wood shavings
is about 10% of consumption in the traditional smokehouse. Combustion chamber type smoke generators (either internal or external to the oven) may create higher smoke densities than the hot-plate type, but may have the disadvantage of not being automated.

A number of authors have reviewed smoke chambers that are used for smoke curing in different parts of the world (Cutting et al., 1965; Mathur and Bhatia, 1967 and Zaitsev et al., 1969). Cement chambers were also constructed to produce good quality product with an increased operation efficiency and reduced fire wood consumption (Rogers et al., 1975). An economically profitable experimental fish drying and smoking kiln was designed and constructed at Volta Lake (Watanabe, 1975). An electro thermal smoking kiln was developed to get a superior quality product (Chakraborty, 1987).
2.9.6. Liquid smoking treatment

In addition to hot and cold smoking methods, dip in concentrated wood smoke solution or condensate of flavour components of wood smoke from which the polycyclic aromatic hydrocarbons have been removed or reduced or by spray injection into the smoking oven in which the droplets are electrically charged in a high voltage electric field and rapidly deposited on the fish were also employed (Watts and Faulkner, 1954; Zaitsev et al., 1969; Seno, 1973) and electrostatic smoking (Hamm and Rust, 1947; Foster and Jason, 1954; Hanley et al., 1955; Foster, 1956; Zaitsev et al., 1969; Seno, 1973). The degree to which smoke constituents are taken up by the fish depends on the wetness of the surface and the concentration of the smoke constituents in the vapour. These two methods of smoking offer higher antioxidant property to the product and involve relatively less time and labour. In electrostatic smoking, about 50-60% of the smoke particles gets trapped due to presence of subcutaneous fat layer, scales, thick skin.

The liquid smoke helps in uniform distribution of flavour and color and there is less environmental pollution associated with its use (Potthast, 1978). Generally, the liquid smoke treated product has less pronounced aroma and flavour and also phenolic compounds (Makarova et al., 1985). The color produced by liquid or natural smoke is intensified when the surface is heated and dried. It is more difficult to get dark smoke colors with cold smoke processes than with hot smoke processes.

2.9.7. Production of smoke

Smoke can be produced from a smoldering, saw-dust fire. In some regions of the world, wood is burned instead of sawdust, and this produces a hotter fire with less smoke so that the fish is charred rather than smoked. Wood smoke is composed of millions of microscopic particles which rise like a fog, and by vapours. The fog is mostly water, carbon and trace solids. The vapour contains volatile oils which are released from the wood and furnish the characteristic flavours and preservative qualities. Hard wood from deciduous trees, especially oak, that when burned produces smoke that imparts a more desirable flavour to fish than soft wood. Oak is generally regarded as producing an excellent flavour but mahogany, hickory, cherry, apple and beech, amongst others, are also used. According to Shewan (1945) teak and pitchpine impart acrid flavour, whereas mahogany, redwood and Oregon pine give a good colour and desirable flavour. Each kind of dried wood produces a burning smoke of its own definite characteristics (Freixo, 1958). Chandrashekar (1986) have obtained good odour, flavour, golden yellow coloured smoked oil sardine using mixture of coconut husk, wood shavings and sawdust in the ratio of 2:2:1 proportion. Cocoanut husk alone can impart good flavour and odour to the product but not the colour (Solanki et al., 1970). Wood contaminated with moulds and
fungus should not be used to avoid contamination of the product (Borgstrom, 1965; Hall, 1997) and presence of wood preservatives may produce harmful smoke which might make smoked fish dangerous to eat.

There is some evidence that a relative humidity of 60% at a temperature of 160°F produces maximum smoke deposition in some species. However, other factors require that drying and heating rates be controlled to other temperatures and humidity at various stages of the smoking cycle. While smoke density can be increased by reducing air ejection from the system (closing dampers), the same action will raise relative humidity and therefore, reduce the drying rate. It is useful to be able to generate high smoke density even at high ejection rates. Modern automatic hotplate-auger smoke generators are capable of producing large quantities of smoke if properly operated.

The composition and characteristics of the smoke depend on the type of wood, the water content of the wood, the temperature to which it is heated to produce the smoke, and the manner in which it is heated.

2.9.8. Chemical constituents of wood smoke

Wood smoke is a mixture of aliphatic and aromatic vapours (which contribute the flavours), and condensed tarry droplets, and it has a very complex composition comprising among other components, acids, phenols, aldehydes and ketones, alcohols, and aliphatic and aromatic hydrocarbons. Phenols and aromatic compounds present in wood smoke are mainly responsible for reducing bacterial spoilage in smoked fish. Measurement of phenols can serve as a rough check on a smoking process. Any preservative effect of the smoke is probably largely due to the presence of a range of phenolic compounds, formaldehyde and acids. Gilbert and Knowles (1975) have reported that more than two hundred chemical compounds were identified in curing smoke.

