

**FROZEN STORAGE STUDY OF SURIMI FROM
ROHU (*LABEO ROHITA*) AND ITS UTILIZATION
FOR VALUE ADDED PRODUCT**

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A Thesis

Submitted to the

*West Bengal University of Animal and Fishery Sciences,
in partial fulfilment of the requirements for the degree of*

Master of Fishery Science

in

Fish Processing Technology

By

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2002

DEDICATED
TO
MY PARENTS

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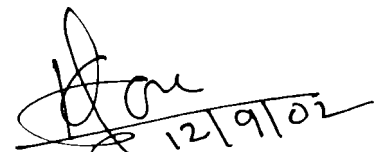
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CERTIFICATE

This is to certify that the work recorded in the thesis entitled "**FROZEN STORAGE STUDY OF SURIMI FROM ROHU (*Labeo rohita*) AND ITS UTILIZATION FOR VALUE ADDED PRODUCT**" submitted by **Mr. Chandraval Dutta** 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.

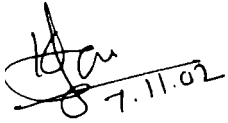
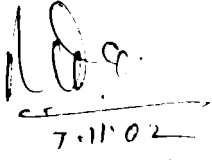
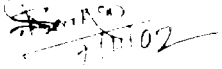
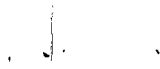
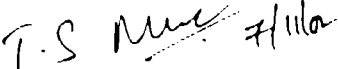
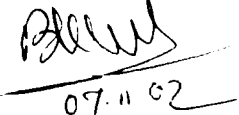

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We, the undersigned, having satisfied with the performance of **Mr. Chandraval Dutta** in the viva - voce examination, conducted today, the, recommend that the thesis be accepted for award of the degree.

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ACKNOWLEDGEMENT

I wish to record my deep indebtedness to Professor K. C. Dora, Dean, Faculty of Fishery Sciences for his valuable guidance and encouragement during the period of this study and in preparation of my thesis.

My grateful thanks are due to Sri Srikanta Sarkar, Head, Department of Fish Processing Technology, Dr. Uday Saha, Lecturer, Sri B.K. Chand, Farm Manager, to Sri T.S. Nagesh, Lecturer, who as members of advisory committee extended necessary guidance during the study.

The Co-operation received from Sri Saikat Banerjee, Krishnendu Manna, Prabir and Biresch of the Department of Fish Processing Technology and also the help received from Sri Monoj Das, Librarian is gratefully acknowledged.

I am extremely thankful to Dr. D.P. Sen, Former Scientist, CFTRI, Mysore for his valuable advise in the final stage of my thesis work.

I extend my indebtedness and heartiest thanks from the deep core of my heart to my batchmates Supratim Chowdhury, Rakesh Majumder, Subhodeep Ghose, Golam Ziauddin, Anupam Roy, Sabyasachi Bonerjee, Piyal, Dilip Karmakar, Kaushik Maity and Nina Das and to my juniors like Prasanta Manna, Dilip Mondal, Banhisikha Dhar and to my seniors like Angshuman Chakraborty, Pratik Mondal. for their extreme co-operation and constant encouragement. Nevertheless, without their help and inspiration I would not have been able to complete my thesis work.

I also take this opportunity to acknowledge once again my debt of gratitude to the binders, 'Dhar Publication' and Rajib Saha, Samit Pal for the

excellent co-operation that I have been getting from them. Lastly but not the least without the assistance, patience and encouragement from my parents, sister and other relatives it would not have been possible on my part to achieve this success.

Dated : 12. 09. 2002.

Place : Monirampur, Barrackpore

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Analytical chemist
BS	Breaking Strength
CCP	Critical Control Point
DMA	Dimethyl Amine
DWM	Dewatered minced Meat
EEZ	Exclusive Economic Zone
EIC	Export Inspection Council
FAO	Food and Agricultural organization
FDA	U.S. Food and Drug Admistation
FFA	Free Fatty Acid
HACCP	Hazard Analysis Critical Control Point
ICMSF	International commission on microbiological specification for food
JAS	Japan Agricultural Standrad
JSA	Japan surimi association
LDPE	Low Density Polyethylene
MSG	Mono Sodium Glutamate
NPN	Non protein Nifrogen
PER	Protein Efficiency Ratio
RDU	Refrigerated Display Unit

