PREPARATION OF SURIMI FROM COMMON CARP (*CYPRINUS CARPIO*) AND ITS QUALITY CHANGES DURING FROZEN STORAGE

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in

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By

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 # 2002 #

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled **"PREPARATION OF SURIMI FROM COMMON CARP (*CYPRINUS CARPIO*) AND ITS QUALITY CHANGES DURING FROZEN STORAGE"** submitted by **Mr. Pratik Mandal** in partial fulfilment of requirement for the Degree of **Master of Fishery Science (Fish Processing Technology)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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INTRODUCTION

1. INTRODUCTION

The world's consumption of fish could be more than doubled if its great-unused resources were fully exploited. These resources remain unused not through a lack of catching technology, but through an inability to transform the raw materials into stable, acceptable products and to distribute these products at an affordable price. Development in minced fish technology could make a major contribution to increase exploitation. Mince has potential application as an ingredient in different foods (Martin, 1976). Surimi is an intermediate food material that has been used in Japan for centuries to make several foods (Lee, 1984). In Japan surimi means "minced meat". Mincing fish flesh, thoroughly washing the fish flesh and then refining and dewatering it make surimi. Traditionally surimi was mixed with ingredients such as salt and spices, kneaded, and then steamed, fried or boiled to make Kamaboko, Tempura and Chikuwa, respectively (Sonu, 1986).

The traditional surimi production was run on day-to-day basis, depending on the supply of fresh fish. Consequently surimi industry could not expand to any great extent and remained in a limited capacity. However in 1959, surimi industry took a new turn when a group of scientist headed by Nishiya and Takeda at Hokkaido fisheries laboratories discovered a technique to stabilize frozen surimi. This discovery was made from an incidental finding of a cryoprotectant which keep the surimi from freeze denaturation during frozen storage. This technique enabled the Japanese manufacturer to develop the stockpile surimi.

Surimi seafood is highly nutritious and contains less than 1% fat. It has 85 to 90 calories per 3 ounce of serving. It also contains very low amount of cholesterol. The indispensability of surimi as an intermediate for kneaded product is proved by the fact that it assures a stabilized supply of raw material and enables planned production. Surimi represents only about 20% of raw material, thereby reducing the storage and transportation costs. The long shelf life and its versatility as a functional material possess unlimited potential for the product developer. Hence surimi finds no substitute.

The use of frozen surimi in recent years has increased significantly world wide, although the Japanese market still plays a leading role. The product is most successful today accounting for almost one third of the entire fish consumption in Japan. Demand of surimi-based product is ever increasing but the availability of surimi is not sufficient to fulfill the demand of consumers. For this purpose Japan and other surimi product consuming countries started importing surimi from other countries like Taiwan,

Korea, Malaysia, US etc. This gave rise to the development of surimi markets. In India surimi concept was introduced in 1992. From that point, till now many surimi processing plants are working in India.

The bulk of world surimi is produced from Alaska pollock, which are abundantly available almost year round at a comparatively lower price. Apart from this Pacific whiting, Blue whiting, Atka mackerel, Croaker, Flying fish, Jack mackerel, Shark, Dog fish, New Zealand hoki, Threadfin bream and others available at lower prices are used for surimi. Frozen Alaska pollock surimi is most widely used by surimi product manufacturer due to its very high gel forming ability and white colour. Currently Indian surimi plants mainly use the Pink perch (*Nemipterus japonicus*) as raw material. Species such as sardine and mackerel also have ample amount of source along the Indian coast. But the main problem with these species in producing surimi is high amount of fat and dark muscle content. However, the other species that are used for surimi production in India are Croaker (*Johnius* sp) and Bull's eye (*Priacanthus* sp).

In India seafood are preferred in fresh condition. Surimi based products are not introduced in India. Many attempts have been made to yet popular this type product. Probably the bland taste of surimi-based products is the main reason. Recently a world wide changing trend is observed in the fish utilization pattern and consumers are beginning to accept prepared seafood. Such trend has also been observed in India. For this reason products such as Surimi cutlet, Fish ball, Prawn wafer, Fish bakarwadi, Kamaboko are made using Indian spices. Thus surimi based product manufacturing in India and export by considering the current demand of product is providing good opportunity.

In the recent years the catch of marine fish especially Alaska pollock has decreased tremendously (1998 - 4,049,223 mt and 1999 - 3,362,473 mt) (Anon, 2001). However, till to date, marine species are the exclusive source or commercial surimi. On the other hand, the demand of surimi and surimi based Kamaboko and analogue products continues to increase as the increase in world population as well as increasing concern of the people on the relationship between nutrition and health (FAO, 1992; Tucker, 1992). Freshwater fish contributes considerably in the world food production. They contain, compared to meat, less lipid but more unsaturated fat and ω-3 fatty acids, more moisture and highly digestible protein and are devoid of fishy flavour and odour due to little amount of volatile bases (Karmas and Lauber, 1987; Aggelousis and Lozos, 1991). For these reasons utilization of these fish species would be desirable.

Common carp *(Cyprinus carpio)* is a fast growing aquaculture species and has been cultivating for centuries in the continents of Asia, Europe and Africa. It has tasty light coloured meat with highly variable fat content. Aquaculture production of common carp was mushroomed in recent years. In 1998 the world aquaculture production of common carp was more than 2.4 million tons and in terms of money value was about US $2.88 million (FAO, 2000). The yield of common carp is also considerable in India. In the present study an attempt has been made to prepare surimi from common carp keeping in view the following objectives.

- Standardizing the processing steps for producing an acceptable surimi from fresh Common carp.
- To compare the changes in quality of whole fish, dewatered mince and surimi during storage.
- To identify the critical control point in surimi production.
- To study the acceptability of surimi by using organoleptic tests.

Chapter-II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Recently surimi and surimi-based products have caught worldwide attention. Lots of literature is available on surimi preparation, its utilization and production of surimi-based products. Important aspects of surimi related to present investigation are reviewed, hereunder.

2.1. Fishes used for production of minced meat and surimi

In principle, bone separation process can be applied to any species of fish, crustacean or mollusc. In practice, this process can be best justified for those species where significant added value will accrue. The fish should have good gel forming ability, which makes for an elastic texture, good taste and whiter appearance. For an industrial scale operation it is necessary that the supply of raw material should be abundant and low priced (Dora, 1992). According to Suzuki (1981), any species of fish may serve as material for fish paste product but the resilient texture called "ashi " varies depending on the fish species.

Fish species such as Alaska pollock, Croaker, Lizardfish are ideal for surimi preparation due to their good gel forming ability and 60% of world's surimi is made by utilizing these white fishes (Ishikawa, 1996). The bland taste of white fishes allows incorporation of crab, shrimp and lobster flavour (Flick *et al.*, 1990).

The uses of pelagic species as a replacement ingredient have been tried (Hasting *et al.*, 1992). Borderias and Tejada (1981) have discussed the utilization of small pelagic fishes in the preparation of fish paste products like ' Kamaboko' and ' Fish fingers '. Major pelagic fatty species studied for surimi production include Mackerel (Kotah *et al.*, 1989; Shimizu and Koguri, 1986; Langmyhr *et al.*, 1988; Spencer *et al.*, 1988), Sardine (Nonak *et al.*, 1991; Suzuki and Watable, 1986; Tsukamasa and Simizu, 1990; Tokunaga and Nishioka, 1988; Suzuki, 1981; Russel and Cheftel 1988, 1990; Saeki *et al.*, 1991; Sarkar, 1997), Capelen (Langmyhr *et. al.*, 1988; Spencer *et at*, 1988) etc. Besides, a wide variety of fish species such as Pacific pomfret (Numakura *et al.*, 1983), Pacific whiting (Groninger *et al.*, 1985), Red hake (Lee, 1986), Silver hake (Lanier, 1984), Shark (Chen, 1995), Menhaden (Bimbo *et al.*, 1988; Leinot *et al.*, 1992) and the marine mammals such as Seal (Shahidi and Synowieek, 1990) have also been tried for the production of surimi.

2.1.1. Utilization of underutilized fish

The world's consumption of fish could be greatly increased if presently underutilized or unused resources were brought into the human food chain. This is a great incentive to apply mince technology to majority of these species because of intractable problems in processing and marketing them by other means (Flick *et al.*, 1990). Different types of fish product have been prepared using minced meat as the basic material from several species which have little or no commercial value including Grandier (*Coryphaenoides*), Smooth head (*Alepocephalus*) and Rabbit fish (*Chimaera* spp) (Burges, 1975; Bailey, 1976).

Mince has been successfully produced from Caribbean sheepshead (*Archosargus),* Black drum *(Pogonias*), Tilapia (*Tilapia*) (Finne *et al.*, 1980; Nickelson *et al.*, 1980). The by-catch species such as Croaker (*Micropogon*), Sand trout (*Cynoscion*) and Mullet (*Mugil*) are also used in the production of minced meat (Rasekh and Melza, 1977; Finne *et al.*, 1980).

Other important fishes used to prepare minced products are Grunt (*Pomadasys argyreus*), Pony fish (*Leiognathus splendens*], Catfish (*Arius*_spp), Threadfin bream (*Nemipterus japonicus*) and Cutlass fish (*Trichiurus lepturus*) (Bremner and Snell, 1978). Ribbonfish was used by Badonia and Devadasan (1980), Dhoma (*Sciaenid* sp) and Lactarius (*Lactarius lactarius*) by Agarwal *et al.* (1986).

2.1.2. Utilization of freshwater fish

Commercially significant freshwater species are generally more consistent in their properties than the marine resources. Technology for producing mince from these species is widely acceptable. The delicate bonestructure of most fresh water fish make filleting difficult, and mincing can markedly improve recovery (King *et al.*, 1971). Carp (*Cyprinus carpio*) minces have been produced in U.S.A from both cultured and natural resources (Dassow, 1980; Dassow and Steinberg, 1973; Dawson and Price, 1979; Mai and Kinsella, 1979). Less work has been done on mince and surimi from tropical and subtropical species, although acceptable products have been made from the ubiquitos Tilapia (Dassow and Steinberg, 1973; Zain, 1980), Silver carp (Siddaiah *et al.,* 1999). Some investigations have also been attempted on the quality of minces of freshwater fishes for the manufacture of surimi (Ismond and Tonogai, 1994; Lin and Morrissey, 1995; Kim *et al.*, 1996; Onibala *et al.*, 1997).

2.2. Mechanical separation of fish meat

Japanese fish processors have extensively used the mechanical fish flesh separators for many years in the production of minced meat. Japanese fish processors are credited for the advent of mechanically separated fish mince (Miyauchi *et al.*, 1973). With the development of mechanical deboner, the seafood industry could obtain edible fish from raw material used previously for inedible purpose. Miyauchi and Steinberg (1970), Carvar and King (1971) and Noble (1972) reported that by the means of meat separating machines significant quantities of edible flesh can be recovered from fish filleting wastes and underutilized species. The development of mechanical deboner is a milestone in the commercial production of fish mince and surimi (Suzuki, 1981).

Though various types of deboners have been developed (Grantham, 1981; Keay 1979; King *et al.*, 1971), the basic operation principle involves mechanically squeezing and pounding the primary material through a honeycomb of narrow orifices (i.e. perforated drum or plate) remains same. This action results in the extraction of softer textured flesh, leaving behind most of the tougher skin and bones. The belt and perforated drum system are commonly used because they can be easily dismantled and cleaned.

High quality surimi is ensured by an efficient removal system of flesh from the bone and skin (Pigott and Tucker, 1990). The efficiency of deboning is defined with respect to higher flesh yield and lower bone content (Lanier and Lee 1992). However, the yield of meat and the amount of bone content is largely dependent on the size of perforation. The drum with smaller perforation (1-7 mm) reduces the bone/fin content (Flick *et al.*, 1990). A drum perforation of 5 mm is suitable for bony fish with reasonable texture and good yield of mince (Wood and King, 1985; Wong *et al.*, 1978). The yield of minced meat also depends on the different speed of drum and belt at which they move. As a result the shear rate is increased and consequently the yield of meat also increased (Carvar and King, 1971). There is no information available on the effect of particle size on texture of minced fish flesh. In picked meat the presence of viable bones and calcium content should be less than 0.5% (USDA, 1975; Yamanoto and Wong, 1974).

2.3. Chemical composition of minced meat

The moisture, protein, fat and ash are four basic constituents of minced fish meat. Apart from these, it also comprises of cholesterol, calcium and other fat degraded products (Krzynowek *et al.*, 1984). Nearly 80% of the composition is of moisture. While, protein and fat account for the rest portion.

However, the proximate composition of minced meat may vary depending on the species and season of catch. The salt soluble protein i.e. myofibrillar protein in minced meat constitute on an average 90% of the total nitrogen (Webb *et al.*, 1976). Non-protein nitrogen (NPN) also constitutes the major nitrogen fraction and the proportion of NPN to total nitrogen is highly species dependent. Phospholipid and free fatty acids (FFA) are the major constituents of the lipid fraction. The fatty acid profile of minced carp muscle indicated high quantity of W_3 ($C_{18:3}$) fatty acids (Mai and Kinsella, 1979).

2.4. Leaching / Washing

Washing of mincemeat is one of the most important steps in manufacture of surimi. Many mince processes employ post separation washing to remove inorganic salts, water soluble proteins, pigments, visceral contamination, fat, bacteria and decomposition product (Flick *et al.*, 1990). Such washing not only improves the colour and odour but also improves the texture of the final product (Miyauchi *et al.*, 1973).

The technique of washing and mechanical dehydration of minced fish meat is carried out to produce a white, odourless and bland flavoured product. According to Pacheco-Aguilar *et al.* (1989) the washing removes substances that promote protein denaturation during frozen storage and enhances functional properties of protein. Washing results in increased concentration of myofibrillar protein namlely, actomyosin and reducing water-soluble protein (Lee and Chung, 1989; Lanier and Regenstein, 1986). Improvement in gel strength is due to increased concentration of myofibrillar protein (Babbitt, 1986). Washing of the meat aided in the removal of the pigments substantially and reduction of extractable nitrogen (Tableros and Young, 1981). Washing has significant effect in reducing the oxidative changes and enhancing the frozen shelf life of minced meat of dark fleshed species (Grantham, 1981; Rodger *et al.*, 1980; Lee, 1986).

The degree of washing required to produce good quality surimi depends upon the type composition and freshness of the fish (Flick *et al.*, 1990). The number of washing cycles and water/meat ratio employed for washing vary among the surimi manufacturers. Ishikawa (1978) and Ishikawa *et al.* (1979) reported that fatty fish require a minimum of four washing with five parts of cold water to one part of mince. It is possible to reduce the number of washing by increasing the quantity of water in the initial step. The increase in gel strength is achieved within two washing (Montiel *et al.*, 1987).

Apart from many advantages washing has many disadvantages like loss of soluble micronutrients including vitamins, minerals and free fatty acids (Sidwell, 1980) and protein, which can be substantial, up to 50% of total protein (Adu, *et al.*, 1983; Pacheco-Aguilar *et al.*, 1989; Yang and Froning, 1992). Lin and Park (1996) pointed out the problem of disposal of wastewater generated in the surimi processing plant. Almost 30 Litre of wastewater per kg of surimi produced were generated onshore processing plats (Lin *et al.*, 1995). In early studies it was shown that the gel strength of surimi continued to increase as the number of washing cycle increased (Nishioka, 1984). However, Lin and Park (1996) indicated that the major portion of the sarcoplasmic protein is soluble and removed during the initial washing step. Subsequent washing removes the residual sarcoplasmic protein and small amount of myofibrillar protein. After sarcoplasmic proteins are completely removed, further washing causes severe loss of myofibrillar protein. Improper water-mince ratio and long time washing results in absorption of water by the meat, which causes problem during dewatering steps.

2.4.1. Effect of water quality

According to Suzuki (1981) the hardness of water, pH, temperature, salinity and mineral content of the water are important factors affecting the quality of surimi. The quality of water plays an important role in determining the quality of surimi.

2.4.1.1. Hardness

Tamoto (1971) has studied the effect of hard water on surimi of Alaska pollock. Hard water can damage the texture of surimi and act as catalyst for fat degradation (Flick *et al.*, 1990). Saeki *et al.* (1986) pointed out that hard water results in increase in myosin cross-linking which leads to drip loss and texture loss.

2.4.1.2. pH

The pH of the water should be optimum in order to ensure maximum functional performance of fish protein (Lanier and Lee, 1992). Gill *et al.* (1979) observed a reduction in colour, water uptake, protein loss and TMAO level when pH of washing water was reduced. Lanier and Lee (1992) recommended the use of sodium bicarbonate in washed water for pH adjustment as fish flesh showed a drop in pH. However, a pH between 6.8 and 6.9 is ideal which not only increases the efficiency of washing but also improves the gel strength (Regenstein and Regenstein, 1991).

2.4.1.3. Temperature

Warm wash water is more conducive to dewatering than cold water. Although the cold water is desirable to preserve product quality, this benefit may be outweighed by loss of efficiency in dewatering procedure when the water temperature is very low. An ideal temperature of water used in washing procedure is 3^0C to 10^0C (Lanier and Lee, 1992). Japan's Surimi Association recommended that the water being used for the last washing cycle should be about 10^0C in order to achieve a reasonable dewatering efficiency. Lanier and Lee (1992) pointed out that high water temperature induces protein denaturation and microbial proliferation in mincemeat. Thermostability of the functional myofibrillar protein based on Ca++ ATPase activity differs from species to species (Hashimoto *et al.*, 1982). The extraction of sarcoplasmic protein is a function of temperature. Therefore, water temperature is one of the critical factors in manufacturing high quality surimi since thermostability of protein is overriding factor.