2.9.9. Antioxidant effect.

Shelf-life extension of smoked fish compared with fresh fish is due to a combination of lowered water activity and the uptake by the product of bactericidal and antioxidant components of wood smoke. It has been shown, for example, that traditional smoking can increase the induction period (i.e. up to a level of 20 milliequivalents of oxygen per kg) of autoxidation from 4 days in unsmoked controls to approximately 50 days. This antioxidant effect, however, is mainly associated with the particle phase of wood smoke (the vapour phase showing little or no antioxidant activity) and increases with increasing temperature of the oxidation stage of smoke production, but it is unaffected by the pyrolysis temperature or air supply. Kasahara and Nishibori (1983) have observed many volatile compounds such as phenols, acids, alcohols, and hydrocarbons in the smoked products of cuttlefish and octopus which are also found in smoke tar. The phenols of
smoked-cuttlefish and octopus were important aroma components and considered to come from the volatiles of wood smoke. A few aliphatic hydrocarbons which seemed to come from meat constituents of cuttlefish were found only in commercial smoked cuttlefish.

2.9.10. Imparting of smoked flavour

Smoking has been a popular way to preserve fish for centuries. The applications of salt, smoke and, in some products, nitrate imparts a characteristic texture and flavor. With the advent of refrigeration, these products now contain less salt and smoke and have higher content of moisture. In addition, packaging systems such as vacuum packaging with high barrier films have extended shelf life. The smoke flavour can be added as a mixture of natural or synthetic flavor chemicals. For example, so called liquid smoke is a solution or condensate of flavour components of wood smoke from which the polycyclic aromatic hydrocarbons have been removed or reduced. It is sometimes called a smoke solution and is used as a smoke dip, or as a spray.

Species of wood will affect smoke deposition and flavor. Most producers have their own preference based on their markets. However, moisture content of the wood, time of cutting (seasoning and sap content), and presence of bark will also affect smoke flavor and density within a species of wood. Consistent smoke flavor and density is easier to achieve if consistent wood character is maintained.

2.9.11. Colour development in smoked product

Colour imparted to the fish by the smoking process is due to carbonyl-amino reactions of the Maillard type and has been correlated with a quantitative decrease in carbonyl groups in the smoke. Brown pigments forming in the surface tissues, however, were said by Ziemba (1969) to inhibit further penetration of carboxyllic groups and other smoke components to underlying tissues. Ribose, from the degradation of nucleotide and ribonucleic acids and free amino compounds, such as anserine and taurine, in the fish muscle extractives also contribute to browning at the surface of drying fish. The color produced by liquid or natural smoke is intensified when the surface is heated and dried. It is more difficult to get dark smoke colors with cold smoke processes than with hot smoke processes. Natural smoke has both water soluble and oil soluble components. These components will selectively deposit on wet or dry surfaces, giving different colors and flavors. Avoiding wet spots on an otherwise dry surface will help avoid color spotting and inconsistent flavor intensity.

2.9.12. Effect on nutritive value of foods

Smoking has several effects on nutritive properties of processed foods. It is generally accepted that phenols and polyphenols present in smoke tend to react with sulphydryl groups of the protein and the carbonyl groups in smoke tend to react with
amine groups. The preserving effect of smoking on fishery products is credited to a combination of surface drying, salting and deposition of antioxidant (phenolic) and antimicrobial constituents on the fish (Gilbert and Knowles, 1975). Smoke contains carbonyls which react with lysine and reduce protein quality, the more smoke the greater is the effect. Whenever smoking is used, the length of smoking and the concentration of smoke should be kept to the lowest possible level to reduce the loss of protein quality (Opstvedt, 1988). Motohiro (1988) has extensively reviewed the Japanese studies on effect of smoking and drying on the nutritive value of fish, different cured products and he has briefly explained the procedure for the preparation of seasoned, smoked squid or octopus meat.

**2.9.13. Carcinogenic compounds of wood smoke**

Many Polycyclic aromatic hydrocarbons (PAHs) have been found in smoked fish. Not all of them are carcinogenic but the Benzo (a) pyrene (BP) is highly carcinogenic. PAHs are complex group of aromatic hydrocarbons containing 2 to 6 benzene rings that may have aliphatic and / or alicyclic hydrocarbon constituents. They are found in many foods as environmental contaminants at low concentrations, including fish and shellfish. Lipid rich tissues may have concentrations up to 10 times more than that in lean muscle. PAHs are produced during pyrolysis, and hence the methods of cooking and preparation of food such as barbecuing, grilling, broiling and frying tend to increase the concentration of PAHs.