SPI	Soy Protein Isolate
SPC	Soy Protein Concentrate
STPP	Sodium Tri Polyphosphate
SSN	Salt soluble nitrogen
TMAO	Tri- Methyl Amine Oxidase
TPC	Total Plate Count
TVBN	Total Volatile Base Nitrogen
UN	United Nations
W/W	Wet weight basis
WBA	Water Binding Activity

Chapter-1

INTRODUCTION

1. INTRODUCTION

India is world's second largest producer of food and has the potential to become number one in due course of time with sustained efforts. The growth potential of food processing sector is enormous and it is expected that the food production will double in the next 10 years in due course and the consumption of value added products will grow at a fast pace. This growth of the Food Processing Industry will bring immense benefits to the economy, to the agricultural and aquaculture yield, to the productivity, in creating employment and in raising the standard of living of a large number of people throughout the country, especially, in the rural areas (International Conference on processed food for 21st Century, 2001.)

India has the seventh largest marine area in the world with an extensive coastline of 7500 km. an exclusive economic zone (EEZ) of 2 million sq.km., a 29,000 km. stretch of rivers and canals, 1.45 million hectares of reservoirs and 0.75 million hectares of tanks and ponds (Devadas & Balakrishnan, FOOD & PACK, 2001) whereas, in West Bengal, the length of coastline is 158 km. (Excluding Hoogly-Matlah Estuary). The inland water resources of West Bengal are (i) Tanks/Ponds- 2,76,201.90 ha, (ii) Beel/Baor-41,781.65 ha, (iii) River- 1,72,586.36 ha, (iv) Reservoir: 16,738.8 ha, (v) Sewage fed- 4,083 ha, (vi) Canal/Creek - 80,085.71 ha, (Fisheries Department, Govt. of W.B., 2000)

World fish production consisting of marine fisheries, inland fisheries and aquacultural has touched a figure of 122 million tonnes and India produced 5.96 million tonnes (Warrier, S.B. 2000). The potential fish production from Indian EEZ is estimated as 3.9 million tonnes and as against level of harvesting as 2.87 million tonnes in 2001 (International Conference on Processed Food for 21 Century, 2001)

West Bengal occupies a place of primacy in fish consumption and production in the country. As the years go by, more and more people are getting themselves involved in fisheries. The domestic demand for fish is also the highest in the country. As fish constitute the staple food of the people, efforts are being made to augment fish production. The total fish production of West Bengal during 1999-2000 was 1045.70 tonnes of which 865.70 tonnes came from inland water resources and 180,000 tonnes from marine sector (Fisheries Department, Govt. of West Bengal, 2000) The fish production of West Bengal from 1979-1980 to 1999-2000 is given in table-1

Fish is a cheap source of protein, which has great demand in developed countries.

The nutritional properties vary from species to species. Animal proteins are better in quality than plant protein as the protein efficiency ratio (PER) for eggs, fish, cow milk and beef are 3.9, 3.5, 3.0 respectively where as incase of soyabean, rice and wheat are 2.5, 2.0 and 1.5 respectively (Damodaran, S. 1996). Domestic per capita consumption of fish in India is only 5kg. where as world average is 12kg. per annum. India's per capita consumption is much lower than that of Asian maritime states (eg, Japan, 86kg). (Devadas & Balakrishnan, 2001).

Processing of products into canned and frozen forms are carried out almost entirely for the export market in India. There are 258 freezing units with a capacity of 2170 tonnes, 23 canning units, with a capacity of 84.5 tonnes, 131 ice making units with a capacity of 1820 tonnes, 24 fish meal units with capacity of 419 tonnes and 297 cold storage units with a capacity of 20,348 tonnes in India.

This sector in India also attracted the attention of investors, both domestic and foreign and an investment as high as U.S. \$ 702.8 million has been approved in the last 6 years of which foreign investment was around U.S. \$ 153.3 million (Devadas & Balakrishnan, 2001).

The infrastructural facilities in West Bengal for the preparation of value added products and fish processing has been gaining attention day by day. There are 65 landing centres, 2 minor fishing harbours, 130 ice plants, 32 processing plants with a capacity of 269 MT/day, 36 number of cold storage with a capacity of 2468 MT in West Bengal. (Fisheries Department, Govt. of W. B., 2000).