1.4.1.4. Salt concentration

The gel-forming component of fish proteins varies widely in water-holding characteristics as a function of salt concentration. A very low salt content causes the water holding tendency to rise and the meat tends to hydrate and swell. (Flick *et al.*, 1990). Increase in salt concentration causes a decline in moisture content, but high salt level leads to solubilization of myofibrillar protein causing the premature setting of protein sol (Lee and Kim, 1985). It is advisable to use water-containing salts such as chlorides of magnesium, calcium (Ofstad *et al.*, 1993) and sodium (Flick *et al.*, 1990). To improve dewatering, salt can be used during the last washing step at concentration level of 0.1 to 0.3% (Pigott and Tucker, 1990).

2.5. Straining and dewatering /Refining

Intermediate dewatering should be carried out effectively and efficiently after each washing or leaching in order to prevent the hydration of mincemeat which may otherwise cause problem in final dewatering (Lee, 1986). Screw press is universally used in surimi plant today for dewatering (Flick *et al.*, 1990). The main objective of straining is to remove the connective tissue, scales, fragment of bones, skin, ligament etc. from minced meat and also to separate white meat from dark meat (Lee, 1986). The quality and yield of surimi appear to increase by this process (Swafford *et al.*, 1985). Rotary screen is more economical and commonly used for this purpose. During straining the perforation of sieve should ideally be about 2 mm, which decides the quality and yield of surimi (Lee, 1986).

Partial dewatered fish meat is subjected to straining and further dewatering, which is usually referred as refining (Lee, 1986). A refiner is nothing but a straining machine, which could work on wet slurry. However, the refiner is not used widely (Flick *et al.*, 1990). During straining it was found that the temperature increase of about 2^{0}C to 4^{0}C which caused protein denaturation and thereby reduction in quality (Suzuki, 1981; Lanier and Lee, 1992). Use of self-cooling strainer can eliminate this problem to some extent, but the best remedy is to use a refiner (Flick *et al.*, 1990).

2.5.1. Final dewatering

The introduction of screw press as the standard dewatering machine was one of the most significant break-through in surimi production. The product could flow continuously through the screw press, hence the name "Continuous dewatering machine" used by some manufacturers. It purges water from the meat slurry by squeezing it into a progressively reducing chamber with the aid of a rotating screw, while allowing the pressurized water to escape through tiny drain holes (Flick *et al.*, 1990).

The moisture content of washed meat ranges between 80 to 85%, therefore, it is necessary to reduce it to less then 80% (Pigott and Tucker, 1990). The main objective of dewatering is to optimize the factor keeping in view the protection of protein and to ensure that final moisture content does not exceed 80% (Lanier and Lee, 1992; Flick *et al.*, 1990; Pigott and Tucker, 1990; Lee, 1986). However, the extent of dewatering depends on the freshness of fish, length of screw barrel, compression ratio and the resident time.

2.6. Use of additives in surimi

2.6.1. Cryoprotectant in surimi

The changes in conformation, aggregation and cross-linking of the myofibrillar proteins are known to be accelerated by mincing (Tsuchiya et al., 1975). Alteration in myofibrillar proteins and their functionality have been observed in frozen muscle and isolated protein system in terms of protein solubility (Park *et al.*, 1987) and decreased gel forming ability (Shenouda, 1980; Kim *et al.*, 1986; Park and Lanier, 1987). A high quality surimi can only be prepared from fish whose myofibrillar protein have not been denatured (MacDonald *et al.*, 1990). A surmi that can be stored while retaining its gel-forming capability becomes a reality only with the discovery of additives that can be mixed into the raw surimi to protect the myofibrillar protein from freeze denaturation. Consequently, surimi destined for cold storage is mixed with antidenaturant/cryoprotectant additives before being frozen (Flick *et al.*, 1990). According

to Suzuki (1981), in order to avoid freeze denaturation of minced meat it is necessary to speed up freezing time and control storage temperature apart from using appropriate cryoprotectant.

The addition and mixing of cryoprotectants such as sugar, sorbitol and polyphosphates with the minced meat in order to stabilize the fish proteins from freeze denaturation during frozen storage is known as blending (Lanier and Lee, 1992). Because the mixing procedure may generate heat, the mixer may be equipped with a self-cooling device. A vacuum-mixing chamber may help purge air bubbles from the product. A silent cutter can also be used for mixing the cryoprotectant additives into raw surimi (Flick *et al.*, 1990).

Cryoprotectants are chemical compounds that prevent or stabilize the product during freezing, frozen storage and thawing (Pigott and Tucker, 1990). They act on biological material effectively and have characteristic features such as low volatility (Nash, 1966), considerable solubility in water at molecular level and ability to form a multihydrogen bond, ability to penetrate membranes (Doebbler and Rinfret, 1962) and capacity to dissolve electrolytes. Noguchi (1974) surveyed about 150 compounds, which have cryoprotective effect on carp actomyosin *in vitro* model system and found that about 30 compounds are markedly effective. Compounds such as amino acids, di-carboxylic acids, hydro carboxylic acids, polyalcohol and polyphosphates were found to be effective cryoprotectant. Sodium glutamate at 0.025 M was highly effective, where as lysine, histidine and cyrine had moderate effect. He concluded that the cryoprotectants will have the following structure: (1) a molecule has to posses an essential group either OH, COOH or H_2 and more than one supplementary group (OH, COOH, NH_2SH, SO_3H or OPO_3H_2, (2) the functional group must be suitably placed and properly oriented with each other and (3) the molecule size have to be comparatively small with a low molecular weight. However, most commonly used cryoprotectants are, sucrose (4%), glucose (4.5%), sorbitol (5%), polyphosphate (0.3%) and salt (0.3%) (Pipatsattayanuwong *et al.*, 1995; Noguchi, 1974).

Traditionally, in Japan, compounds like sugar, sorbitol or dextrose have been used as cryoprotectant (Noguchi, 1974). The undesirable sweetness and/or browning tendency caused by the addition of sugar and/or sugar alcohol to the surimi can be eliminated by the use of polydextrose as an alternative cryoprotectant (Lanier and Akahane, 1984). Park *et al.* (1988) reported that poly dextrose could be a substitute for sucrose and sorbitol in Alaska pollock surimi. This was confirmed by Sych *et al.* (1990 a, b, c,) for frozen cod surimi. Polyphosphates are most extensively used in seafood industry (Brotsky, 1980). Sugars synergistically enhance the effect of phosphates particularly under alkaline conditions (Sorenson, 1980). According to Flick *et al.* (1990) the content of polyphosphate ranges

between 0.2 and 0.3% of frozen surimi. Although a higher content is more effective in preserving water retaining and gel-forming capability of surimi, polyphosphates adversely affect the taste of Kamaboko and must be held below 0.3 percent. Cryoprotective effect of sorbitol (Ueno Seiyaku, 1979), hydrolyzed starch (MacDonald and Lanier, 1991;Grabowska *et al.*, 1975), protein fat emulsion (Ajinimoto, 1978) and some amino acids like glutamate, lysine (Fujiwara, 1976) have been reported. Lactitol and palatinit^{R} have cryoprotective effect when incorporated with Cod surimi. It was demonstrated that lactitol dihydrate [D-gelactosyl-B (1-4)-D-Glucitol] possesses cryoprotective properties at 8% level concentration in frozen Cod surimi as well as the level of lactitol in surimi can be maintained up to 6.4% without alteration of stabilizing effect (Sych and Carrier, 1991; Sych *et al.*, 1990 a, b, c). Another cryoprotectant glyceride reduced the size of ice crystal in frozen surimi through its emulsifying action and provides a soft, fine texture to Kamaboko (Flick *et al.*, 1990).

The most common surimi formula is 92% washed minced meat, 4% sugar and 4% sorbitol and this product can be stored up to a year without loss of gelling properties (Regenstein and Regenstein, 1991). A mixture of sucrose (4%), sorbitol (4%) and polyphosphate (0.3%) was fairly effective in surimi (Lee, 1984). Park *et al.* (1986) reported that a mixture of 5.6% sucrose and sorbitol (1:1) have an effective cryoprotective property. Extended frozen storage of surimi is made possible by the addition of 4% sucrose, 4% sorbitol and 0.2% polyphosphate, which inhibit the freeze denaturation of myofibrillar proteins (Noguchi, 1974; Akahane, 1982). Uijttenboogaart *et al.* (1993) reported that sodium ascorbate (0.2%) and sodium tri-polyphosphate (0.2%) and propyl gallate (0.02%) protect the mackerel surimi. Ascorbate acts as reducing agent and free radical scavenger and polyphosphate acts as chelating agent and thus it improves the gelation properties of surimi (Nishimura *et al.*, 1990). Good quality surimi could be made from Pacific whiting with substantial increased gel strength if it is made with beef plasma protein (Morrissey *et al.*, 1992; An *et al.*, 1992). Simpson *et al.* (1994) worked on Pacific whiting using sucrose (4%) and sorbitol (4%) as cryoprotectant along with 0.2% w/w brifisol-S-1 including 1% w/w beef plasma protein as enzyme inhibitor and their results showed that surimi can be stored up to 6 months at –20^{0}C to –50^{0}C with high textural quality.

The activity of proteolytic enzyme in fish muscle causes softening of surimi gel. Cystein protease of Pacific whiting and Arrowtooth flounder seemed to degrade the surimi myofibrillar proteins (Morrissey *et al.*, 1993; Wosson *et al.*, 1992). Stoknes and Rustad (1995) pointed out that proteolytic activity in muscle of Atlantic salmon was optimum at a temperature of 65^{0}C with pH 8. Seymour *et al.* (1994) illustrated that protease activity is high at temperature of 55^{0}C and minimum at 70^{0}C. Chum salmon contains cathapsin and alkaline protease with proteolytic activity during spawning (Konogaya,

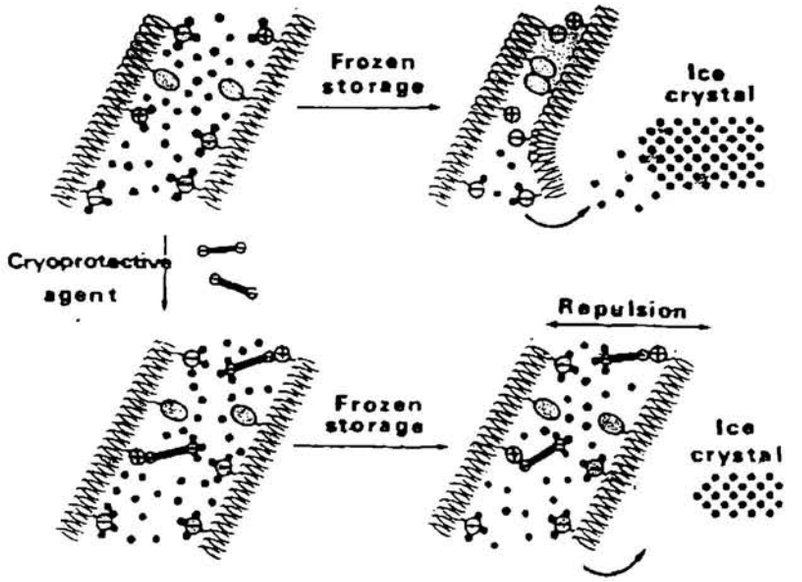
1985; Nomata *et al.*, 1985; Yamashita and Konagaya, 1990). High proteolytic activity occurs in whiting muscle and mince, such activity continues in surimi (Chang-Lee *et al.*, 1989; Morrissey *et al.*, 1992).

Several scientists have used various ingredients along with cryoprotectant as enzyme inhibitor, such as beef plasma protein, egg white, potato etc. (Chang-Lee *et al.*, 1989; Hamann *et al.*, 1990; Proter, 1992; Morrissey *et al.*, 1993). Water-soluble derivatives from potato reduce proteolysis in surimi (Reppond and Babbitt, 1993; Lanier *et al.*, 1981). Porter *et al.* (1990) reported that derivative from potato could be used as an inhibitor for the successful production of Pacific whiting surimi. Pacific whiting can be processed into high quality surimi, having the ability to produce strong and cohesive gels with the use of protease inhibitors (Chang-Lee *et al*, 1990; Morrissey *et al.*, 1992). Ueno *et al.* (1984) reported that addition of beef plasma protein could improve the gel forming ability. Nagahisa *et al.* (1981) studied the effect of several compounds to inhibit the protease in Pacific hake muscle tissue and found that dried egg white powder, oxyacidic salts, peroxide and water extract of potato are effective protease enzyme inhibitors. Arrowtooth flounder could be used to produce surimi if dried beef plasma or egg white were used as an inhibitor of protease enzyme (Wasson *et al.*, 1992).

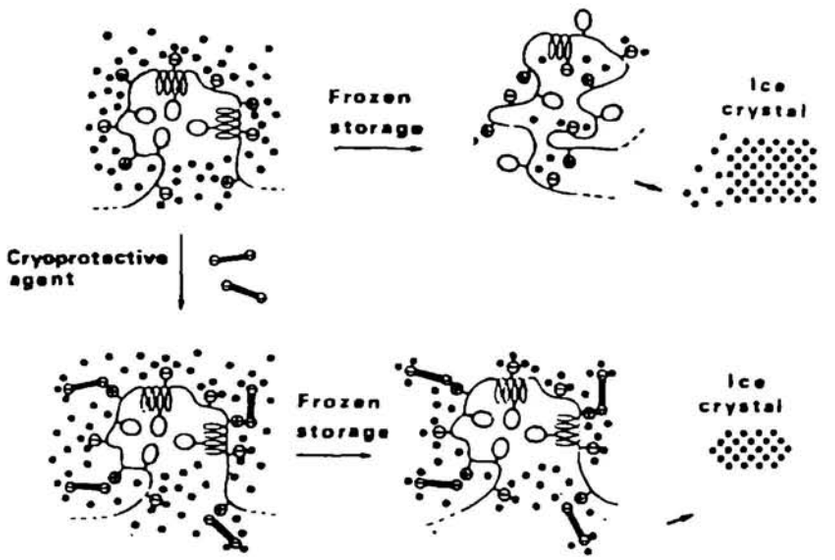
2.6.1.1. Mode of action of cryoprotectant in surimi

Denaturation of protein, rancidity of fat, toughening of texture and dehydration are some of the basic problems, which may cause severe deterioration of fish and fishery product during frozen storage. Uses of cryoprotectants successfully reduce this problem to a great extent. In order to maximize the functionality of surimi the use of cryoprotectants was first proposed by Nishiya *et al.* (1961). Extensive research works have been carried out to evaluate the influencing factors of protein denaturation and also to explain the mode of action of cryoprotective agents (Fennema, 1973; Sikorski *et al.*, 1976; Park, 1985). How these cryoprotectants protect fish protein from freeze denaturation has not been fully answered. Several workers pointed out that surface tension and water binding capacity of cryoprotectants are responsible for preventing the protein denaturation (Buttkus, 1970; Matsumoto, 1979; Sun and Wang, 1984; Salde and Levina, 1988 and Baute, 1994). Matsumoto (1980) has illustrated diagrammatically the prevention of denaturation by cryoprotectant (Figure-I).

Chemically reactive substances are not suitable as cryoprotectants. The effectiveness of cryoprotectants mainly depends on the solubility and melting point of these compounds. The molecular structure influences the hydration capacity and increased hydration decreases the aggregation of proteins. From this theory the effectiveness of monosodium glutamate can also be understood



A schematic model of denaturation (aggregation) of alpha-helical proteins during frozen storage and its prevention by cryoprotectants. —⊕, Cationic side change: —⊖ , Anionic side chain: —○, Nonpolar side chane; •, Water molecule: ⊖—⊖ , Dianionic cryprotectant molecule.



A schematic model of denaturation (unfolding) of nonhelical (globular) proteins during frozen storage and its prevention by cryoprotectants.(Symbols are same as above figure).

Fig. I. Mode of action of cryoprotectants in surimi.

(Matsumoto, 1980). The repulsive force exerted on protein molecules by the anionic group of amino acids and other dicarboxylic acids increase the hydration. The increased hydration of protein molecule, i.e., preferential hydration (Arakawa and Timasheff, 1982), results in slower growth of ice crystal, thereby reducing the degree of protein denaturation (Matsumoto, 1980).

2.6.2. Use of protein additives in surimi

The seafood industry uses a variety of gelling and non-gelling additives in order to maintain the functionality of frozen muscle proteins and to modify the internal structure of actomyosin gel. Sugar, sorbitol, salts, calcium carbonate and protease inhibitors are some of the most commonly used non-gelling additives. Whereas the properties of protein additives, interaction of protein-protein, protein-water and lipid-water are very important in the formation of stable gel network (Regenstein, 1984).