PAHs are invariably found in smoked fish products at greater concentrations depending on the degree of exposure to smoke. They are deposited from the smoke in which more than 200 PAHs have been identified at concentrations and combinations that depend on the type of wood and the temperature of smouldering. Some of the PAHs are human carcinogens, co-carcinogens, teratogens and mutagens, and hence these compounds are subject to surveillance in foods as part of food safety and environmental programmes. About 16, two to five ring PAHs have been identified as priority environmental pollutants. Presence of benzo[a]pyrene in fish products together with dibenzo [a,h]anthracene, represents the most potent of the probable human carcinogens. The highest concentrations of PAHs are found in heavily smoked traditional products; up to 10 times more benzo[a]pyrene during hot smoking compared with cold smoking and up to 10 times more benzo[a]pyrene on the product (about 1 mg / kg) in a traditional smoke house compared with a modern kiln in which the smoke is recirculated (about 0.1 mg / kg). The use of liquid smoke products in which the PAHs have been removed or reduced is effective in reducing the PAH concentrations of smoke flavoured foods.
PAH, being soluble in fat gets accumulated in adipose tissue and liver in man. Though not directly implicated in the induction of cancer, they are either known or suspected carcinogen and contribute to carcinogenic pressure in people. Besides, PAH are implicated in damage to hematophorotic and lymphoid systems. Degeneration of the spleen, thymus and mesenteric lymph nodes and inhibition in the development of bone marrow have also been observed. Nitrosamines have been detected in smoked fish, but at far below the carcinogenic levels. The productions of nitrosamines and nitrates in smoked foods have been linked to the presence of nitrous oxide in the smoke vapour phase (Daun, 1979). In case of fishery products polycyclic aromatic hydrocarbons and Nitrosoamine compounds mainly detected in cured and smoked products among which Benzopyrene (BP) a polycyclic aromatic hydrocarbon is the principal compound detected many a times (Mossanda.1980; Potthast,1978; Daun.1979; Lenges et.al, 1976 and Steining, 1976.). The use of fitters and modification of smoking process help in the reduction of Benzopyrene content to a level lower than the IUPAC level (Chandrasekhar, 1986).

2.9.14. Product storage

After completion of smoking process, smoked fish removed from the kiln is allowed to cool before grading and packing. Packing of the smoked fish in hot/warm condition results in the deposition of moisture on the inside of the packaging, which is conducive to premature mold growth. Both cold and hot-smoked fish are stored in the refrigerator, although the keeping quality at a given temperature is not as good as that of unsmoked fish. The practice of dispatching to retailers in the frozen state which obviously helps to minimize spoilage prior to consumption is growing. Frozen storage of these products extends the shelf-life up to several months. Vacuum-packed and seasoned smoked-dried products of red squid (Ommastrephes bartrami) stored at room temperature was found in good condition for 90 days though they developed pale brown color during storage (Lee et al., 1985).
2.10. Battering and breading of fishery products

Batter is highly viscous liquid prepared from ground cereals, spices, salt, sugar and other ingredients and / or additives for coating. Typical batter types are: non-leavened batter and leavened batter. Breading means final coating of fishery products with dry breadcrumbs or other dry preparations mainly from cereals with colorants and other ingredients. The amount of breading material pick-up is determined by viscosity of the batter. Typical breading types are: free flowing breading, coarse breading, flour-type breading. The process of battering and breading makes the fried products more attractive. The colour of the finished products mainly depends on the type of breading materials. Wheat based breading mixes give a golden brown colour and those based on bread crumbs a reddish brown colour. In commercial battering step, materials are pre-treated to improve adhesion of the batter, in this, materials are first passed through a falling curtain of batter (flour and water paste which may contain ingredients for adding flavour) which coats the whole surface. If necessary, excess batter is blown off with a current of air and the coated product is passed through a bed and curtain of dry crumbs (bread crumbs) to attain overall coverage. The product may be flash fried to set or fix the coating and then refrozen to –30 °C before or after packaging, for storage and distribution. Many coated products are available with a variety of cooking options, such as baking in a conventional oven, grilling, shallow fat frying, deep fat frying, microwave cooking etc.

Holston (1956) has given a good summary of the properties of batters and their materials and factors controlling them. He was of the opinion that most of the commercial batter mixes are prepared from ground corn flour and corn meal and contains spices and non-fat dry milk solids. Similarly, breading material may be used singly or combinations of the following ground, soft, wheat cereals, cracker meal, potato or soya flour; dried bread crumb and starch or hydrolysed flour. Batter used for fishery products should have several important characteristics such as, complete coating of the product with uniform thickness and it should not distract the original flavour of the product and must retain the desired amount of breading on the product (Stansby, 1963).