The fisheries resources of India are grossly underutilized. Marine catch landed in India include, prawn, shrimps, tuna, cuttlefish, squid, octopus, red snapper, ribbonfish, mackerel, sardine, lobsters, catfish and countless other varieties and inland fish include Indian major carps (Rohu, Catla, Mrigal), catfish, hilsa, pabda, koi, tilapia, parse, bhetki etc. As a consequence of technological innovation as well as international competition, many commercial marine species are over harvested leading to depletion of their stocks. However, out of 3000 fish species that contribute to the total marine catch, consumer preference is restricted to only a few selected species. The rest which forms a significant portion of the total catch termed as "by catch" is under utilized and used as feed or manure.

Large quantities of trash fish/shellfish(*squilla*) are discarded at sea because it is currently uneconomical to preserve and bring them ashore (Basu, S. 2001).

Preparation of value added product using the low priced, under utilized fish is a sure way of better utilization. Now, a days with improved standard of living, nuclear family system, demand for convenience products eg: value added products are likely to increase in developing countries like India, especially state like West Bengal and also have a good export potential. There is vast scope for increasing fish consumption in the country by the development of 'convenience foods' or 'ready to cook' and 'ready to eat' products. Fish and fish products in frozen can be marketed to urban centres, where these convenience foods would be welcomed. The need for diverting the low valued fish for human consumption and the necessity for diversification of fish and fishery products need no emphasis

In some districts of Andrapradesh, there is huge production of Indian major carps but as such there is no much local demand for these fishes and available at a very reasonable rate. The availability of these fishes from inland sector at a reasonable price can form a good source of raw material for the preparation of convenience and novel food for the urban population.

Surimi is mechanically deboned, washed and stabilised minced fish flesh, widely used as an intermediate product for a variety of fabricated seafoods. Surimi is essentially pure protein with extremely high nutrients and ability to form gel. Surimi based foods usually contain other ingredients such as starch, egg white, flavourings and seasonings and occasionally vegetable oil and natural shellfish meat. (Dora & Hiremath, 1991). At present fishes like pink perch, croakers, Indian halibut have been successfully used in the commercial production of surimi in our country. Surimi was successfully produced from fatty fish like mackerel (Sarkar, 1997).

According to the data available, the fishes used for the production of surimi is decreasing and hence the companies are on the lookout for the alternative resource (Patil & Tandale, 2001). Alaskapollock harvest has declined due to stricter fisheries management from a harvest high of 6.76 million metric tons in 1987 to 3.56 million metric tons in 1997 which has opened the door for the use of new species in the surimi industry (Morrisey & Tan, 2000).

Hence, in the present investigation an attempt has been made to assess the frozen storage stability of surimi and to develop value added product from a fresh water fish namely Rohu (*Labeo rohita*) instead of using marine fish

Past efforts on fresh water fish utilization include production of fish products similar or analogous to those prepared from sea fish, because of the consumer's preference for such products. Minced or ground fish meat may be the best way to use fresh water fish. A variety of products made from minced fish, like fish frankfurters, "sea dogs," canned and frozen minced fish, nuggets, and other products have been developed and marketed in the USA, Japan, and Europe (Zaitsev et. al., 1969; Daily et. al., 1978; Baker and Bruce, 1982; Moulard and Voigt, 1990; Crapo and Himelbloom, 1993; Delaquis et al, 1993).

Compared to meat, freshwater fish contains, less lipid but more unsaturated fat and n-3 fatty acid, more moisture, highly digestible protein and devoid of fishy odour and flavor due to small amount of volatile bases (Karmas and Lauber, 1987, Aggelousis and Lazos, 1991). Pruthiarenun et. al. (1985) found freshwater fish more promising than marine species in producing minced meat products, Indian major carp Rohu (*Labeo rohita*) produced good quality gel. Next superior species in the order were Catla > Mrigal > Silvercarp (Nowsad et. al. 1999). Other fishes like Tilapia could be processed into surimi, fish burger, fish balls, fish crackers, fish fingers, fish sausages etc. (Irianto and Irianto, 1997). Utilization of fresh flesh of freshwater carp, bream and other species of the cyprind family as well as salmon, white fish, perch, sturgeon, and plaice has been suggested for the preparation of cutlet, fish ball, and croquette by Zaitsev et. al. (1969). Fish ball was successfully produced from minced meat of freshwater bream (*Abramis brama*) (Lazos, 1995)

Surimi is widely used as an intermediate product for a variety of fabricated seafoods including kamaboko, sausage, imitation shrimp products and different other forms of highly appetising products such as fish ball, fish burger, fish sticks, fish crackers, fish nuggets, fish patties, fish rolls, fish cakes, chikuwa etc. (Dora & Hiremath, 1991).