Several researches have been carried out on the protein additives. Chung and Lee (1990) reported that non-muscle proteins such as lactoalbumin, whey protein, egg white, wheat gluten, bovine serum albumin, starches, alginates and carbohydrate gums are principal protein additives used in see food industry in order to increase the gelling properties of minced product. Burgarella *et al.* (1985) studied the effect of adding egg white and whey protein concentrate on attaining the rigidity of croaker surimi and their result showed that mixing myofibrillar protein with whey protein concentrate was better than mixing with egg white. Gnanasambandam and Zayas (1992) reported that in comminuted meat product wheat germ protein, corn protein and soya flour are mainly used as protein additives. Addition of egg white and beef plasma protein increases the shear strain (Park, 1994). Egg white improves the whiteness, texture, glossiness (Akahane, 1983; Lee, 1984; Okada, 1985) and inhibits Modori (Hamann *et al.*, 1990). Apart from these egg white also enhances the gel strength (Alvarez *et al.*, 1992). Rockower *et al.* (1982); Iso *et al.* (1985); Yoo and Lee 1993); Lemmers (1991) have also studied the effect of milk protein, soya protein isolate, whey protein concentrate, dried egg white, soya flour and texturized soya protein on fish.

Use of additives in order to increase the strength may have adverse effect on the texture of surimi. Little information is available in this regard. However, Chung and Lee (1991) reported that the state of water in hydrated biopolymer ingredients and the equilibrium state of moisture distribution between the ingredients (dispersion phase) and the protein gel matrix (continuous phase) have a strong effect on textural properties of heat set gel.

2.6.3. Use of antioxidant in surimi

A major problem with minced fish products is the loss of quality attribute to rancidity (Deng *et al.*, 1977). This problem is becoming especially important with the advent of mechanically separated fish (Silberstein and Lillard, 1978). The washing stage is, therefore, considering as critical as it eliminates major portion of lipid during manufacture of surimi. The residual lipid is the main cause of quality deterioration of surimi during frozen storage. The large proportion of highly unsaturated fats in many fish and the prooxidant contribute to the ease with which the fatty acids undergo various chemical reactions with protein and thereby induce formation of lipid oxidation products, which develop off flavours (Shimizu *et al.*, 1992; Hultin, 1988; Decker and Hultin, 1990 b). Freezing and frozen storage do not completely arrest all possible quality changes and reactions that lead to oxidative changes which can proceed at low temperature, although slowly. However, this problem can be solved satisfactorily by the use of suitable antioxidants in surimi.

Antioxidants not only prevent development of off-odour and off-taste and also protect the texture. Ozilgen and Ozilgen (1990) proposed the action of antioxidant. According to them, the antioxidants block the propagation process, donate a hydrogen atom to free radical and the free radicals of antioxidants are stabilized by resonance and enter termination.

Butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT), tertiary butylated hydroxy quinone (TBHQ), propyl gallate and tocopherol are some of the commonly used antioxidant (Sarkar, 1997). They can be used either directly or in combination. There is a growing concern over the use of synthetic antioxidant and certain spices are known to possess antioxidant properties (Barbut *et al.*, 1985). Joseph *et al.* (1989) reported that use of clove at 0.2% level extends the self life of mackerel mince and it is more effective than pepper and cinnamon. Sodium erythrobats, EDTA and sodium tri-polyphosphate were found to be effective in controlling rancidity (Iredale and York, 1977). The use of EDTA and citric acid along with antioxidant possess a synergistic effect. Vynche (1978) reported that 0.5% tochopherol and 0.01% BHQ reduce lipid oxidation in Mackerel. BHA and BHT increased the storage life of frozen fish (Hudson, 1990; Hultin *et al.*, 1992; Labuza, 1971). But Ke *et al.* (1977) pointed out that propyl gallates and TBHQ are more effective than BHA and BHT in fish and fishery products. Mestiri *et al.* (1992) have reported the effectiveness of tocopherol and polyphosphate.

2.7. Quality characteristics of fish surimi

2.7.1. Composition

Although the Japan Surimi Association (JSA) quality standards for surimi were revived in 1978, in practice the 1974 standards continue to be influential among most of the country's surimi manufacturers. The manufacture of surimi alters its composition from the original mince (Flick *et al.*1990). Flesh from of typical white-flesh species of a fish contains about 80% water and less than 1% carbohydrate by weight (Watts and Merrill 1963). Commercial surimi could contain water between 75 and 85% and carbohydrate between 5% and 10% depending on its intended use (Lee 1986; Miyauchi *et al.*1973; Sonu 1986). The moisture content and pH were considered as the prime criteria to judge the quality of surimi (Suzuki, 1981). According to Regenstein and Regenstein (1991), a good quality surimi should contain about 79 to 81% of moisture and a pH value of 6.8 to 6.9.

Surimi contains proportionally less protein than mince. The protein content of surimi lies between 12 and 15% but the quality of the surimi protein is very high. The washing step of minced meat is responsible for the decrease of protein content due to removal of sarcoplasmic protein and NPN fractions. Many authors have reported the decrease in protein content during washing (Bligh and Regier, 1976; Grantham, 1981; Roussel and Cheftel 1988).

Flick *et al.* (1990) reported that the fat present on the underside of skin in dark fleshed fish is liable to oxidation resulting in fishy odour and discoloration of flesh. Most of the lipid present in species typically used in surimi making is removed when wash water, lipid and blood are decanted from the washing tanks (Miyake *et al.*, 1985). Pigott and Tucker (1990) and Suzuki (1981) reported that in raw Pollock surimi fat content is less than 1%. Nettleton (1985) pointed out that loss of water-soluble vitamin-B complex is quite expected during washing.

Most surimi produced today contain very little sodium chloride and is referred to as 'low-salt surimi'. The small amount of sodium chloride present in most surimi comes from a very dilute sodium chloride solution used at the end of the washing step in order to aid in subsequent dewatering (Lee, 1986). However, the colour, texture and flavour are important quality aspects of surimi (Park and Morrissey 1994).

2.7.2. Functional properties of surimi

Rhee (1985) reported that the physical and chemical properties like gel forming ability, emulsifying capacity, foaming capacity, water holding capacity, solubility, viscosity, fat binding capacity

etc. are some of the important functional properties of surimi. The behavior of myofibrillar protein in the food system is the function of these properties (Lanier, 1986). Because of the dynamic structure and amphiphilic nature of fish protein, they have varying functional properties.

Protein is one of the most important classes of functional ingredients as they show versatility during processing. Functional properties can be defined as an effect induced by an ingredient on either the organoleptic properties of a food such as flavour, odour, texture, appearance or on the mincing properties of the food during its processing like extrudability, resistance to tear or breakage etc. Therefore, functional properties not only depend on ingredient added to the food but also affected by the processing (Lanier and Lee, 1992).

Denaturation of the functional salt-soluble proteins of surimi prior to the point of final use will decease their effective functionality. Kim *et al.* (1985) noted that denaturation of surimi proteins induced by cyclic freezing and thawing caused a dramatic drop in the elasticity of gels prepared from this surimi, while the rigidity of the same gel actually increased as a result of the treatment.

2.7.2.1. Gelling properties

Montejano *et al.* (1984) reported that a gel is a semisolid material, which has certain degree of rigidity, plasticity and brittleness. It is becoming increasingly recognized that each of the functional properties like water fat and particle binding and texturization are linked to the formation of a stable gel network structure in the food (Acton *et al.*, 1983). However, "gelation is a process of protein aggregation, resulting in well balanced territory network which trap within a large quantity of water" (Schmidt, 1981).

Both myosin and actomyosin have dominant role in surimi gelation and show species specificity with regard to properties of gelation (Miyake and Hayashi, 1956; Shimizu *et al.*, 1981). The first process of formation of gel is turning the myofibrillar protein into a sol state by addition of salt. Sol formation is due to loss of microstructure of myofibrillar protein in water with help of salt (Niwa, 1992). It is found that 2.5 to 3% NaCl produces an optimum gelling effect in terms of gel strength and compliancy (Lanier *et al.*, 1985). Stronger and more elastic gels can usually be obtained by preheating mince fish paste at a temperature near 40^{0}C for short time or by refrigerating overnight prior to further heating. (Okamura, 1961; Ueda *et al.*, 1968 a; Suzuki, 1981; Lanier *et al.*, 1982). The former phenomenon is referred to as "high temperature setting" while the latter is termed "low temperature setting" (Wu *et al.*, 1985 a, b). Degradation of the gel structure sometimes occurs at temperature near

60°C extensively due to the activity of heat-stable proteolytic enzymes (Ikeuchi and Simidu, 1963; Makinodan and Ikeda, 1971; Lanier *et al.*, 1981; Shimizu *et al.*, 1981).

The characteristic texture and elasticity of certain surimi-based products depend on cold setting. The fish muscle protein can set at lower temperature as compared to the other animal protein (Wu *et al.*, 1985 a, b). Niwa *et al.* (1980) and Numakura *et al.* (1987) reported that myosin is the most important protein affecting the gel forming ability of fish during lower temperature setting. The Ca^{++}ATPase associated with the myosin molecule enforces the gel strength of low temperature-set surimi (Funatsu and Arai, 1992; Numakura *et al,* 1989). Excessive heating results in tough, rubbery analog (Lee, 1986). Thus gel-based product is influenced by cooking temperature and length of time during processing (Lee 1984, Wu *et al.*1985 a, b; Douglas – Schwara and Lee, 1988; Montejano *et al.*, 1984).

Protein concentration, ionic strength, pH, type of meat and heating condition influence gelation (Asghar *et al.*, 1985). The temperature for optimum gelation is species dependent (Shimizu *et al.*1981; Kamath *et al.*, 1992). Fish muscle proteins also differ in their critical gelling temperature compared to proteins of egg white, whey, pork or beef muscle and chicken or turkey muscle (Lanier *et al.*, 1982; Montejano *et al.*, 1984). However, surimi analogs are traditionally made from heat-set gels at temperatures approaching 90°C (Lee, 1984). Gel formation is mediated by disulfide linkage and hydrophobic interaction (Chan *et al.*, 1993; Itoh *et al.*, 1979). In addition the formation of network also depends on salt linkage and hydrogen bond (Beas and Crupkin, 1990; Roussel and Cheftel, 1990; Niwa, 1992).

Chen (1995) reported that ionic strength (salt concentration) has great influence on the gel forming ability of surimi. He also found that the sensitivity to the ionic strength is species dependent. Sardine surimi gel gets affected by salt concentration, blending, operation, heat treatment and moisture content (Alvarez *et al.*, 1995). The effect of high hydrostatic pressure (HHP) on gel strength of Pacific whiting and Alaska pollock surimi has been studied by Chung *et al.* (1994). He pointed out that there is three fold increase in strain and stress for HHP treated whiting gels made without inhibitor. Decrease in microorganism, enzymatic activity and change in rheological characteristics in several food systems are caused by high hydrostatic pressure. (Farr, 1990; Okamoto *et al.* 1990; Hoover, 1993).

Shiba (1992) and Shiba and Numakura (1992) studied the advantage of ohmic heating over ordinary heating with respect to higher gel strength. They also reported that the gel strength of Walley

pollock, White croaker, Threadfin bream and Sardine was improved by ohmic heating compared to heating at 90°C in a water bath. Ohmic heating not only inactivates the endogenous enzyme but also improves the gel without enzyme inhibitor and produces a surimi with high shear stress and strain (Yongsawatidigul *et al.*, 1995; Yongsawatidigul and Park, 1996).

Two-step heating is much more effective in order to improve the gel strength than that of one step heating. The effect of two-step heating is more at lower temperature from 25°C to 30°C. Nowsad *et al.* (2000) observed the species variation in the disintegration of the gel. The association or disassociation between actin and myosin and changes in species conformations of myofibrillar protein is referred as Modori (Sano *et al.*, 1988). The proteolytic degradation of fish myofibrillar protein by heat stable proteinase groups is attributed to Modori phenomenon (Makinodan *et al.*, 1987; Toyohara *et al.*, 1990). Klesk *et al.* (2000) reported that the optimum heat treatment for tilapia surimi appeared to be 40°C for 1 hour followed by cooking at 90°C for 15 minutes.

Proteolytic degradation of fish proteins can also impair their ability to gel, thus effectively reduces functionality. In particular, a heat activated alkaline protease activity is often found in surimi (Lin and Lanier, 1980; Lanier *et al.*, 1981). Several physical factors such as freshness, fishing method, season, size and species also determine the gel forming ability of fish muscle protein. In general white-fleshed fish possess a better gelling ability than fatty fish (Shimizu *et al.*, 1981).

2.7.2.2. Viscosity

Bourn (1982) defined viscosity as the internal friction of fluid or its tendency to resist flow. It is usually denoted by $\eta=\delta/\gamma$, where η is the viscosity, δ is the shear stress and γ stands for shear rate. Protein denaturation and aggregation during frozen storage can be measured by viscosity (Colmenero *et al.*, 1988). Viscosity can be used as an index of fish protein quality and it is more reliable than protein solubility and emulsifying capacity (Colmenero *et al.*, 1988; Gandhi *et al.*, 1968; Groninger *et al.*, 1983). Viscosity also provides important information on physico-chemical interaction of the protein molecule (Kinsella, 1976; Rha and Pradpasena, 1986).

Tejada *et al.* (1984) reported the role of myofibrillar and sarcoplasmic protein on viscosity of marine fish muscle homogenate. Ueda *et al.* (1968 a,b) studied the mechanism of actomyosin heat denaturation by viscosity measurement and reported that actomyosin of some fish species are more easily denatured than those of others. The changes in apparent viscosity of muscle homogenate are

related to change in actomyosin (Borderias *at al.*, 1985). Depending on the origin of myosin and type of meat, the protein functionality differs significantly. (Matsumoto, 1980; Colmenero and Borderias 1983; Turgut 1984; Asghar *et al.,* 1985; Whittle *et al.*, 1988). The viscosity is also influenced by protein concentration, pH and ionic strengh. Thus the viscosity plays an important role in food and it depends on intrinsic environmental and processing factors (Cofrades *et al.*, 1993).

2.7.2.3. Water holding capacity (WHC)

The water holding capacity is defined as the water absorbed by a dried protein powder after equilibrium against water vapour of known relative humidity (Kinsella, 1976). Differences in water holding capacity are characteristic of different muscle (Bouton *et al.*, 1971). The water retention properties of protein include expressible moisture (EM) and water binding potential (WBP). These factors have influence on the texture, juiciness and flavour of meat (Gerald *et al.*, 1983; Regenstein and Regenstein, 1984).

Protein source, composition and presence of carbohydrate, lipid, pH and salt are considered as important factors which have significant influence on the water holding capacity of protein (Hermansson *et al.,* 1972). The change in water holding capacity may take place due to a change in value of myofibrillar protein (Gerald *et al.,* 1983). Shindo *et al.* (2000) established the relationship between the critical water content and denaturation of myofibrillar protein in fish surimi. The ability to hold the added moisture can be predicted on the basis of original proportion of moisture and protein content (Swift and Berman, 1959). Hamm and Deatherage (1960) reported that water retention is inversely related to protein content and increase in pH enhances the water holding capacity.

Kosiba and Jawarek(1979) found that mechanically deboned cooked meat had a very limited water holding capacity when compared to the mechanically deboned raw meat because of denatured proteins. The thermogravimetry analysis (TGA) showed that the surimi and surimi/tapioca/carrageenam had higher water retention ability when heated (Barreto *et al.*, 2000).

2.7.2.4. Protein solubility

Proteins are widely used as functional ingredients in manufacture of processed foods. The functionality of protein influences the sensory properties of food (Hung and Zayas, 1992). The protein solubility (PS) is an excellent index of protein functionality. PS has a great influence on the *textural* properties of fish indicating the potential applications (Kinsella, 1976). According to Hermansson and

Akesson (1975), the knowledge of solubility of protein is a good index about its application and also helps to optimize the processing condition.

Kinsella (1982) reported that the extractibility of protein is a function of pH. Therefore, it is better to study the solubility of that protein in different ionic strength (Mattil, 1971). The denaturation of myofibrillar protein can be measured by the quantity of extracted myofibrillar protein from the muscle by salt solution with 0.45-0.6 ionic strength (Bate and Smith, 1948; Zender *et al.*, 1958; Migita, 1961). pH and temperature of meat have great influence on the solubility of myofibrillar protein. Konagaya and Konagaya (1979) reported that low pH and high temperature cause protein denaturation resulting in low solubility.

Bigelow (1967) pointed out that protein with lower average hydrophobicity and higher charge frequency would have higher solubility. Some scientists found a decrease in the solubility of myofibrillar protein during the rigor process. Whereas some other found a higher solubility during rigor in the case of muscle of Red sea bream, Yellow tail and Goby (Suzuki and Migita, 1962).

2.7.2.5. Foaming

Foam can be defined as a two-phase system, consisting of air cells separated by thin liquid lamellar phase due to its large liquid gas interfacial area. Nakai (1983) reported that protein surface properties are mainly responsible for foam development. Energy is required during the formation of foam and it is fundamentally unstable (Halling, 1981).

Foaming properties at different level of protein have been investigated (Cherry and McWatters, 1981; Kasaric and Ng, 1983; Multilangi and Kilara, 1985; Patel and Fry, 1987). Foaming properties of protein can be determined by various methods based on shaking, spurging and whipping (Hailing, 1981). The effect of pH (Bera and Mukherjee, 1989; LeMeste *et al.*, 1990; Phillips *et al.*, 1990), presence of additives (Phillips *et al.*, 1990; Poole, 1989) and ionic composition and strength (Richert, 1979) on the foaming properties of protein has also been studied. Changes in foam expansion with increasing concentration are similar in most animal proteins (Brittle and Lavoie, 1992). In Japan surimi obtained form shark are utilized for the production of marshmallow textured surimi based product because the shark muscles have an excellent foaming properties (Suzuki, 1981).