The shelf-life of battered and breaded smelt is extended up to 5 months at -23 °C (Baker and Darfler, 1979). Torres et al., (1985) have studied the viscosity of the batter and method of breading, effect of frying temperature, colour and crispness scores of coated fillets, the absorption, net weight loss, moisture loss and residue to fat ratio of breaded fish fillets. Grodner et al., (1991) have analysed the proximate composition of batter and bread to establish value added protein guidelines for use in food service.

Salvador et al., (2002) have studied the effect of corn flour, salt, and leavening on the texture of fried, battered squid rings. Although salt-containing formulations had
significantly lower viscosity values than the other samples, the batter pickup values were not significantly different. Replacement of wheat flour by corn flour decreases significantly the oil content of the battered squids. The batter formulation influences the degree of oxidation of the lipid fraction of the deep-fried products; the battered product with leavening in its formulation showed less oxidation (Springer-Verlag, 2003). Llorca et al., (2003) have examined the effect of batter formulation on lipid uptake during frying and lipid fraction of frozen battered squid.

2.10.1. Equipments used for coating of seafoods

Commercially different equipments are used for the production of coated / enrobed products viz., predusting, battering, breading, double coating, frying, freezing and storage. Here, all the steps are automated for continuous and large scale production. Predusting equipment is useful in achieving more uniform application of dry batter powder over the product. Modern predusters ensure consistent coating and eliminate the transfer of dust to the atmosphere.

In battered and breaded products, quality assurance with reference to the ratio of seafood to coating level is of much concern and therefore it is essential to select a flexible and versatile machine for each step which is suitable for a wide range of battered and breaded products. Simplicity of design for easy dismantling and cleaning is very important. Tressler et al., (1968) reported use of semiautomatic scale equipment for battering and breading. Johnson (1982) has stressed the need for flexible machines for a range of battered and breaded products, continuous inline equipments including fryers. Wheaton and Lawson (1985) have mentioned a simple battering machine for fish sticks. An account of enrobing equipments is given by Fellows (1988). The enrobers are classified into liquid enrober which is simple battering device and solid enrober which otherwise is called as breading machine. Roessink (1989) has given an account of coating equipments suitable for low value fish. Dikhoff (1990) has described liquid and solid enrobers used for fishery products. He is of the opinion that good enrobes should be designed in such a way as to permit the reuse of batter and bread crumbs.

Coated products are popular due to introduction of modern coating equipment. Wheaton and Lawson (1985) have described use of small scale fryers for battered and breaded products. Fryers may be either batch or continuous type. In large scale product lines, continuous deep fat fryers are more important which aims at reducing the amount of oil absorbed by the product, which recirculates the oil and also have facility for removing food particles that could burn and spoil the appearance and flavour of the products (Fellows, 1988; Mallett, 1993).
2.11. Microbial changes during processing and storage

2.11.1. Processing and icing

Primary contamination of fish and shellfish can occur up to the time of catching, then secondary contamination begins at the start of handling and processing. Processing necessarily involves handling with the inevitable introduction of bacteria of human origin. During processing, the number and types of bacteria present in products will fluctuate within a given sequence. Counts generally fall at washing, cooking and rise during sorting, grading, breading and packing (Cann, 1977). The shelf life of fish products is markedly extended when products are stored at low temperatures. In industrialized countries it is common practice to store fresh fish in ice (at 0 °C). The mesophilic flora initially present in tropical shellfish will not grow at the temperature of melting ice. Consequently, spoilage will be delayed until a psychrophilic flora develops. Chinnamma *et al.*, (1970) have reported that the bacterial count and the count of pathogenic organisms like *E. coli*, fecal *Streptococci* showed considerable variation during ice storage of mussel. Total plate count during ice storage showed steady increase. Prafulla *et al.*, (2000) have noticed similar increase in TPC of squid and cuttlefish stored under chilled condition following different methods of icing.