In the present study, an effort has been made to assess the frozen storage stability of dewatered minced meat and surimi in terms of biochemical and microbiological quality. Besides, the other objectives of this investigation are :—

- ☆ To standardise processing steps for the production of an acceptable surimi from Rohu (*Labeo rohita*).
- ☆ To develop a value added product like ball from surimi of Rohu.
- ☆ To study the frozen storage study of fish ball prepared from treated and

untreated dewatered minced meat in terms of biochemical, microbiological and sensory evaluation at -20°C .

- ✧ To identify the critical control point in the production of fish ball from surimi.
- ✧ To study the refrigerated storage study of fish ball prepared from treated and untreated dewatered minced meat in terms of biochemical, evaluation at $10\pm 2^{\circ}\text{C}$ in Refrigerated Display Unit.
- ✧ To determine the production cost of each unit of the product (Fish Ball).

FISH PRODUCTION OF WEST BENGAL

Table Fish Production of West Bengal

Unit: ' 000 tonnes

TABLE — I

Year	Quantity	Inland	Marine
1979-80	337	315	22
1980-81	370	340	30
1981-82	378	350	28
1982-83	271	218	53
1983-84	355	319	36
1984-85	402	370	32
1985-86	424	384	40
1986-87	471	412	58
1987-88	505	443	62
1988-89	523	458	65
1989-90	601	512	89
1990-91	680	555	125
1991-92	732	592	140
1992-93	757	612	145
1993-94	806	653	153
1994-95	820	669	151
1995-96	893	740	153
1996-97	937	765	172
1997-98	950	786	164
1998-99	995	823	171
1999-2000	1046	866	180

Source : (Fisheries Deptt., Govt. of W. B., 2000)

Chapter-II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The minced meat is currently prepared from fresh (unfrozen) fish, either at sea or on land. Land based processing is limited because it depends on the availability of fresh fish. Moreover, land based processing plant should be near to the fishing ground. If frozen fish could be used for minced meat manufacture, then the potential year-round supply of fish could improve the economics of land based minced meat manufacture. Nevertheless, it may allow processing plant to be sited more distance from the catching ground but closer to the minced meat analogue plants. The availability of surimi raw material round the year to sustain healthy production line is as important as the quality standard of the raw material fish. A large number of literatures of research on this aspect have been accumulated in the last 20 years (Suzuki, 1981; Pigott and Tucker, 1990; Flick *et. al.*, 1990; Regenstein and Regenstein, 1991; Fillipi *et. at.*, 1992; Lanier and Lee, 1992; Ofstad *et. al.*, 1993; Ishikawa 1996; Klesk *et. al.*, 2000; Shindo *et. al.*, 2000).

2.1 Minced meat

Owing to modernization of fish processing equipments and new technologies have also opened the way for increasing utilization of underutilized, low valued fishes in minced meat technology. Minced meat is the flesh which is separated in comminuted from the skin, bones, scales and fins from the fish. During separation of meat from fish, any type of species of fish can be applied but low cost fish, should be taken into consideration. Basically, minced meat can be used as a base material for the preparation of different types of products which have good demand.

Surimi is mechanically deboned fish flesh washed with water and mixed with cryoprotectants for good frozen shelf life (Lee, 1984). Surimi is water washed, refined mince prepared from fish, which is relatively stable and can be frozen and cold stored and still retain the necessary functional properties for making Kamaboko. The first element to highlight is that surimi is an intermediate product.

Minced meat is an inexpensive source of quality protein for food provided it can be incorporated into acceptable products, (Delay and Deng, 1978). A comprehensive review on minced meat technology and products developed all over the world has been documented (Grantham, 1981).

Minced fish represents a significant advance in an effort aimed at improving utilization of fish proteins in human food (Murray *et. al.*, 1980). Jiang *et. al.*, (1986) described the new approaches to improve the quality of minced fish products from freeze-thawed Cod and Mackerel.

2.1.1. Fishes utilized for preparation of minced meat and surimi.

During selection of fish species, the following points should be taken into consideration.

- i) The fish should have good gel strength.
- ii) The abundant supply of fish throughout the year.
- iii) Low price.

Development of various physico-biochemical techniques to up grade the gel quality of the fish flesh having poor gel forming ability (Suzuki and Watabe 1987; Seki *et. al.*, 1990; Sakamoto *et. al.*, 1994) has led to search various prospective fish species as surimi raw material other than Alaska Pollock (Kim *et. al.*, 1996).