2.7.2.6. Emulsifying capacity

Kinsella (1982) defined the emulsifying capacity as the volume of oil (ml) that can be emulsified by protein before phase inversion or collapse of emulsion occurs. The emulsifying capacity of protein is usually determined by emulsion stability (ES), Emulsion capacity (EC) and emulsion activity index (EAI). The emulsion stability refers to the ability of protein to form an emulsion that remains unchanged for a particular duration, under specific condition. However, emulsification is one of the important functional properties in wide variety of food proteins.

David (1978) reported that emulsification is brought about by homogenetion of two immiscible phases normally oil and water. In the first stage, for oil-water emulsion, small spherical droplets are formed by homogenetion. In the second stage, for a stabilized emulsion, the oil droplets formed during homogenetion must remain suspended in the continuous phase.

Tejada *et al.* (1984) studied the role of myofibrillar and sarcoplasmic protein on emulsifying capacity of marine fish muscle homogenates. Salt soluble proteins are more suitable for emulsification than that of watersoluble protein (Saffles, 1968; Schut, 1976). Gaska and Regenstein (1982 a, b) reported that watersoluble proteins do not take part in emulsion formation. Emulsifying capacity of fish meat largely depends on protein solubility (Grabowska and Sikorski, 1976). A defined correlation exists between the water oil absorption index (WOAI) and the emulsifying capacity and on the basis of this relationship it is possible to predict emulsifying capacity (Dekantrewiez *et al.,* 1987). Strength compactness, elasticity and electrical properties of the interfacial film around the oil droplets also influence emulsion stability (Powrie and Tung, 1976). The protein molecules have high molecular weight and they possess both hydrophilic and hydrophobic properties simultaneously, which enable the protein to be absorbed at the oil-water interface and decrease interfacial tension between the two phases. This reduces the mechanical energy required to produce a given emulsion droplet size (Cante *et al.,* 1979). The use of microscopical methods for quick and accurate measurement of EC has been suggested by Swift *et al.* (1961). But this may consequently affect the other parameters such as pH, ionic strength, concentration of a disperse phase and added surfactants in emulsion.

2.8. Changes in surimi during frozen storage

Oxidative rancidity of fat, denaturation of protein, ice crystallization, dehydration, changes in intramolecular conformation, changes in pH and ionic strength are some of the common deterioration processes which take place during frozen storage of minced meat and their products. Crawford *et al.*

(1972) studied the change in protein and fat of minced fish meat during frozen storage. The denaturation of protein during frozen storage is responsible for decrease in gel forming ability, water holding capacity and fat emulsifying capacity (Park *et al.*, 1988; Matsumoto, 1980; Hsu, 1990). Simpson *et al.* (1994) reported that the loss of salt soluble protein, gel functionalities, stress an strain decrease notably during frozen storage of surimi because of he use of cryoprotectant in surimi. However, the quality changes, which occur during frozen storage, are of great economic importance (Mills, 1975).

2.8.1. Degradation of fat

Washing step of minced meat significantly reduces the level of lipid in surimi. Tiwari (1995) has reported a reduction of 73.5% fat after 3 washing cycle. The residual lipid is the main cause of quality deterioration of surimi during frozen storage. The large proportion of unsaturated fats in many fish (Standal *et al.*, 1975) is responsible for oxidative rancidity of the products and thereby develops an off-flavour. The lipid oxidation is influenced by the factors such as the amount of lipids, their susceptibility to autooxidation (Ke *et al.*, 1982), the level of heme compounds (Castell and Bishop, 1969; Fischer and Deng, 1977), the level of microsomal system associated with lipid oxidation present (McDonand *et al.*, 1979) and the presence of metal ions (Allen *et al.*, 1979). Non-enzymatic oxidation is predominat in frozen stored mince (Broderias *et al.*, 1978). Polyphosphates are added to the minced meat to enhance the protein functionality and water binding capacity. They also have antioxidant properties, particularly in combination with other additives (Morris and Dawson, 1979; Tableros, 1980).

2.8.2. Changes in protein

Frozen storage is associated with the denaturation of fish meat protein functionality (Matsumoto, 1979, 1980; Park *et al.*, 1987). The structural and chemical properties of muscle proteins are profoundly affected by the extended frozen storage. As a result the quality attribute of muscle food products are affected significantly. Fish mince has a shorter frozen storage life than intact fillets or whole fish (Babbitt *et al.*, 1972; Hiltz *et al.*, 1976).

Protein denaturation occurs due to the formation of ice crystals, surface dehydration and cell rupture (Mishra and Srikar, 1989). Protein denaturation affects the textural quality of breaded sticks or portion made form the affected blocks. It also reduces the functional property (binding), which is a critical factor for manufacturing heat-gelled products (Sorenson, 1975). According to Anon (1976), the storage changes in frozen minced fish can be suppressed considerably at low temperature, but it is not maintained commercially.

Lanier *et al.* (1980) reported that the solubility and gel forming ability of myofibrillar proteins are found to be optimum when they are in an undenatured state. Fluctuating temperature during frozen storage reduces the gel forming ability of fish proteins (Suzuki, 1981). At freezing temperature the myosin of fish muscle protein denatures at a faster rate (Suzuki 1981). The aggregation of denatured fish muscle proteins occurs mainly on myosin (Sikorsky *et al.*, 1976), actin (Jiang, 1984), tropomyosin and troponin (Irisha *et al.*, 1978). Wagner and Anon (1986) reported that the denaturation of protein occurs during freezing and frozen storage due to the unfold of myosin head region by weakening of the actomyosin which result in loss of protein functionality. The solubility of the myofibrillar protein also depends on the freezing rate, frozen storage temperature and prefreezing conditions (Dyer *et al.*, 1956; Reddy and Srikar, 1991). High frozen storage temperature causes insolubilization of myofibrillar protein (Fukuda *et al.*, 1981).

The changes in conformation, aggregation and cross-linking of the myofibrillar proteins are known to be accelerated by mincing (Tsuchiya *et al.*, 1975). Solubility in 0.6 M NaCl or KCl and ATPase activity are used to measure the extent of myofibrillar protein denaturation (Matsumoto, 1980; Wagner and Anon, 1985). Koning *et al.* (1985) reported that there was a considerable reduction in soluble protein with a simultaneous decrease in organoleptic rating from 4.5 to 0.5. They also pointed out that the formation of free fatty acids (FFA) and denaturation of protein are related phenomenon. Infact myosin molecules are prerequisite to produce strong and elastic gel and such characteristics are affected by the degree of intra and inter strand cross linkage (Schmidt, 1981).

Myofibrillar protein of fish is less heat stable than mammalian muscle (Takashi, 1973; Arai *et al.*, 1973). Johnston *et al.* (1975) reported that myosin from tropical fish is mare stable than cold water fish. Park *et al.* (1988) and Sych and Carrier (1991) have reported a loss of salt soluble protein in cryoprotected surimi during frozen storage. The aggregation of protein during frozen storage results in firmer fillets with low water holding capacity (Sikorski *et al.*, 1976). However, the stability and shelf life of surimi depend on the frozen storage condition (Matsumoto and Noguchi, 1992) and freshness (Lin and Morrissey, 1995).

Trimethylamine oxide (TMAO) is found in the flesh of Gadoid variety (Dingle *et al.*, 1974). It breaks down further in the presence of kidney tissue, blood and subcutaneous brown flesh to produce dimethylamine (DMA) and formaldehyde (FA) (Tokunaga, 1974; Castell et al., 1971; Bremner, 1980). FA then attacks the myofibrillar muscle protein rendering it insoluble and thereby toughening the texture

and reducing water holding capacity. (Sikorski *et al.*, 1976; Matsumoto, 1979). Toughening of the flesh due to protein denaturation has been suggested to be caused by cross-linking of proteins produced by FA during the enzymatic degradation of TMAO to DMA and FA. (Gill *et al.*, 1979). This reaction is more pronounced in Alaska pollock mince compared to its muscle (Babbitt *et al.*, 1984).

The morphological changes of Common carp (*Cyprinus carpio*) during frozen storage have been studied by Okada *et al.* (1986) by measuring the ATPase activity, viscosity and total -SH groups in myosin B in the presence of 0.1 or 0.6 M KCl when stored at –20^{0}C. The deformation of the filaments observed is attributed to denaturation. Shaban *et al.* (1985) reported a decrease in the ratio of myofibrillar protein to total protein from 80% at the beginning to 30% after 12 months in case of Alaska pollock surimi during frozen storage at –20^{0}C, while the levels of sarcoplasmic protein and NPN substances remained almost constant throughout the one-year period of storage.

Verma and Srikar (1994) suggested a significant inverse correlation between salt soluble nitrogen (SSN) and peroxide value (PV), free fatty acid (FFA), trimethyl amine (TMA) as well as total volatile base nitrogen (TVBN). Emulsifying capacity and protein solubility have significant relationship to the native structure of protein (Srikar and Reddy,1990; Valkert and Klein,1979). A relationship between change in viscosity, protein solubility (PS) and emulsifying capacity (EC) are influenced by time elapsed before freezing, type of freezing and seasonal variation of fish. Denaturation induced by freezing process reduces the EC (Grabowsaka and Sikorski, 1974). High correlation was found between EC, viscosity and PS in frozen fish during frozen storage (Colmenero and Borderias, 1983). Salt soluble proteins are responsible for emulsion formation (Gaska and Regenstein, 1982 b). Temperature, pH, protein source, solubility type of oil used, salt, equipment design and shape of container, speed of blending and rate of oil addition are some of the critical factors which influence the EC. Change in the viscosity is minimum at –30^{0}C and maximum at -10^{0}C in presence of KCl whereas in the presence of NaCl the value is maximum between –20^{0}C to -10^{0}C (Takahashi *et al.*, 1993).

A loss of salt soluble proteins during frozen storage of minced meat obtained from Cucumber fish was observed (Bremner, 1977). Abdullah and Yu (1985) observed a similar reduction in the solubility of muscle protein in 5% NaCl solution in Chub mackerel. Agarwal *et al.* (1986) observed a rapid decrease in salt soluble nitrogen during the first 16 weeks, water-soluble nitrogen during the first 24 weeks of storage (at -20^{0}C) in the minced meat of Lacterius and Dhoma, following a gradual decrease in the subsequent period up to 32 weeks.

2.9. Microbiology of surimi and surimi based seafoods

The initial step in the transformation of mince to surimi is a thorough washing. This step can have several microbiological effects. The wash water removes significant number of bacterial cells from the mince (Licciardello and Hill, 1978). Traditionally, the temperature of the wash water is no less than 10^0C. Thus, psychrotrophic bacteria present in the mince may be capable of growth during the wash step.

Another step in surmi making that has microbiological ramification is the addition of carbohydrate antidenaturant compounds. These compounds may occasionally be contaminated with bacterial spores and constitute an abundant carbon source in addition to the proteins and amino acids already present. It is well known that many bacteria grow best in carbohydrate-rich media, and it has been found that the APC of Atlantic pollock surimi rises slightly faster than that of Atlantic pollock mince during storage at 5^0C and 13^0C (Ingham and Potter, 1987).

The native microbial load of surimi varies depending on length of time the fish is held before processing. About 90% of the bacteria isolated form the sample of surimi were Gram-negative and the predominat genera were *Pseudomonas, Acinetobacter* and *Moraxella* (Elliot, 1986). Similarly, Japanese researchers reported that total viable cell counts for blocks of frozen surimi were 10^5-10^6 cfu/g. One prolific strain of *Enterobacter cloacae* was found to cause browning of surimi (Fujita *et al.*, 1974). Genera of bacteria isolated from kamaboko include *Pseudomonas, Flavobacterium, Corynebacterium, Lactobacillus, Bacillus* and *Micrococcus* (Sasayama 1973).

One type of spoilage reported to affect Kamaboko was softening and slime production resulting from growth of *Bacillus licheniformis*. The slime was a levan made from sucrose in the surimi (Mori *et al*, 1973 b). The source of *B.licheniformis* was found to be potato starch used in the preparation of the Kamaboko (Mori *et al.*1973a). *Leuconostoc* has also been found to produce dextran slime on kamaboko (Uehiyama and Amano, 1959). *C. botulinum* toxin production was found to occur in inoculated Kamaboko stored at 30^0C (Sasajima *et al.*, 1978).

Several preservation techniques for surimi-based foods have been studied. Modified atmosphere packaging (MAP) may find use with surimi-based foods. An additive mix of refined alcoholic rice fermentation products and amino acids was tested as a preservative for smoked

Kamaboko and was found to inhibit growth of coiiforms (Ishida and Watanabe 1981). Dipping of Kamaboko in lysozyme solution has also been found to increase shelf life (Akashi and Oono, 1972).

2.10. End products of surimi

Surimi is the intermediate raw material from, which the end products called NERISEIHIN (Surimi-based products) are manufactured (Wu, 1992). About 90% of surimi-based products are various types of fish cakes called KAMABOKO. Less than 10% of surimi-based products are represented by fish sausage, fish ham, and fish bur-gers. Imitation crab and other surimi-based shellfish analogs may be included under Kamaboko type. Such products are mainly known as analogs and imitation products. In addition to white colour and bland taste, gel-forming properties of surimi is the most important quality attribute for such products (Flick *et al.*, 1990).

Kamaboko products are divided among three major categories: steamed, boiled and fried. Typical streamed Kamboko is called ITATSUKI (board-mounted) KMABOKO, but the variety also includes imitation seafood, naruto, hapen and spongy marshmallow-like products, which contain entrapped air. The typical boiled Kamaboko is CHIKUWA, which has the shape of a hollow bamboo stem. Typical fried Kamaboko products are SATSUMA AGE and TEMPURA. Kamaboko is also given various names depending on product shape, such as SASA (bamboo leaf shaped), SOBA (noodle shaped), DATE-MAKI (whirled or rolled) and so on (Flick *et al.*,1990).

The main ingredient of Kamaboko is about 85% of surimi (Miyake *et al.*, 1985). The raw or frozen surimi with salt (Ka-en) or without salt (Mu-en) is grounded into a paste with sugar, potato-starch, sodium glutamate and egg white to obtain Kamaboko (Suzuki, 1981). Surimi based imitation scallop products contain 60% surimi, 25% water, 5% egg white, 4.3% starch, 1.5% scallop essence, 1% salt, 0.5% sweet sake and 2.7% chemical sea soning (Flick *et al.*, 1990). Surimi-based products are prepared by extruding the surimi paste into various shapes resembling such shell fish meat as king or snow crab legs, crab claws, lobster tails, scallops or shrimp. The closer the analog resembles the natural product, the greater the extraction sophistication (Flick *et al.*, 1990). According to the fabrication and structural features, Flick *et al.* (1990) divided surimi-baesd products into four categories as follows.

1) Molded products

Molding the chopped surimi into the desired shape and allowing it to set and form an elastic gel makes molded products. Molding may be accomplished by either a single extrusion or a co-extrusion. Co-extrusion gives a meat like texture, where as the single extrusion results in a uniform and rather rubbery mouth feel (Flick *et al.*, 1990). The molding process of lobster and shrimp can be carried out under atmospheric pressure (Kawana, 1988) or reduced pressure (Nishimura *et al.*, 1986) or elevated pressure (Hice and Webb, 1985). Restructured shrimp from broken or odd-shaped shrimp of low value and shrimp flavored surimi-based products are included in this category.

2) Fiberized products

Fiberized products are made by extruding the paste into a thin sheet through a rectangular nozzle having a narrow opening. The extruded sheet is then partially heat set and cut into strips of desired width by a cutter, similar to a noodle cutter. Surimi used in this process should be of top grade so that the paste remains sufficiently cohesive and elastic while it is stretched, cut and pulled. Fine strips are preferred for the fibrous crab-leg product, whereas wider strips are more suitable for the simulated shellfish in the form of sea flake or chunk. The crab-leg product is produced by a straight cut, but the flake and chunk types are formed by an oblique cut (Miyake *et al.*, 1985; Flick *et al.*, 1990; Wu, 1992). Crab analog and scallop analog are two main examples of fiberized product.

3) Composite-molded products

For composite-molded products, the strings of desired length are mixed with or without surimi paste and extruded into a desired shape. Strings are produced by slicing a block of surimi gel into thin rectangular sheet, followed by stripping into a desired width. This type of product gives a better bite than the strictly molded variety, which tends to be rubbery and uniform in texture. Composite-molded products are found in chunk form and sold mixed with fiberized products. Another type of composite-molded product called "fish ham" is prepared by mixing the dice of cured tuna and pork into fish paste before extrusion (Flick *et al.*, 1990; Wu, 1992).

(4) Emulsified products

To make the emulsified type of product, surimi is treated similarly to meat when it is processed for emulsion products. The level of fat added is usually less than 10%, and the type of fat used is not limited to animal fat. In fact, vegetable oil is often added, because, unlike mammal and bird meat, fish

meat readily produces a stable emulsion with oil. Sausage-type and wiener-type of products mainly belong to this category (Flick *et al.*, 1990).