2.11.2. Freezing

The effect of freezing on the bacterial population is erratic and difficult to predict. There is generally, some reduction in the count and the number will continue in most cases, to fall during frozen state. In general gram-negative bacteria are more sensitive to freezing than gram-positive bacteria and bacterial spores are highly resistant (Raj and Liston, 1961). *Salmonella* and other members of the *Enterobacteriaceae* are among the more sensitive bacteria (Raj and Listen, 1961) but there are great variations in the response of these organisms present in fish. Chinnamma *et al.*, (1970) have reported that the viable counts come down by 99% and reduction of pathogenic organisms like *E. Coli* and faecal *Streptococci* during frozen storage of *Perna viridis* at the end of storage period of 44 weeks at -23 °C. Similar reduction in bacterial count during freezing and subsequent frozen storage has been noticed by number of researchers (Dhananjaya, 2000; Nagaraj, 1994; Sunilkumar, 2002; Nagesha, 2002; Sastry, 1981 etc.).
2.11.3. Prepared products

There are an increasing number of prepared frozen seafood products in the world market. These include both raw and pre-cooked seafoods that range from relatively simple products such as breaded fish portions and smoked product. Batter and breading introduce different kinds of microorganisms to the seafood particularly Gram-positive bacteria and mould (Raj, 1968).

2.11.4. Smoking

Quite large amount of fish are still treated by smoking processes. In the industrialized countries this process is primarily designed to produce a product of desirable appearance, odour and flavour, but in some parts of the world smoking is used as a preservative process. Preservation is achieved by drying. Prior to smoking fish products are given brine treatment to have desired texture and flavour. The brining will bring about an initial change in the microflora. Generally, smoking will cause a shift in the microflora from gram-negative to gram-positive. Coryneform bacteria, Micrococci and Bacilli are frequently encountered in the hot smoked products. In cold smoked products, a typical Pseudomonad spoilage flora develops during subsequent storage. In the case of hot-smoked products, internal temperature of the fish during smoking is important. It has been observed that chub smoked at 140°F for 30 minutes had a microflora dominated by Cocc (61%) and non-sporing rods (34%). When smoked at 160°F, the product showed 81% spore forming rods with Cocci and non-spore forming rods composing only 9% and 10% respectively of the microflora (Liston, 1980).

The danger of botulism due to growth of Clostridium botulinum type E in smoked fish is now very widely recognised. Botulism from smoked products is traceable to improper holding practices. Unless the products are dried to a water activity (aw) below 0.93 during smoking or by heavy salting, they should be considered potentially capable of supporting the growth of C botulinum. Those products should be kept under refrigeration or consumed shortly after preparation to overcome this problem. Dried and smoked products are normally spoiled by moulds. Philips and Wallbridge (1977) isolated Aspergillus (six species), Wallemia (Spirendonema), Penicillium (five species), Acremonium and Rhizopus in smoked-dried products from tropical region. Chandrashekar (1986) reported that in case of smoked Indian sardine that smoking for 3½ hours at 45 °C followed by 1 hour at 70 °C is lethal to the bacteria and fungi present in the fish.
2.12. Sensory evaluation

Since the consumer is the ultimate judge of quality, most chemical or instrumental methods must be correlated with sensory evaluation before being used in the laboratory. However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, etc., may be reduced. In sensory analysis appearance, odour, flavour and texture are evaluated using the human senses. Sensory evaluation scores generally decrease as a function of storage period in different products and different storage methods at different temperatures.

Sufficient literatures are available on the sensory evaluation of different fishery products during ice storage and also during frozen storage. Joseph et al., (1977) have reported that organoleptic qualities of the control squid sample became unacceptable after 15 weeks of storage, whereas the treated samples remained in good condition up to 19 weeks. Sastry (1981) has studied the sensory analysis of frozen stored squid, treated with polyphosphate and it was preferred over untreated control samples. Iced cuttlefish can be stored in an acceptable condition up to 7 days. Krivchenia and Fennema (1988a) studied the sensory analysis of frozen white fish fillets for 18 weeks, revealed that STP treated sample (-12 °C) were significantly preferred over untreated control samples. Verma (1992) has reported that sucrose treated oil sardine is acceptable up to 4 months, whereas control is only up to 3 months. Selvaraj et al., (1992) have reported that trisodium polyphosphate treated frozen stored squid showed better overall acceptability compared to control.

Joseph and Sherief (2003) have recorded decreased sensory score during ice storage treated cuttlefish using sodium chloride, citric acid and BHA in different concentrations and combinations. The salt + citric acid treated sample retained original quality and colour and acceptance for longer time than other treated samples. In another study, the dressed cuttlefish subjected to direct icing and in ice-water slurry packed in polystyrene container was found to be in acceptable condition even on the tenth day of storage; but whole cuttlefish subjected to the same storage condition was found to be acceptable only up to eight day (Anon, 1994). As the storage progresses reduction in sensory scores of squid treated with sodium tri-polyphosphate, sucrose and mixture of STPP + sucrose during 105 days of frozen storage has been observed by Nagaraj (1994). The latter treatment could retain better quality and acceptance for longer time than other treatments.