Commercial scale surimi production from most of the species has not been successful because of their limited stock size, seasonal variations of catch, dark color of flesh, highly unstable fat content or high level of proteolytic enzymes form parasites in the muscles (Kim *et. al.*, 1996). However, still to date, marine species are the exclusive source of commercial surimi or surimi based kamaboko and analogue products and continues to increase with the increase in world population and the concern for nutrition and health (FAO, 1992; Tucker; 1992). Fish species such as Alaska Pollock, Croaker and Lizard fish possessing a good gel forming ability are ideal for surimi production and 60% of world's surimi is made by utilizing these white fishes (Ishikawa, 1996). According to Flick *et. al.*, (1990) Crab, Shrimp and Lobster flavour could be effectively incorporated to the bland taste of white flesh mince meat. The use of pelagic fish as a replacement ingredient has also been tried (Hasting *et. al.*, 1992). Besides, Saurida and Sarines, Cat fishes, Ribbon fish, Perches, Carangids and deep sea fishes Groninger *et. al.*, 1985 ; Nonaka, *et. al.*, 1989 ; Leinot *et. al.*, 1992)

Priacanthus hamrur, *Diplacanthopoma nigripinnis*, *Chlorophthalmus agassizi* and *Centrolophus niger* could be used as raw material for production of surimi (Dora & Hiremath, 1991)

2.1.2. Utilization of underutilized fish

The fish species which have little or no commercial value, high bone content, unpleasant smell, less taste can be use as the basic material for the production of surimi as Grandier (*Coryphenoides spp.*). Smooth head (*Alepocephalus spp.*) and Rabbitfish (*Chimeraspp*) (Burges, 1975; Baily, 1976)

Other under utilized fishes used for preparation of minced products are Grunt (*Pomadasys argyreus*), Lizard fish (*Saurida tumbil*), Bony fish (*Leognathus splendens*), Cat fish (*Arius spp*), Thread fin bream (*Nemipterus japonicus*) and Cutlass fish (*Trichirus lepturus*) (Bremner and Snell, 1978) Dhananjaya et. al., (1986) repoted on the acceptability and frozen storage stability of Deep sea fish (*Diplacanthopoma nigripinnis*). Successful production of mince has been found from Carribbean sheeps head (*Archosargus*), Black drum (*Pognia*), Tilapia (*Tilapia*) (Finne et. al., 1980; Nickelson et. al., 1980). Hybrid Catfish (female *Clarius macrocephalus* and male *C. gariepinus*) is a popular fish farmed in Thailand, is used for variety of products including surimi (Nongnuch and Raksakulthai, 1996).

2.1.3. Utilization of freshwater fish

Fresh water fish contributes considerably the world food production, both in biomass and diversity. Fresh water fish contains, compared to meat, less lipid but more unsaturated fat and n-3 fatty acid, more moisture and highly digestible protein and is devoid of fishy flavor and odour due to little amount of volatile bases (Karmas and Lauber, 1987; Aggelousis and lazos, 1991). Lazos (1995) opined that due to some reasons such as small size, bony flesh and characteristic flavour which restrict most of the freshwater fish species to be accepted by the consumers widely. Various processing methods of some freshwater species have been developed during the last two decades (Lazos, 1995, 1997), a few attempts have been taken to utilize these species in minced products. Lazos (1996) produced canned fish ball from fresh water bream and suggested that minced or ground fish meat might be the best way to use freshwater fish.

Comparing the storage life of fish balls prepared from by-catch and freshwater fish species Pruthiarenum et. al., (1985) found freshwater fish to be more promising than marine species in producing minced products. Some investigations have been done on the quality of minces of freshwater fishes for the manufacture of surimi (Ismond and Tonogai, 1994;

Onibala *et. al.*, 1997; Siddaiah *et. al.*, 1999)

2.2 Meat Separation

Mechanical fish meat separators, developed by companies in Japan, Germany and USA, are modern sanitary machines that remove virtually all the flesh from the frame of a properly prepared fish. (Morrisey & Park, 2000). According to Pigott (1986) there are several methods to prepare fish for deboning. One is to remove the head, gut and thoroughly clean the belly walls before deboning the carcass. The other is to fillet the fish and then debone the fillet, which contains skin and bones.

The most common types of meat separators are rotating in opposite directions between which the fish is pressed. Miyauchi and Steinberg (1970); Carver and King (1971) and Noble (1972), reported that by means of meat separating machines significant quantities of edible flesh can be recovered from fish filleting wastes and under utilized species.