2.11. Quality assurance of surimi

The producers are committed to create and maintain a system to ensure customers satisfaction through quality product by continuous quality improvement, elimination of wastes and development of professional skills and expertise. Conventionally the quality of surimi is examined against a criteria or specification. However, this method is considered inefficient, impractical and expensive. Corrective or preventive action is applied to the degree appropriate to the magnitude of the problem and commensurate with the risks encountered. To avoid the limitation of end product quality control and to assure microbiological safety of the product, a new concept named Hazard Analysis Critical Control Point (HACCP) has been developed by ICMSF (1988). It is a rational and logical approach to identify and control hazards at various points of production. The key elements of HACCP are the identification of hazards and assessment of risks. ISO 9000 series is the basis of HACCP approach of quality assurance. Huss (1992) studied the developments and use of HACCP in fish processing. HACCP assures the control at source and hence results in a defect free product (Kenney, 1992; Jakobsen1993).

Chapter-III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Raw Material

Fresh and live Common carp (*Cyprinus carpio*) caught off the pond in Kanchrapara, 24 Parganas (N) were used in the present study. The fishes were processed within 3 h of harvesting and the processing was carried out under hygienic condition. During processing the temperature of the raw material was kept as low as possible by using sufficient amount of chilled water. The materials were divided into three groups. One group (Control) i.e. whole fish was kept as such in frozen storage at –35^{0}C and its freshness was analyzed for 120 days with an interval of 15 days. From second group, dewatered mincemeat was made and kept at -35^{0}C for similar analysis. From the third group surimi was prepared and also analyzed for its freshness.

3.1.2. Chemicals used

All the chemicals used in the analysis were either of 'Analytical or Guarantied reagent' grades.

3.1.3. Bacteriological media

Nutrient agar obtained from 'Hi-Media', Mumbai was used for estimating total plate count (TPC).

3.1.4. Glasswares

The glassware's used were all 'Borosil' made.

3.1.5. Equipments

3.1.5.1. Processing equipments and accessories

• **Meat picking machine**: To separate meat from skin and bones of dressed fish, single phase motor driven, Roll type (belt and drum type), fish meat picking machine supplied by 'Stadler Corporation' was used. The drum of the machine is made of stainless steel and belt is of food grade synthetic material. The capacity of the machine is 20 kg/h (approximately).

• **Fish meat mincer**: Model S-1, cast-graded stainless steel, 304 housing with double cutting system meat mincer supplied by ' Stadler Corporation' was used. The capacity is 30 kg/h (approximately) with gear drive.

• **Bowl chopper**: Model SB-2, laboratory model with 3 blades in ox, cast stainless steel bowl and covers single speed motor with 2 speed controls bowl chopper supplied by 'Stadler Corporation' with batch capacity of 61 L was used.

• **Hand operated screw press** (Traditional type)

• **Deep freezer**: Horizontal model deep freezer supplied by 'Anonym' with a capacity of 380 L and temperature range of $-35^0 \pm 1^0C$ was used.

• **Vessels and utensils**: All the vessels and utensils were made of stainless steel.

• **Processing tables** (Stainless steel)

3.1.5.2. Analytical instruments:

- Kjeldahl digestion system (Borosil)
- Kjeldahl distillation unit (Borosil)
- Muffle furnace (BITA)
- Hot air oven (Instrumentation India)
- IIC, Bacteriological incubator
- REMI, R8C Laboratory centrifuge
- Microprocessor based spectrophotometer with column dot matrix printer (Systronics)
- Sterilizer (Instrumentation India)
- pH meter (Systronics)
- Electronic single pan balance (Dhona 100 DS)
- Vacuum oven (Anonym)

3.1.5.3. Packaging material

Low-density polyethylene (LDPE) bags were used for packing the fish as well as surimi.

3.2. Methods

3.2.1. Study of Raw material characteristics

3.2.1.1. Physical characteristics

Total length, standard length and weight of 30 fish selected at random, were measured. The yield of picked meat was calculated based on the whole fish and dressed fish separately.

3.2.1.2. Chemical characteristics

Methods used to assess the freshness of the fish are described in the section 3.2.4., 3.2.5. and 3.2.6.

3.2.1.3. Sensory characteristics

Fresh Common carp were evaluated for freshness by using descriptive scoring for appearance, texture, colour and odour of raw fish on 9-point hedonic scale (Shewan *et al.*, 1953). The overall acceptance of the fish was also assessed. The mean score of 8 trained panelists was calculated for each attribute.

3.2.1.4. Microbiological characteristic

The microbiological characteristic of fresh fish was carried out according to the standard method recommended by APHA (Speck, 1976). Appropriate dilution of homogenate were made in a physiological saline (0.85%) and plated into duplicate on nutrient agar, by pour plate method. Incubation was done at 37^{0}C temperature for 48 h.

3.2.2. Standardization of washing procedure

In the present study, the minced meat was subjected to water washing, using chilled water (5^{0}C), in order to remove blood, pigment, fat and components with low molecular weight. Minced meat was subjected to repeated washing and settling. In order to improve the colour, as many as four times washing were done with 5 minutes of soaking in between washings. In the present study the ater to After each wash, meat was gently squeezed in a muslin cloth to remove as much water as possible and the meat was almost devoid of fishy odour after the last washing. 0.1% NaCl solution was used during the last washing in order to facilitate easy removal of water in further processing steps. There after, the meat was subjected to pressing and the moisture content of the product was maintained about 80%.

3.2.3. Production of surimi

After dewatering the partially dehydrated meat was mixed with 4% sorbitol and 0.3% sodium tripolyphosphate in a bowl chopper for 2 minutes at 15-18^{0}C. This washed mince and surimi were packed in LDPE bags and stored at –35^{0}C temperature. The procedure used for production of surimi is given in the Figure-II. The dewatered mincemeat and whole fish were also stored at same temperature.

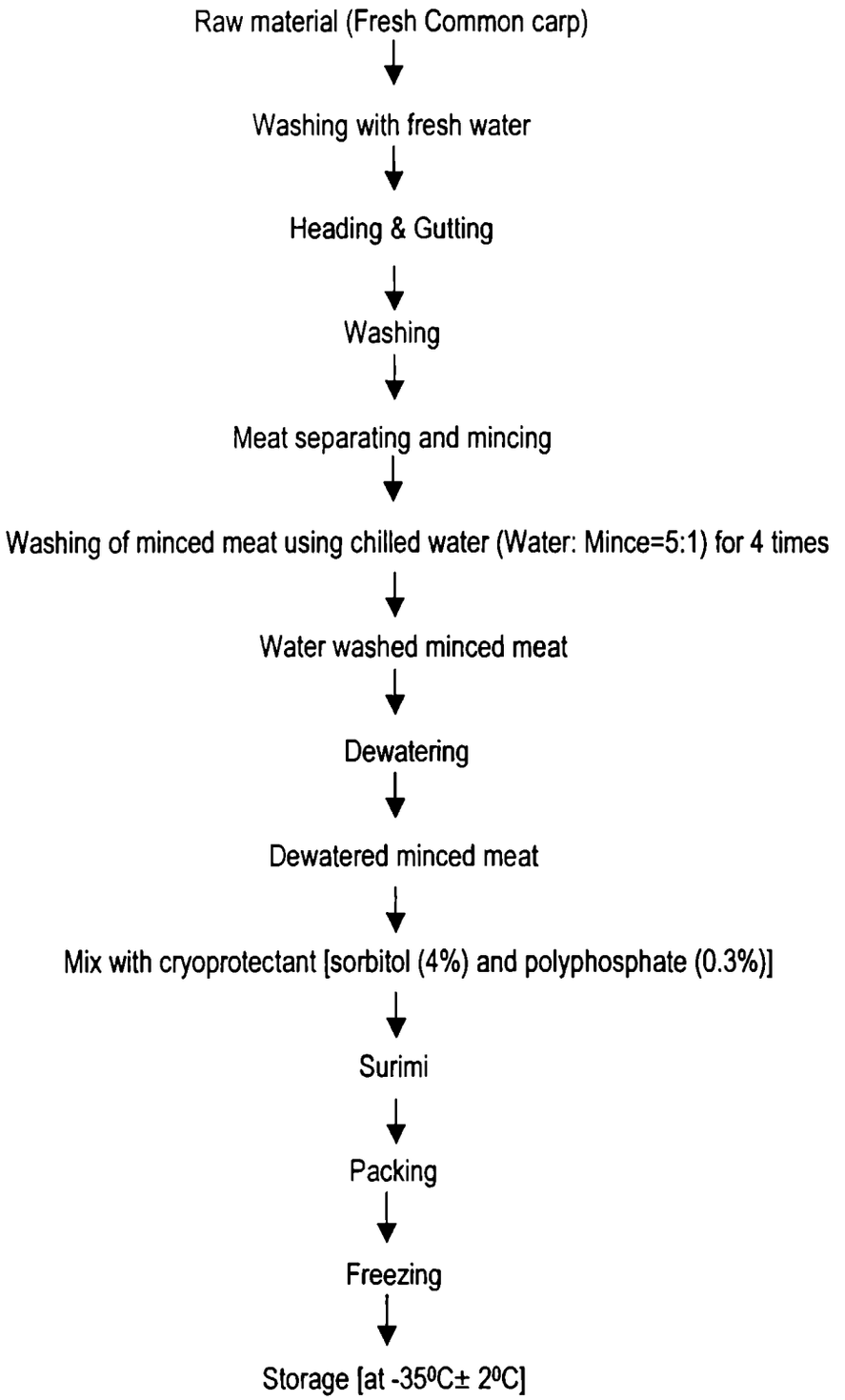


Figure-II Flow diagram of surimi production

The quality characteristics of all the 3 groups were analyzed. The effect of freezing or frozen storage on the quality change of minced meat and surimi, in comparison with whole fish were studied. The beneficial effect of preservatives likes sorbitol, salt and sodium tri-polyphosphate in improving the shelf life of the product was assessed.

3.2.4. Chemical composition

3.2.4.1. Moisture

Moisture content was determined by the standard hot air oven method (AOAC, 1995). About 5 g of sample from whole fish and minced meat were taken in moisture bottles and dried in a hot air oven, maintained at $105^0C \pm 2^0C$ for 12 hours. The weight loss was expressed as moisture content percentage of the samples.

3.2.4.2. Crude protein

The total nitrogen was estimated by Kjeldahl method (AOAC, 1995). Crude protein value was calculated by multiplying the total nitrogen value by a factor of 6.25.

About 1g of meat sample was transferred to 250 ml of digestion tube and 10-12 ml of concentrated. sulphuric acid and 0.2 g of digestion mixture were added-and the sample was digested on digestion chamber till a clear digest was obtained. After cooling, the volume was made up to 100 ml with distilled water. Then 2 ml of digested solution was taken for distillation in Kjeldahl distillation unit with 40% sodium hydroxide solution. The liberated ammonia was absorbed in 2% boric acid solution containing mixed indicator (2% methyl red and 2% methylene blue in 1:1 ratio and dissolved in ethyl alcohol), the pink colour of the boric acid turns green. The boric acid was titrated against N/140 standard hydrochloric acid until the solution turned pink. Total nitrogen was calculated and expressed as g/100 g of sample.

3.2.4.3. Crude fat

Fat content of the moisture free sample is determined by extracting the fat with a suitable solvent as recommended by AOAC (1995). Petroleum ether can be used as the solvent. Extraction is done by using Soxhlet apparatus.

10 g of the moisture free sample is transferred carefully to an extraction thimble. The thimble is placed inside a Soxhlet apparatus along with petroleum ether. The electrical heating unit is adjusted

properly so that the solvent siphons over 5-6 times per h and extract the fat in the solvent for 16-20 h. The extraction thimble is removed and placed in a glass dish. The remaining solvent is evaporated at 100°C in an oven (about 2 h). With the aid of a spatula, the entire residue from the thimble was transferred to a glass dish. The weight of the glass dish containing the fat free residue is measured. The fat content of the sample was expressed as g/100 g of samples.

3.2.4.4. Total ash

The ash content was measured by the method of AOAC (1995).

Moisture free samples were incinerated in a muffle furnace at a temperature of 600°C± 50°C for 4-5 hours and the values expressed on wet weight basis as percentage. As content of the sample was expressed as g/100 g of sample.

3.2.5. Quality changes during storage

3.2.5.1. Total volatile base nitrogen (TVB-N)

The TVB-N an index of spoilage of fish of all the samples was determined by the method recommended by EIC (1995).

100 g of sample was blend with 300 ml of trichloro acetic acid (5%) solution and then filtered to obtain a clear extract. 5 ml of extract was distilled with 5 ml 2(N) sodium hydroxide. The distillate was collected in 15ml 0.01(N) hydrochloric acid containing 0.1 ml rosolic acid indicator. After distillation, excess acid was titrated by using 0.01 (N) sodium hydroxide to a pale pink end point. A blank was also determined. The TVB-N of the sample was expressed as mg/100 g of sample.

3.2.5.2. Alpha amino nitrogen (AAN)

The AAN in the sample was estimated following the copper method of Pope and Stevens (1939). The amino acids react with excess copper present in the form of cupric phosphate and the amount of copper taken into solution by amino acid or similar materials is determined iodometrically. About 20 g of sample was ground thoroughly with 40 ml of 7% Trichloro acetic acid (TCA) solution and filtered and made up to 100 ml in volumetric flask and 50 ml of the extract was taken for estimation. The results are expressed as mg/100 g of sample.

3.2.5.3. Salt soluble nitrogen (SSN)

The SSN was estimated by the method of Dyer *et al.* (1950). 5 g of sample was homogenized at about 4^{0}C in a tissue homogeniser for 3 min using chilled 5% sodium chloride solution, buffered with 0.02 M sodium bicarbonate and pH was adjusted between 7 and 7.5 using 0.1(N) HCl. The total volume of the homogenate was made up to 100 ml. It was centrifuged at 4000 rpm for 10 min and then nitrogen content of 2 ml supernatent was determined by Kjeldahl method and expressed as percentage of total nitrogen.

3.2.6. Determination of lipid quality

3.2.6.1. Peroxide value (PV)

The peroxide value (PV) of the lipid was determined from the lipid extract, as described by Jacobs (1958), iodometrically. 10 g sample was taken and ground well with 15 g anhydrous sodium sulphate. Then transferred to a 100 ml stoppered flask and 30-50 ml of chloroform was added and placed in dark place for about 15-20 min with occasional shaking. 10 ml of chloroform extract, 25 ml of solvent (2 volume of glacial acetic acid and 1 volume of chloroform), 1ml of potassium iodide solution and 35 ml of water were added. The liberated iodine was titrated against standard sodium thiosulphate solution and expressed as mili equivalent of peroxide/kg of lipid.

3.2.6.2. Thiobarbituric acid (TBA) value

This test, developed by Bernheim *et al.* (1948) was refined and adopted by Yu and Sinnhuber (1957) and was considered to correlate more closely than the peroxide number with sensory measurements of oxidative rancidity.10g of blended fish was mixed with 50 ml distilled water in a blender. It was then transferred into a flask. Then blender was washed with 47.5 ml distilled water and washing was also transferred to the flask. 25 ml of 1:2 HCl was added to the solution and then distilled. 1 ml of distillate was taken in a test tube and 1 ml of TBA reagent and 5 ml of distilled water were added and then heated in water bath for 30 min. Then cooled and red color developed was measured at 538 nm using a spectrophotometer. TBA value was expressed as mg of malonadehyde/Kg of sample.

3.2.7. pH

The pH of the sample was determined by method described by Suzuki (1981). About 10 g of the sample were blended with 90 ml of distilled water and pH of the blend or homogenate was measured directly by the help of a pH meter.

3.2.8. Changes in sensory quality

Sensory attributes like appearance, texture and odour of whole fish, dewatered mince meat and surimi were analyzed during four months of storage with an interval of 30 days on 9-point hedonic sacle (Shewan *et al.*, 1953). The mean scores of 8 trained panelists were calculated for each attribute.

3.2.9. Statistical analysis

Analysis of variance (ANOVA) (Snedecor and Cochran, 1962) were calculated to find out the significant difference in the sensory characteristics of samples. The Duncan's Multiple Range Test (DMRT) was followed to identify the homogenous subsets of treatemt. To find out the relationship between various methods used to assess the quality changes during storage, correlation co-efficient (r) was also calculated.

3.2.10. Development of HACCP concept in surimi

The Hazard Analysis Critical Control Point (HACCP) was applied to the surimi processing line. The strategy as outlined in ICMSF (1988) was used for the identification of critical control point in surimi production.

Chapter-IV

RESULTS

4. RESULTS

Fresh Common carp (*Cyprinus carpio*) used in present work were analyzed for physical, chemical, microbiological and sensory characteristics. Experimental trials were conducted on quality of whole fish, dewatered mincemeat and surimi to assess the changes of the above mentioned samples stored over a period of four months. The results of these analyses are given in following sub-sections.

4.1. Characteristics of raw material

4.1.1. Physical characteristics

The physical characteristics of fresh fish studied are shown in Table-I. The average weight and average total length of the Common carp were found to be 535.38 g and 28.8 cm respectively. The average standard length of fish was measured to be 24.3 cm. The yield of dressed fish was found to be 62.53% and yield of picked meat was 35.88% from whole fish and 57.38% based on dressed fish.