Lanier and Lee, (1992) opined that efficient deboning is based on the higher meat yield and lower bone content. High quality of minced meat is achieved by an effective removal system of flesh from bone and skin (Pigott and Tucker, 1990). Perforation size ranges from 1 to 5 mm (Regenstein, 1986), with smaller sizes giving fine mince with good color but possibly leading to losses during rinsing According to Flick *et. al.*, (1990) the drum with smaller (1 – 7mm) perforation reduces the bone content of fish, 5 mm drum orifice is suitable for bony fish with reasonable texture and good yield of mince (Wood and King, 1985; Wong *et. al.*, 1978).

2.3 Chemical composition of minced meat

The moisture, protein, fat, non-protein nitrogen and minerals are the basic constituent of minced fish meat (Dora, 1992). Besides it also consists of cholesterol, calcium and other fat degraded products (Krzynowek *et. al.*, 1984). Depending on type of the species and season of catch approximately 80% of the composition is composed of moisture, protein and fat. On an average the myofibrillar protein or salt soluble protein in minced meat constitutes more than 90% of the total nitrogen (Webb *et. al.*, 1976). Non-protein nitrogen is also a major nitrogen fraction and is highly species dependent. Phospholipid and Free fatty acid are the major constituents in lipid fraction and the fatty acid of minced carp muscle indicates its

quality of w_3 fatty acids (Mai and Kinsella, 1979)

2.4 Washing of minced meat

The most important step of surimi processing to ensure maximum gelling as well as colorless and odourless surimi, is efficient washing. Many of the problems with color, taste, and odour that develop in minced meat are minimized or eliminated when washed. Park *et al.*, (1990) found greater ease of dewatering and higher yield of protein recovery in processing tilapia surimi from pre-rigor fish. The degree of washing required to produce good quality surimi depends upon the type, composition and freshness of fish. A water/meat ratio ranging from 4 : 1 to 8 : 1 is often employed by on shore processors, (Lin and Park, 1996). Increased water usage for washing usually resulted in more protein loss and waste water disposal (Lin and Park, 1996).

The effect of added water content and chopping method on physical properties of surimi and Kamaboko made from Spotted shark was studied and showed that the tensile strength and cohesiveness increased up to 76% water content but decreased thereafter (Lee and Chen, 1997).

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, a recent report, Lin and Park (1996) indicated that most sarcoplasmic proteins are fairly soluble and removed during initial washing steps. In Rohu, a distinct variation of Breaking Strength in two wash gel over one wash gel at 40–50°C was observed (Nowsad *et al.*, 1999).

Washing developed the color from 'gray- yellowish' or 'grayish white' in unwashed minces to 'white' in washed minces ($P < 0.05$). However, the color quality did not vary ($P > 0.05$) between one wash and two wash minces of all carp species (Nowsad, *et al.*, 1999)

The effect of number of washings operation, pH and kind of washing solution and washing technique on freezing denaturation of protein in Silver Carp surimi was studied by Wang and Wang (1999). It was suggested to wash the minced meat 3 to 5 times by using 0.1% Citric acid solution first and Calcium Chloride (0.15%) solution depending on the following factors eg. economy, efficiency and quantity of product.

2.5. Straining and dewatering/refining

After each washing or leaching intermediate dewatering should be performed properly in order to avoid problem in final dewatering (Lee, 1986). Screw press is universally used in surimi plant for dewatering purpose (Flick *et. al.*, 1990). A refiner is nothing but a straining machine, which could work on wet slurry. The purpose of refining is to remove bones, skin and perhaps dark muscle if this is objectionable. During straining process many researchers have observed a slight temperature increase of about 2 to 4°C. Suzuki, (1981); Lanier and Lee, (1992) opined that the increase in temperature could lead to protein denaturation. The use of self cooling strainer can eliminate this problem (Flick *et. al.*, 1990)

2.5.1. Final dewatering

Innovative processing technologies applied to surimi processing over the past decade have enhanced process efficiency, resulting in product quality improvement. The moisture content of washed meat ranges between 80 - 85% and therefore, it is necessary to reduce it to less than 80% (Pigott and Tucker, 1990). The use of screw press has achieved significant success as it can be used continuously and suitable for large scale production. The main purpose of dewatering is to optimise the factor for protection of protein and also to ensure the moisture content of the final product should not exceed 80% (Lee, 1986; Pigott and Tucker, 1990; Flick *et. al.*, 1990; Lanier and Lee 1992).