4.1.2. Chemical characteristics

4.1.2.1. Freshness evaluation

The result of freshness evaluation such as TVB-N, AAN, SSN as well as lipid quality of fresh fish such as PV, TBA values are outlined in Table-II.

4.1.2.2. Proximate composition

The proximate composition of the fillet and dewatered mincemeat are delineated in Table-III. The moisture and protein contents of fillet were found to be 76.90% and 17.78%, respectively. The dewatered mincemeat had higher moisture and lower protein content. The lipid content of the fillet was measured to be 3.16% on w/w basis. The lipid content was also less in dewatered mincemeat as compared to the fillet. As the fillets was used for analysis, the ash content was less than 1%.

4.1.3. Microbiological characteristics

Only the TPC of the raw material was determined. The result is shown in Table-IV. The TPC of the raw material was 5.2×10^5 / g of sample.

4.1.4. Sensory characteristics

Results of the sensory evaluation for all attributes of both uncooked and cooked fishes depicted in Table-V. The results of both samples were between 'like very much' and 'like moderately' based on 9-point hedonic scale.

4.2. Analysis of samples

The surimi prepared from fresh fish was analyzed to compare with dewatered mincemeat (DWM) and whole fish with an interval of fifteen days for four months.

4.2.1. Nitrogenous compounds

The various nitrogenous compounds analyzed during frozen storage are TVB-N, AAN and SSN. The results are delineated in Table-VI, VII, and VIII, respectively.

4.2.1.1. TVB-N

The Table-VI and Figure-III represent the TVB-N value of all samples during storage. The TVB-N value of all the samples increased gradually during storage. TVB-N ranged from 2.78 mg% to 16.64 mg% in whole fish after four months of study. The DWM and surimi also exhibit similar kind of characteristics like whole fish. The TVB-N value of surimi (13.95 mg%) was less as compared to the whole fish (16.64 mg%) and DWM (17.38 mg%) after four months of storage period.

4.2.1.2. Alpha amino nitrogen (AAN)

The AAN increased significantly during storage in all the samples (Table-VII and Figure-IV). During four months study the AAN content increased from 5.54 mg % to 28.41 mg% for whole fish. After four months the AAN contents of surimi and dewatered mincemeat was 26.58 mg % and 32.41 mg %, respectively.

4.2.1.3. Sait soluble nitrogen (SSN)

SSN contents of all the samples during storage period are outlined in Table-VIII and Figure-V. In fresh fish the SSN content was about 73.34% of the total nitrogen with a steady decrease to about 40.92% after four months. The DWM showed slightly lower value (60.34%) at the end of fifteen days than whole fish (64.82%). But the rate of decrease during storage was much lower in surimi as

compared to whole fish and DWM. After four months the SSN content of surimi was found to be 49.20%.

4.2.2. Lipid characteristics

4.2.2.1. Peroxide value (PV)

The peroxide values for whole fish dewatered minced meat (DWM) and surimi are presented in Table-IX and Figure-VI. PV ranged from 17.1 to 44.48 milliequivalent of peroxide per Kg of total lipid after three months and showed decline at the end of four months in whole fish. The DWM (49.03 milliequivalent/Kg of fat) and surimi sample (42.23 milliequivalent/Kg of fat) also showed similar kind of characteristics like whole fishafter four months of storage.

4.2.2.2. TBA value

The changes in TBA value of whole fish, dewatered mince meat (DWM) and surimi are depicted in the Table-X and Figure-VII. The highest TBA value of 6.95 mg of malonaldehyde per Kg sample was observed in DWM after three months. The increase in TBA value was more in dewatered mincemeat than surimi. After four months of study the TBA value of DWM and surimi were 3.82 and 3.13, respectively.

4.3. pH

Table-XI and Figure-VIII show the pH value of whole fish, dewatered mince meat (DWM) and surimi during frozen storage of four months. pH values are almost constant during the entire storage period. The range of pH values in whole fish, DWM and surimi are 6.78 to 6.88, 6.68 to 6.86 and 6.84 to 6.85, respectively.

4.4. Sensory characteristics

Table-XII gives the average panel mean score for whole fish, dewatered mince meat (DWM) and surimi. The average mean score for appearance, texture and adour showed sudden decline after 90 days. The DWM sample was acceptable when it was initially prepared using fresh fish but the score decreased with increase storage period.

4.5. Statistical analysis

To know the relationship between the various methods used for quality assessment, correlation coefficients were calculated and shown in the Table-XV. The mean panel scores of different treatment

for various attributes on different sampling days were analyzed using two-way analysis of variance technique (ANOVA). Table-XIII depicts the results of ANOVA. No significant variation ($p>0.05$) was observed between days and attributes. But significant difference ($p<0.01$) was observed between days and treatment and between treatment and attributes. The DMRT of sensory scores due to treatment showed dewatered mince meat and whole fish as homogenous subsets as they did not show any significant difference ($P>0.05$). In contrary, surimi showed significant difference ($p<0.05$) with whole fish and dewatered mincemeat. The results of DMRT are shown in Table-XIV.

4.6. Hazard Analysis Critical Control Point (HACCP)

The critical control point in the production of surimi is presented in Figure-IX.

Table-I Physical characteristics of raw material

SL.NO.	CHARACTERISTICS	MEAN
1.	Average length (cm)	28.80
2.	Average standard length (cm)	24.30
3.	Average weight (g)	535.38
4.	Yield of dressed fish (%)	62.53
5.	Yield of picked meat (Based on whale fish) (%)	35.88
6.	Yield of picked meat (Based on dressed fish) (%)	57.38

Table-II Chemical characteristics of raw material

SL.NO.	CHARACTERISTICS	MEAN
1.	TVB-N (mg %)	2.78
2.	AAN (mg%)	5.54
3.	SSN (g%)	73.34
4.	PV (milliequivalent /Kg of fat)	19.10
5.	TBA (mg of malonaldehyde/Kg of sample)	1.14

Table-III Proximate composition of fresh common carp and dewatered mincemeat

PROXIMATE COMPOSITION (%)	FILLET	DEWATERED MINCE MEAT.
Moisture	76.90	80.34
Protein	17.78	14.57
Total lipid	3.16	1.12
Total ash	0.93	0.75

Table-IV Microbiological characteristics of raw material.

TPC/ gm of sample	5.2×10^5

Table-V Sensory characteristics of raw and cooked meat of Common carp.

SENSORY ATTRIBUTES	RAW	COOKED.
Appearance	8.2	8.6
Odour	7.4	8.2
Texture	8.4	7.5
Colour	8.3	8.8
Overall acceptability	8.0	8.4

Table-VI Change in total volatile base nitrogen (TVB-N mg%) in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	2.78		-
15	3.50	3.85	3.27
30	5.93	6.10	5.45
45	7.31	8.94	6.46
60	8.57	10.31	7.32
75	10.16	12.27	9.60
90	12.78	13.35	10.20
105	14.98	15.83	11.32
120	16.64	17.38	13.95

Figure-III Change in total volatile base nitrogen (TVB-N mg%) in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage.

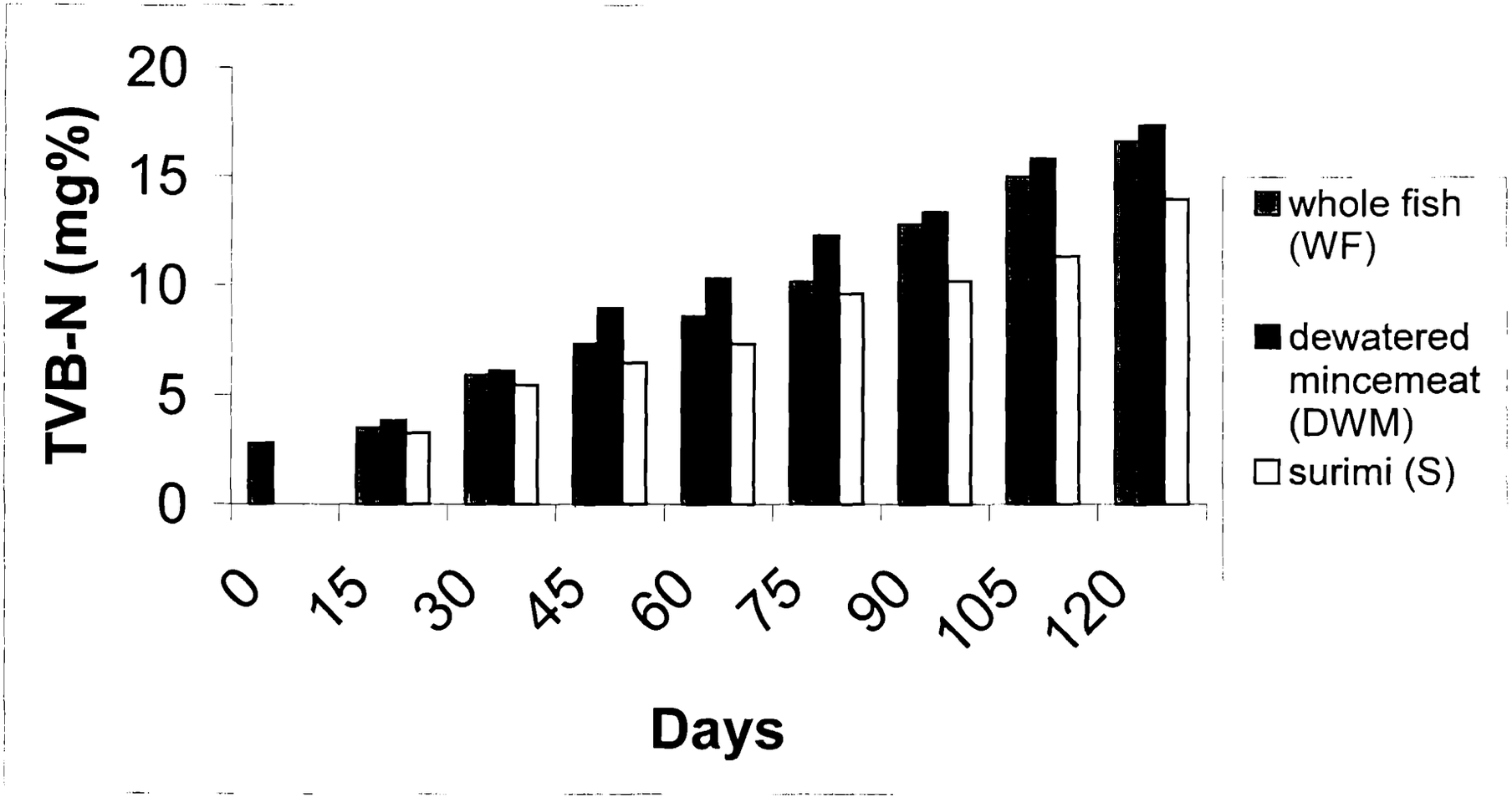


Table-VII Changes in α-amino nitrogen (AAN mg%) content in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	5.54	-	-
15	9.33	11.50	9.10
30	13.12	15.33	12.73
45	16.10	18.76	15.62
60	19.13	22.20	18.71
75	21.13	25.50	20.60
90	23.10	27.83	22.60
105	25.75	29.10	24.54
120	28.41	32.41	26.58

Figure IV Changes in α -amino nitrogen (AAN mg%) content in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage.

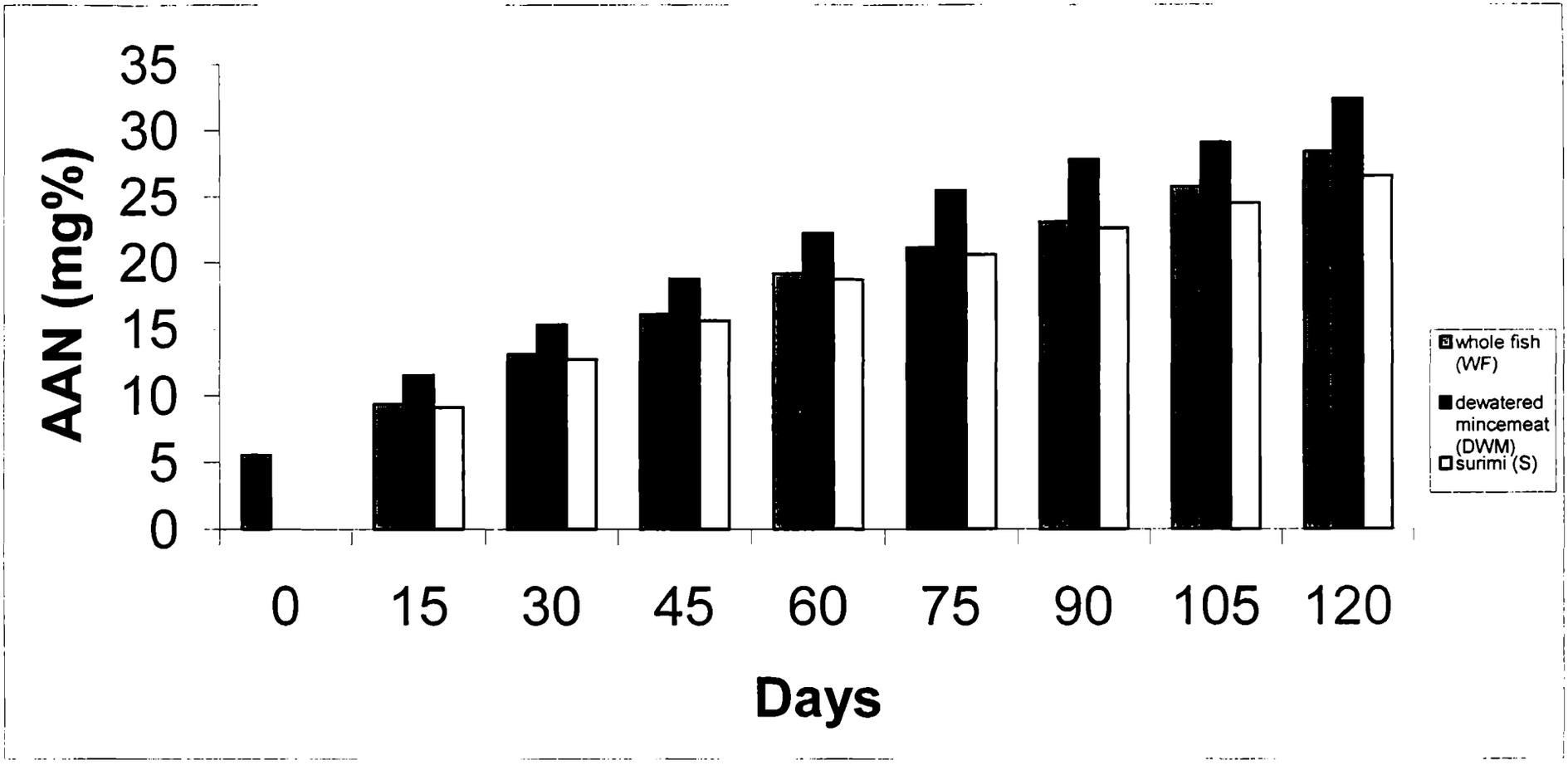


Table-VIII Changes in salt soluble nitrogen (SSN g% of total protein) in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM).	SURIMI (S)
0	73.34	-	
15	64.82	60.34	69.84
30	57.31	53.95	67.43
45	55.75	51.32	64.48
60	52.86	48.93	61.32
75	51.05	46.82	59.88
90	49.43	42.46	58.43
105	45.89	40.37	53.31
120	40.92	38.31	49.20

Figure-V Changes in salt soluble nitrogen (SSN g% of total protein) in whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage.

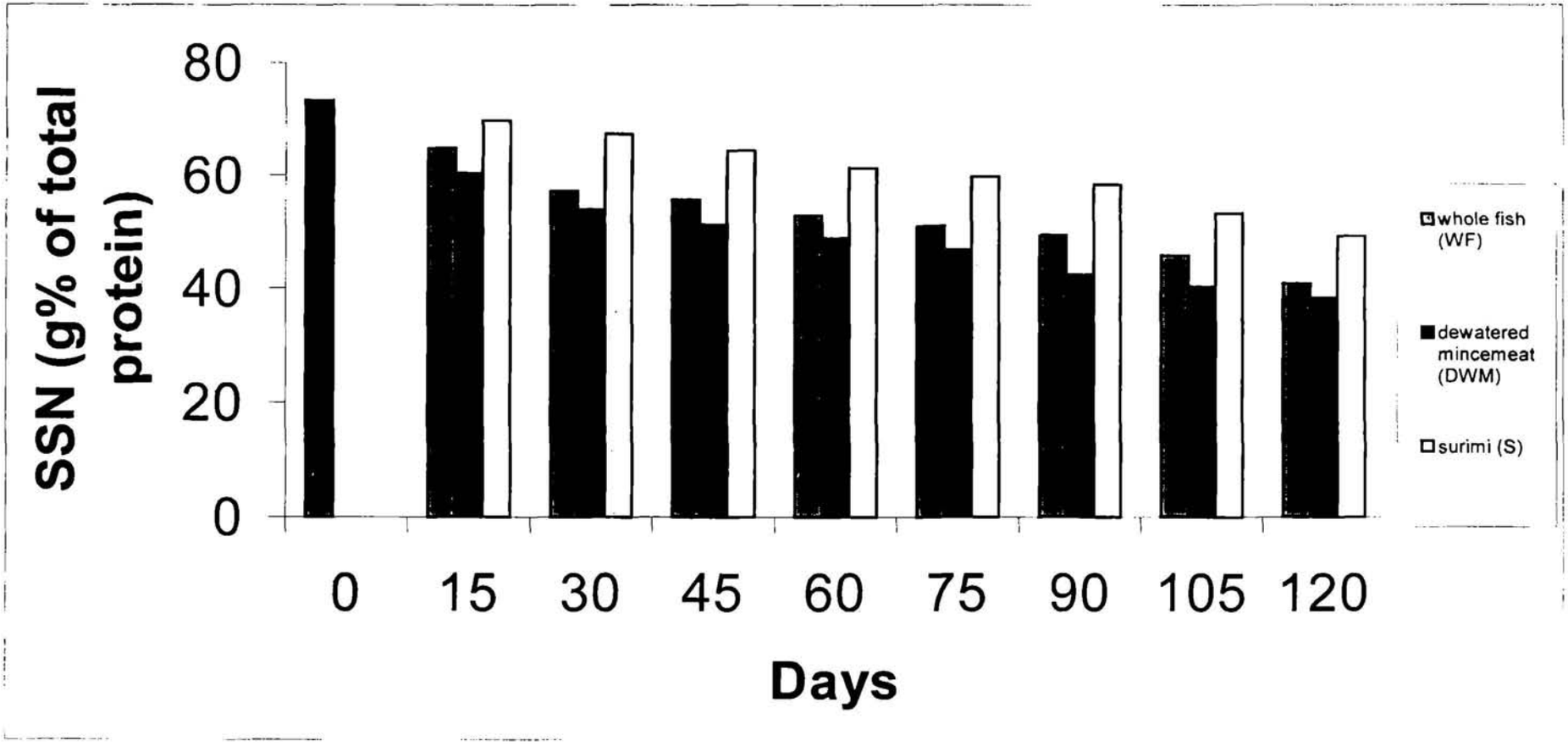


Table-IX Changes in peroxide value (PV Milliequivalent/Kg of fat) in whole fish (WF), dewatered mincemeat (DWM) and surimi(S) during frozen storage.

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	17.1	-	-
15	26.38	30.63	28.57
30	33.23	38.23	35.93
45	35.55	42.42	37.82
60	39.86	46.31	40.84
75	42.02	49.55	45.43
90	44.48	54.67	49.36
105	41.76	51.39	44.17
120	37.15	49.03	42.23

Figure-VI Changes in peroxide value (PV milli equivalent/Kg of fat) in whole fish (WF), dewatered mince meat (DWM) and surimi (S) during frozen storage.

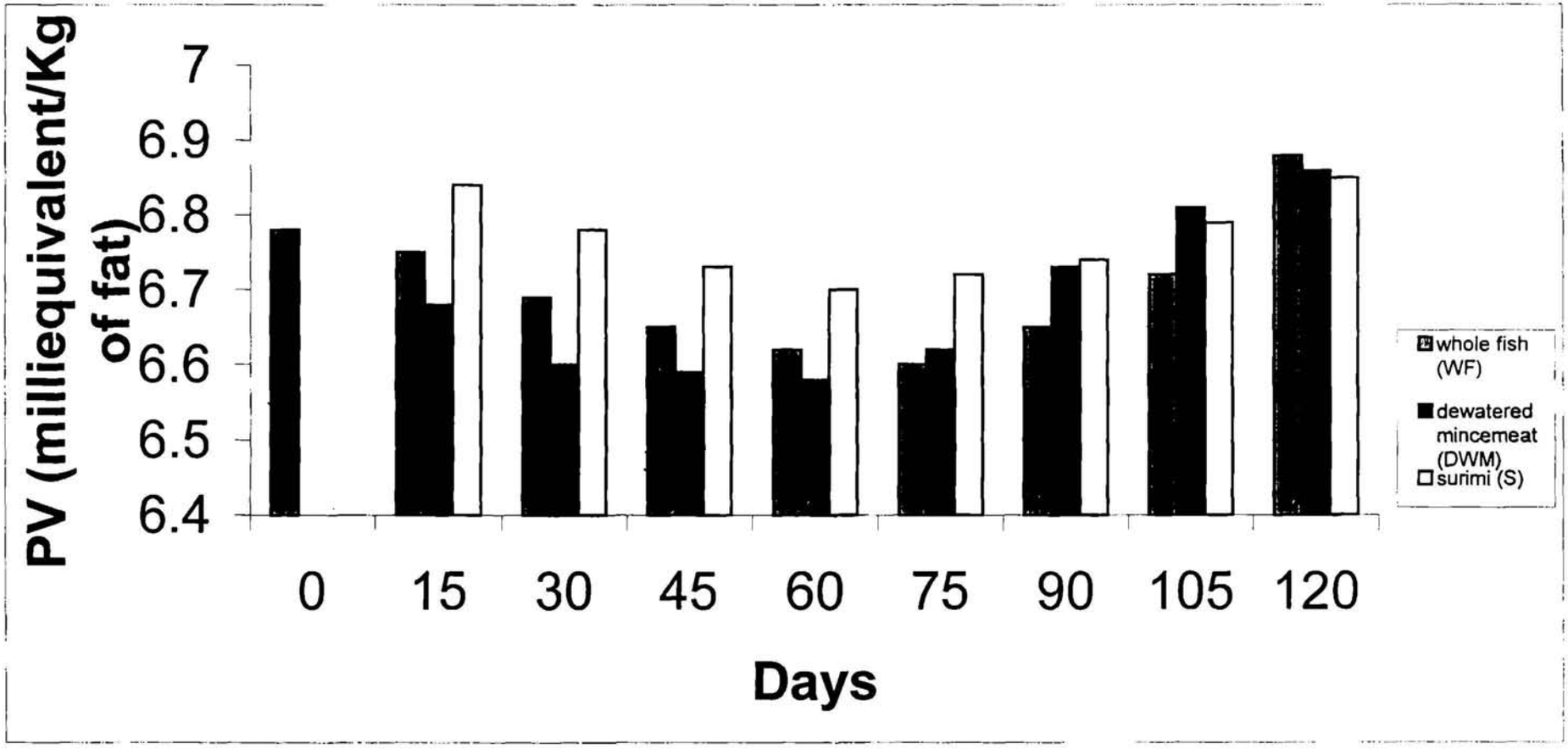


Table-X Changes in TBA (mg of malonaldehyde/Kg of sample) in frozen whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	1.14	-	-
15	1.98	2.62	2.32
30	2.76	4.03	3.05
45	3.86	5.98	3.49
60	4.03	6.35	3.74
75	5.69	6.87	4.08
90	6.03	6.95	4.53
105	5.46	5.08	3.82
120	4.28	3.82	3.13

Figure- VII Changes in TBA (mg of malonaldehyde/Kg of sample) in frozen whole fish (WF), dewatered mince meat (DWM) and surimi(S) during frozen storage.

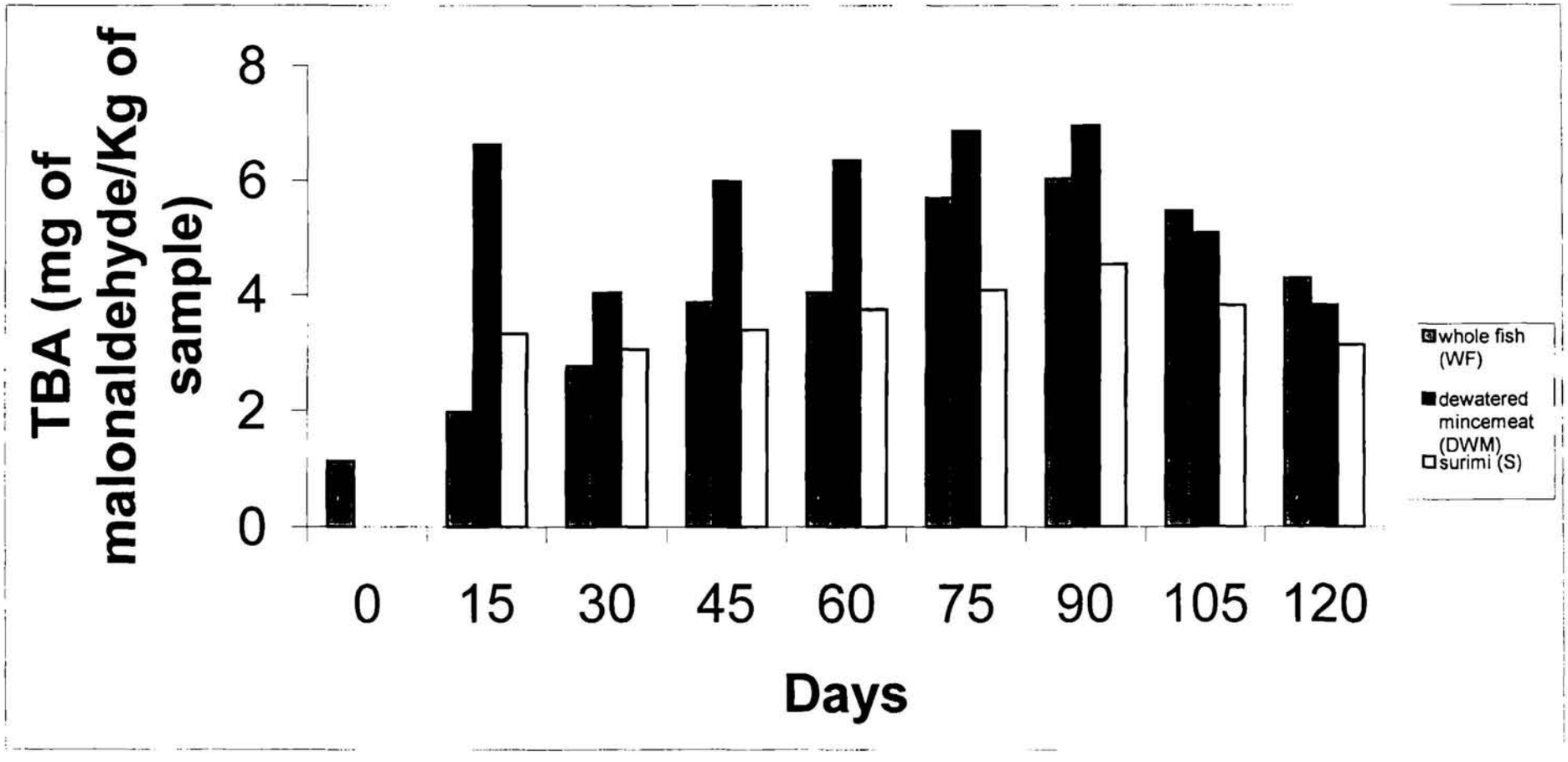


Table-XI Changes in pH in frozen whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage.

STORAGE PERIOD (DAYS)	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	6.78	-	-
15	6.75	6.68	6.84
30	6.69	6.60	6.78
45	6.65	6.59	6.73
60	6.62	6.58	6.70
75	6.60	6.62	6.72
90	6.65	6.73	6.74
105	6.72	6.81	6.79
120	6.88	6.86	6.85

Figure- VIII Changes in pH in frozen whole fish (WF), dewatered mince meat (DWM) and surimi (S) during frozen storage.

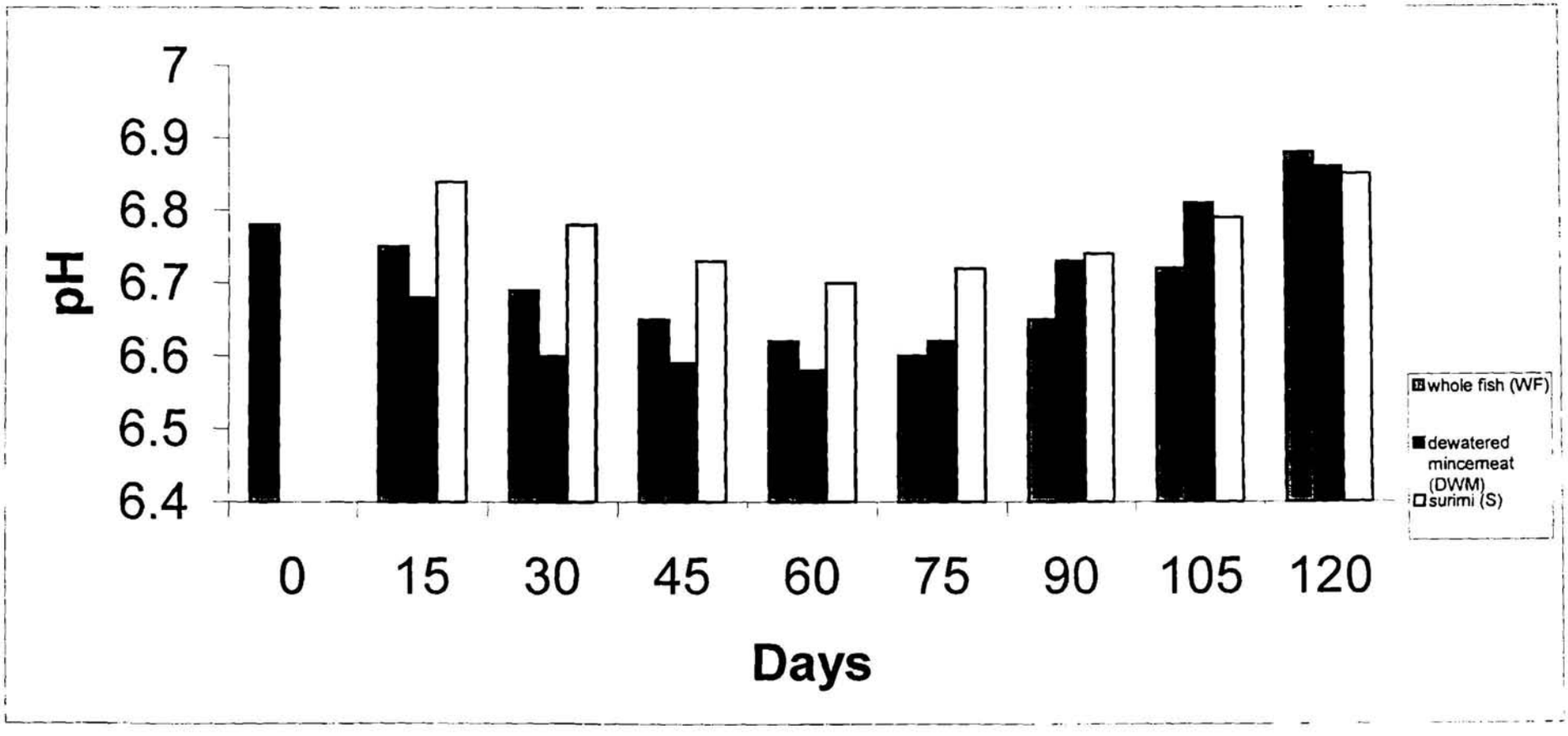


Table-XII Mean sensory score of frozen whole fish (WF), dewatered mincemeat (DWM) and surimi (S) during frozen storage

DAYS	SAMPLE / ATTRIBUTES	WHOLE FISH (WF)	DEWATERED MINCE MEAT (DWM)	SURIMI (S)
0	Appearance	8.2	8.4	8.5
	Texture	8.4	8.6	8.7
	Odour	7.4	8.2	8.4
30	Appearance	8.0	7.9	8.2
	Texture	8.1	8.2	8.3
	Odour	7.1	7.2	8.1
60	Appearance	7.7	7.5	8.0
	Texture	7.8	7.6	8.1
	Odour	6.8	6.9	7.8
90	Appearance	7.4	7.1	7.8
	Texture	7.6	7.2	7.9
	Odour	6.5	6.3	7.3
120	Appearance	6.6	6.2	7.2
	Texture	6.7	6.5	7.4
	Odour	6.1	5.7	6.9

Table -XIII Analysis of variance for whole fish (WF), dewatered mincemeat (DWM) and surimi (S) stored for four months

Source	df	Sum of Squares	Mean Sum of Squares	F value
Days	4	15.504	3.876	331.449**
Attribute	2	3.954	1.977	169.045**
Treatment	2	3.352	1.676	143.335**
Days x Attribute	8	0.140	1.744	1.492
Days x Treatment	8	0.861	0.1081	9.202**
Attribute x Treatment	4	0.540	0.135	11.534**
Error	16	0.187	1.169	
Correlated Total	44	24.538		

* $P < 0.05$
** $P < 0.01$

Table- XIV DMRT

Treatment	N	Subset	
		1	2
DWM	15	7.3000[a]	
WF	15	7.3600[a]	
S	15		7.9067[b]

Values with different superscripts are significant at 5% level.

Table -XV Correlation coefficient between analytical methods.

Samples	Correlation between the analytical methods.									
	SSN x PV	SSN x TBA	SSN x AAN	SSN x TVB-N	TVB-N x PV	TVB-N x TBA	TVB-N x AAN	AAN x PV	AAN x TBA	PV x BA
Whole fish (WF)	-0.873**	-0.834**	-0.983**	-0.946**	-0.766*	-0.804**	-0.981**	-0.857**	-0.866**	-0.952**
Dewatered mince meat (DWM)	-0.913**	-0.394	-0.989**	-0.989**	0.883**	0.360	0.993**	0.913**	0.415	0.717*
Surimi (S)	-0.708*	-0.439	-0.974**	-0.984**	0.780*	0.516	0.982**	0.847**	0.692	0.932**

* $P < 0.05$
** $P < 0.01$

Figure- IX Application of HACCP: The critical control point in the production of surimi.