Decanter centrifuges are now commonly used to recover fine particles lost through the dewatering screens and screw presses (Lanier and Lee, 1992). Babbit (1997) has demonstrated the function of a decanter to replace a conventional screw press in the surimi lines. The decanter surimi process is a simplified operation but it increases yield and gives products of consistent quality (Burns, 1997).

2.6. Storage stability of minced meat

Minced meat has shorter frozen storage life compared to fillets or whole fish (Dora, 1992). It is axiomatic that the quality of frozen fish material will depend on the quality of fish initially frozen (MacDonald *et. al.*, 1997). The adding of bone marrow exudate, catheptic enzymes, enzymes from blood, lipids and inorganic constituents in the minced fish affect texture, flavour and appearance and reduce the frozen storage life (Joseph and Perigreen,

1986). During frozen storage of fish changes in quality is of great commercial importance (Mills, 1975 Crawford *et. al.*, 1979)

Leaching improves frozen storage stability by reducing the activity of Trimethylamine oxidase (TMAO), removing lipids, substances, and by concentrating the myofibrillar proteins (Lanier & Lee, 1992)

2.6.1. Fat degradation

The characteristic feature of lipid oxidation in fish meat are influenced by factors such the amount of lipids, their susceptibility to auto-oxidation (Ke *et. al.*, 1982).

Polyphosphates are added to the minced meat in order to enhance the protein functionality and water binding. They have also been found to have antioxidant properties, particularly in combination with other additives (Morris and Dawson, 1979; Tableros, 1980).

Antioxidants such as polyphosphate at 0.2 - 0.3% are commonly incorporated as synergists to the cryoprotective effect of carbohydrate additive in the manufacture of surimi (Park, & Lanier, 1987; Park, Lanier, & Green, 1988; Kumazawa, Y. *et. al.* 1990). As antioxidant of lipids they may also protect proteins from denaturation induced by hydrolysis or auto oxidation of phospholipids. (Wessels, Simmonds, Seaman, 1981). A wide range of antioxidants and their application to frozen storage, including seafood had been reviewed by Erickson (1997).

2.6.2. Changes in protein

Frozen storage is an important long term storage mechanism for muscle food because it prevents microbial spoilage and minimizes the rate of biochemical reactions in muscle. Never the less, it is inevitably associated with some deterioration of meat protein (Matsumoto, , 1980; Park *et. al.*, 1987) Park, 1994 opined that during frozen storage several changes occurred in muscle protein such as denaturation, ice crystallization, dehydration and changes in intramolecular conformation.

Many proteins have exhibited instability as measured by the partial loss of functionality, at low subfreezing temperatures. Molecular changes in proteins with respect to functionality at low temperatures as a function of oligomer dissociation, rearrangement of subunits

within oligomers, aggregation was studied by Fennema (1982). In general, the tertiary structure of native protein is maintained by several types of interactions, namely, hydrophobic, hydrogen bonding, electrostatic, and vander waals forces. Among these, hydrophobic interactions are recorded as primary importance in most proteins (Edelhoch and Osborne, 1976; Taborsky, 1979) and the strength of these interaction is weakened by lowering the temperature. Hanafusa (1973) stated that the ordered structure of water molecules at a low temperature breaks hydrophobic interactions causing changes in the conformation of protein. Therefore, lowing the temperature will tend to produce structural changes in those protein molecules that are dependent on hydrophobic interactions for maintenance of native structure (Taborsky, 1979). Factors other than temperature have profound effects on the structure of proteins such as p^H , ionic strength, surface tension, protein concentration, concentration of nonprotein solutes. (Fennema, 1982)

The myofibillar proteins exhibit optimum solubility and gel forming ability when they remain in and undenatured form (Lanier *et. al.*, 1980). The gel forming ability of fish proteins is found to be reduced as a result of temperature fluctuation during frozen storage (Suzuki, 1981).

Deterioration of textural quality resulting from frozen storage has been well documented for Cod and Pollock (Kramer *et. al.*, 1977; Kramer and Nordin, 1979; Storey, 1980; Love *et. al.*, 1982; Babbitt and Repond, 1987; Le Blanc *et. al.*, 1988; Sych *et. al.*, 1990). The Trimethylamine oxide enzyme (TMAO), particularly high in the gadoid fish, plays an important role for the breakdown of trimethylamine oxide (TMAO) to formaldehyde and dimethylamine (DMA) (Harada, 1975). It has been suggested that the formation of formaldehyde may accelerate textural toughness through frozen storage (Gill *et. al.*, 1979; Parikin and Hultin, 1982).