Product flow	Hazard	Preventive measure
Raw fish	Contamination	Monitoring environment
Catch handling	Growth of bacteria	Time and temperature control
Chilling	Growth of bacteria	Quality, quantity and time of ice used
Washing	Cross contamination	Use of potable water
Dressing	Cross contamination and growth of microorganism	Personal, hygiene and factory sanitation, time, temperature
Meat separation	Spoilage	Time, temperature control
Washing	Loss of functional properties	Temperature, ratio and number of cycle of water washing
Dewatering		
Addition of cryoprotectent	Loss of functional properties	Selection of efficient cryoprotectant and level of addition
Freezing	Chemical and autolytic changes	Time, temperature of freezing
Packing	Spoilage	Glazing and Packaging
Storage	Dehydration chemical changes	Low and constant temperature

Chapter-V

DISCUSSION

5. DISCUSSION

5.1 Raw material characteristics

The average total length and weight of 30 number of Common carp used in the present study were 28.80 cm and 535.38 g, respectively. The typical size of this species has been recorded 100 cm and 35 Kg (Frimodt, 1995).

Dressing yield of Common carp was 62.53%. Bhat (1983) has reported a dressing yield of 68.04% from medium sized Mrigal. The yield of picked meat was 35.88% based on the whole fish. However, a picked meat yield of 26.1% was observed in Tilapia (Finne *et al.*, 1980). Generally the yield of picked meat directly relate to the size of the fish. The fairly high yield obtained in the present study may be due to the comparatively bigger size of fish used.

The proximate composition of fillet of Common carp is outlined in Table-III. The result of this study showed moisture content of 76.90% and protein content of 17.78%. Similar results were reported from Common carp (Frimodt, 1995). The fat content is highly variable (2 to 25%) in Common carp (Rippen, 1990). In the present study the fat content (3.16%) appeared to be low (5.6%) as compared to the study of Frimodt (1995) but high (1.9 ± 0.6%) as compared to the study of Vacha *et al.* (1993). The variation may be attributed to the feeding and spawning cycle of fish. However, a low fat content is very important to get good quality gelled or emulsion type and analog products (Suzuki, 1981; Flick *et al.*, 1990). The proximate composition of fish depends on the diet, size, sex, physiological state of fish and also the ecological conditions. The results obtained in the present study are in concurrence with above reports within reasonable limits.

All the chemical parameters of the samples such as TVB-N, AAN were within the limit of acceptance (2.78 mg%, 5.54 mg% respectively). Connell (1980) recommended a TVB-N value of 20 mg% as the safe level for the raw material. The SSN content of fresh fish usually varies between 70 and 85% of total protein (Dyer *et al.*, 1950). In the present study the SSN content was 73.34% indicating high quality of raw material. This observation is also reflected in mean sensory evaluation score (Table-V). The overall score of fresh and cooked fish were 8.0 and 8.4, respectively on a 9-point hedonic scale.

The PV is regarded as an useful and reliable method to estimate the extent of auto oxidation in early state or storage (Olley and Lovern, 1960). The PV upto 30 milli equivalent of oxygen/Kg of fat is considered acceptable without any objectionable off taste and odour (Lojolina *et al.*, 1983). The present study showed a PV of 17.1 mill equivalent of oxygen /Kg of fat. In an attempt of confirming this value, the TBA was determined which is an index of secondary oxidation of lipid. However, the TBA value of 1.14 mg malonaldehyde /Kg of fish, was within the acceptable limit. Connell (1980) suggested the safe level of TBA to be 1 to 2 mg malonadehyde/Kg of sample.

The TPC/g of whole fish (including skin, gill and intestine), observed in the present study (Table-IV) was found to be in conformity with the results as described earlier (Liston *et al.*, 1976).

5.2. Standardization and preparation of surimi

The fresh Common carp were processed immediately without any kind of inordinate delay. The whole fish were manually dressed. Particular care was taken to keep the fish in chilled condition during evisceration. The dressed fishes were subjected to mechanical separation of fish flesh. The separated Common carp mince was grayish white in colour. After separation the meat was subjected to repeated washing with chilled water (5°C). Hassan and Mathew (1999) reported three washing cycles with mince to water ratio of 1:5 for Common carp. They also used 0.3% sodium chloride solution for the last washing to ease the removal of water in the further processing steps. Nowsad *et al.* (1999) recommended 0.1% NaCl solution for final washing for tropical major carp minced meat. In the present study the number of washing cycles were increased to four but the proportion of mince to water (1:5) remained same. Last washing was done by using 0.1% solution of sodium chloride. Five minutes of setting time between each washing was found to be adequate for tropical major carp minced meat (Nowsad *et al.*, 1999). The above recommendation was also followed in the present study.

The washing of minced meat enables the removal of sarcoplasmic protein, which inhibits gelation (Roussel and Cheftel, 1988; Shamasundar *et al.*, 1988). Therefore, increase in number of washing cycle was found to be advantageous in removing fat, colour pigments, odour bearing components and also results in the concentration of myofibrillar protein. The serious disadvantage in surimi production is the requirement of large amount of water. Among many alternatives, a 3:1 proportion of water to picked meat was found satisfactory. Babbitt (1986) has made similar observation. Suzuki (1981) has recommended a ratio of 1:2 to 1:8 (mincemeat: water) depending on the species.

In the present study the moisture content of minced meat was increased from 76.90% to 80.34% after 4 washing cycles. Hydrophilic nature of myofibrillar protein is responsible for such an increase in moisture content. The dewatering is an important step to maintain the moisture content of surimi around 80%. Good quality surimi usually contains less than 85% moisture (Lee, 1985). In the present study the screw press was used and moisture content was maintained at less than 81%. Therefore, it can be said that the screw press will serve the purpose of dewatering during small-scale surimi production.

After washing the fat content decreased from 3.16% to 1.12%. Four washing cycles reduced the lipid levels by 64.5%. Lin and Morrissey (1995) reported a 39% reduction of lipid in fresh water squawfish mince after third wash. The high level of lipid reduction in the present study might be associated with the characteristic of mince that contained less fatty muscle.

Washing resulted in the decrease in protein content due to removal of sarcoplasmic protein (Lin and Park 1996). The dewatered sample contained 14.57% protein, which is less as compared to the initial protein content. Many authors have reported the decrease in protein content during washing (Bligh and Regier, 1976; Grantham, 1981; Roussel and Cheftel, 1988).

The unwashed mince was grayish white in colour having a fishy odour. Washing resulted in blunt colour and notable reduction in odour of the mince due to removal of pigments and odour producing components. Similar result was observed by Nowsad *et al.* (1999) in tropical major carp mince.

Surimi is used as frozen material and the denaturation of protein involving loss of solubility, water retention capacity, gelling ability and lipid emulsifying capacity are the major problems. This problem necessitates a stabilization step against the freeze denaturation, which is an irreversible change in the myofibrillar protein namely actomyosin. This occurs due to formation of intramolecular cross-linkage caused by ions and ice crystals, chemical interaction of protein with formaldehyde generated from TMAO, binding of fatty acids and lipid oxidation product and formation of disulphide bond. In the present study sorbitol (4%) and sodium tri-polyphosphate (0.3%) were mixed with the dewatered minced meat in order to control the deteriorative changes.

5.3. Quality changes during storages

5. 3.1. Nitrogenous compounds

5.3.1.1. Total volatile base nitrogen (TVB-N)

Volatile base nitrogen indicates the production of ammonia, mono-di and trimethylamine nitrogen and is found in the common pattern of spoilage. Kimura and Kiamakura (1934) recommended a level of 10 mg% or less for fresh fish, 20-30 mg% at the beginning of spoilage and over 30 mg % for spoiled fish. In the present study the TVB-N of fresh fish (2.78mg%) is in concurrence with the above-mentioned recommendation. It indicates the high quality of raw material. During four months storage the TVB-N content of all samples increased gradually (Table-VI). Siddaiah *et al.* (1999) observed increasing trend in the frozen silver carp mince both untreated and treated with sugar, sorbitol and sodium tri-polyphosphate. In the present study the increase was more pronounced in whole fish (16.64 mg%) and dewatered mince meat (17.38 mg %) than the surimi (13.95 mg %) after four months storage period. However, the above-cited values indicate that all the samples are in acceptable condition.

5.3.1.2. Alpha amino nitrogen (AAN)

The AAN content showed a gradual increasing trend during the storage period in all the samples (Table-VII). In the present study AAN content of all samples varied between 26.58 mg% to 32.41 mg% after four months of storage. Dora (1992) reported that AAN value of Pink perch was 14.33 mg% after four months of storage. The increase in AAN indicates the hydrolysis of protein during frozen storage (Rao, 1989). Free amino acids produced as a result of hydrolysis of tissue protein may be due to the action of muscle proteinase (Myasoedova *et al.*, 1972). The AAN content of surimi is less after four months of study as compared to other samples. The effect of cryoprotectants in surimi may be responsible for this.

5.3.1.3. Salt soluble nitrogen (SSN)

SSN is considered as an index of protein denaturation in fish (Joseph and Perigreen, 1986; Shamasundar and Prakash, 1994). There was a gradual fall in the SSN throughout the period of storage for all the samples (Table-VIII).The decrease was more in the dewatered mincemeat. This decrease in SSN content can be attributed to the aggregation leading to the insolubilization of myofibrillar protein fraction during frozen storage. The beneficial effect of cryoprotectant is indicated by higher SSN content (49.20% after four months) in surimi. Sarkar (1997) reported that SSN value of Sardine surimi decreased from 74.08% to 50% after four months of frozen storage.

The protein denaturation has been effectively minimized by using a combination of sodium tri-polyphosphate and sorbitol. The phosphate treatment reduces driploss and increases tenderness (Ellinger, 1972; Suzuki, 1981) whereas sorbitol protects the ability of surimi to form deformable gels (Park *et al.*, 1988). The preferential hydration of protein molecules results in growth of ice particles and lesser the extent of protein denaturation (Arakawa and Timasheff, 1982).

5.3.2. Lipid

5.3.2.1. Peroxide value (PV)

Lipid deterioration during frozen storage is one of the most important factors, which affects the quality of food directly or indirectly. Auto oxidation by atmospheric oxygen is the major cause of oxidative rancidity in fish products, although enzymatic (lipoxigenase) action and photo oxidation cannot be ruled out (Wheaton and Lewson, 1985). The lipid oxidation during storage is monitored by estimating the PV, which increased markedly in all the samples (Table-IX). Peroxide value reached a peak after three months storage and thereafter decreased. Labuza (1971) stated that the PV is being highly unstable, get decomposed to secondary oxidation products accounting for a decrease in its value.

5.3.2.2. Thiobarbituric acid (TBA) value

The estimation of malonaldehyde is suggested as a dependable index of the further decompostion of peroxides in fish and fishery products (Yu and Sinnhuber, 1957). The TBA value showed an increasing trend in all the samples (Table-X). Sinnhuber and Yu (1958) reported that good quality fish had a TBA number less than 3 whereas products of poor quality had value from 4 to 27. The initial TBA value of whole fish (1.14 mg of malonaldehyde/kg) indicates high quality of raw material. In the present study the TBA value of surimi showed an unacceptable limit along with the other samples at the later stage of storage. The disuse of any suitable antioxidant in surimi probably responsible for this. However, all the samples showed a sudden decrease of TBA value at the end of four months as secondary oxidation products under go further decomposition (Labuza, 1971).

5.4. pH values

pH of the fish muscle is considered as an index of its freshness. However, it is not a very useful index for frozen stored samples (Botta and Richards, 1973). The pH value of all the samples showed an increasing trend after an initial decrease (Table-XI). Similar trend was observed in Silver carp mince treated with sugar, sorbitol and sodium tri-polyphosphate (Siddaiah *et al.*, 1999). The increase in pH was probably due to the production of basic volatiles. The choice of cryoprotectants determines the pH

of surimi. Verma (1992) has recorded a low pH of 5.2 in surimi samples treated with ascorbic acids. Generally pH does not vary during the frozen storage. In the present study the variation of pH was very less during the storage. The slight fluctuation ranged between 0.1 and 0.3 pH units. The use of polyphosphate at 0.3% level did not markedly increase the pH.

5.5. Sensory characteristics

The mean sensory scores of whole Common carp, dewatered mince and surimi decreased gradually in first three months of storage. But, thereafter, the sensory scores of whole fish and dewatered mince decreased abruptly. Whereas, the sensory score of surimi did not decrease significantly during entire period of storage and it was in acceptable condition after the experimental period. Similar observation has been reported by Siddaiah *et al.* (1999) for Silver carp mince treated with sugar, sorbitol and sodium tri-polyphosphate.

5.6. Statistical analysis

The result of ANOVA after four months of storage showed a significant difference ($p<0.05$) between days, attributes and treatment. There was no significant difference ($p>0.05$) between days and attributes. In order to confirm the results of ANOVA, DMRT was performed. Significant difference ($p<0.05$) in sensory scores was found between surimi and other samples (Table-XIV). This might be due to the fact that surimi was prepared using cryoprotectants and it had better quality as compared to others. Negative correlation was found between SSN and other chemical parameters, whereas PV and TBA had positive correlation between them. From the correlation coefficient it can be inferred that it is necessary to use different methods to assess the quality of surimi and it is not safe to depend on a single method for quality assessment. It is also important to compare the chemical characteristics with sensory characteristics to determine the overall acceptance of surimi.

5.7. Use of HACCP concept in surimi production

HACCP is becoming popular as a tool for assurance of quality of fish and fishery product. The hazards of each step of surimi production are analyzed and three preventive measures have been suggested (Figure-IX). The raw material quality, washing and addition of cryoprotectants are the steps that have been identified as critical control point. With respect to product quality the storage temperature and packaging are important. In order to prevent the production of defective product sufficient care should be taken in monitoring water quality, sanitation of plant and personal hygiene.

Chapter-VI

SUMMARY

6. SUMMARY

The fish consumption pattern in many countries is changing and development of technology has helped to produce a variety of fishery products, which can be served after minimum processing. A growing field of application is the manufacture of surimi which originated in Japan. It is made by mincing the fish flesh, thorough washing, refining, dewatering and stabilizing it. Surimi is prepared using mostly white fleshed fish specially Alaska pollock. Still now freshwater fish species have not been used considerably as a basic raw material of surimi. A brief summary of the present investigation is given below.

- The present work was taken up to process Common carp (*Cyprinus carpio*), one of the widely cultured freshwater fish. The physical, chemical, microbiological and sensory characteristics of the fresh fish were analyzed.

- The fishes were of the average total length of 28.8 cm and average weight of 535.38 g.

- The chemical indices such as TVB-N, AAN, SSN, PV and TBA were found well within the limits of acceptance.

- The number of washing cycle, the ratio of water to picked meat and setting time were standardized with respect to Common carp minced meat.

- A repeated washing for 4 times with water to picked meat ratio of 5:1 and a setting time of 5 minutes between each washing was found to be sufficient to get a bland, odour free product and achieve a 64.5% reduction of fat content. The last washing was done with 0.1% solution of NaCl for the easy removal of water during further processing steps.

- Screw press (traditional type) was used to press out as much water as possible preferably to retain less than 80% moisture. The dewatered sample contained 14.57% protein, which is less as compared to the picked meat (17.78%). Removal of water-soluble protein during washing is responsible for this.

- Two cryoprotectants namely sorbitol and sodium tri-polyphosphate were mixed with dewatered mincemeat at a level of 4% and 0.3%, respectively. Since the fat content of the raw material was not very high (3.16%), no antioxidant was used.

- The storage study of whole fish, dewatered mincemeat (DWM) and surimi indicated certain extent of deteriorative changes in nitrogenous constituent resulting in increase of TVB-N and AAN where as the decrease in SSN. The deteriorative changes in lipid fraction ware considerable resulting in increase of PV and TBA.

- Substantial benefit was observed in cryoprotectant treated samples during storage.

- The decrease in sensory score especially flavour can be attributed to lipid changes during storage as no antioxidant was used.

- The statistical analysis of the sensory scores confirmed the above-cited findings.

- The raw material quality, washing, cryoprotectants and condition of freezing and frozen storage have been found to be the critical steps during surimi production.

- The present investigation indicates that production and freezing of Common carp surimi could offer a potential means of processing fresh water fish. Addition of suitable cryoprotectants can improve the stability of Common carp surimi significantly.

Chapter-VII

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7. REFERENCES

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* Not referred to the original.

Chapter-VIII

APPENDIX

8. APPENDIX

ORGANOLEPTIC EVALUATION OF WHOLE FISH, DEWATERED MINCE MEAT AND SURIMI

Please evaluate the given sample placing the appropriate score against each attribute

Sample / Characteristics	Whole fish	Minced meat	Surimi
Appearance			
Colour			
Taste			
Odour			
Overall acceptability			

Score	Like extremely	9
	Like very much	8
	Like moderately	7
	Like slightly	6
	Neither like nor dislike	5
	Dislike slightly	4
	Dislike moderately	3
	Dislike very much	2
	Dislike extremely	1

Comments
Name
Date

(Signature)