Among the various muscle proteins, myofibrillar proteins such as myosin have shown to be more susceptible to freeze damage, while sarcoplasmic proteins such as globulin, myogen and myoalbumin and sarcoplasmic proteins such as collagen and elastin are not affected significantly by freezing (Park, 1994). The frozen storage condition and freshness of fish (Lin and Morrissey, 1995) are important factors that decide the stability and shelf life of surimi. 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.*, 1986; Park *et. al.*, 1987, a, b,) and decreased gel forming ability (Shenouda, 1980; Kim *et. al.*, 1986; Park

et. al., 1987 a). The ATP ase activity of actomyosin or myosin isolated from frozen stored fish meat decreases proportionately to the length of storage period, as does the rate of precipitation of actomyosin (Careche and Tejada, 1991) Matsumoto et. al., (1993) reported the inactivation of myofibrillar Ca-ATP ase during freeze drying Since ATP ase actively is located in the globular head region of myosin, the reduction of ATPase activity during frozen storage of fish meat is caused by conformational change of myosin. (Matsumoto et. al., 1993).

A considerable reduction in soluble protein with a simultaneous decrease in organoleptic rating from 4.5 to 0.5 during frozen storage was studied by Koning et. al., (1985). They proved that the formation of Free Fatty Acids (FFA) and denaturation of protein are related phenomena.

Akahane (1982) also reported the freeze denaturation of active molecules. Matsumoto (1980) detected no significant changes in the number of free -SH groups in frozen carp meat. During frozen storage change in the properties of myosin, actine and actomyosin play an important role in extractability of protein. Freezing rate, frozen storage temperature and freezing conditions are the important factors that contribute significantly on the myofibrillar protein solubility. (Reddy and Srikar, 1991).

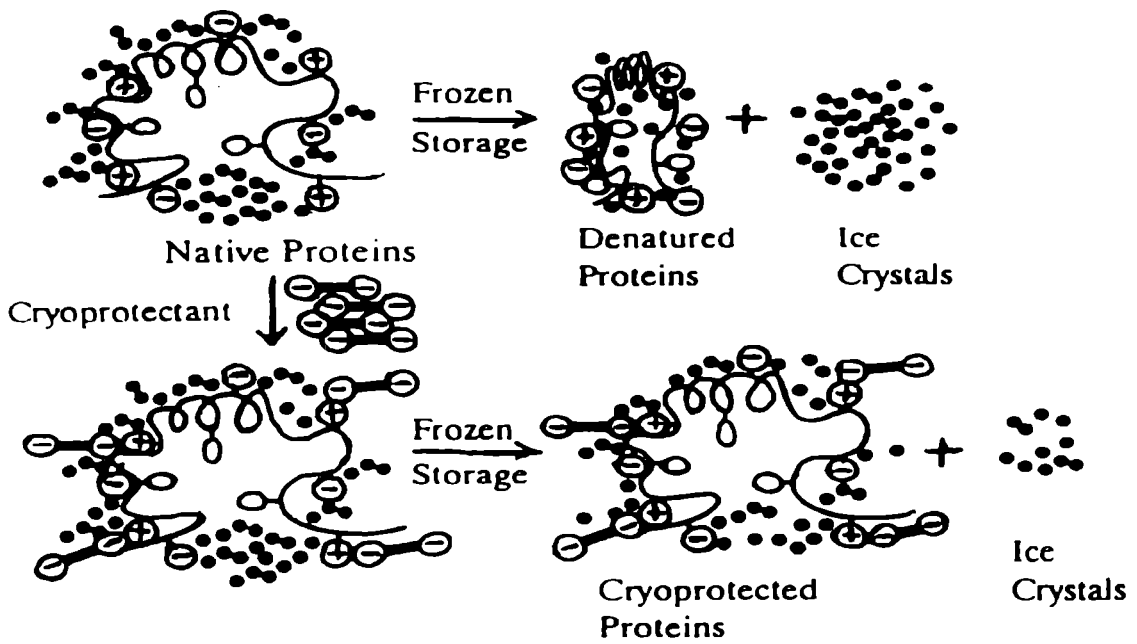
The insolubilization of myofibrillar protein is attributed to high frozen storage (Fukuda et. al., 1981). An inverse correlation between salt soluble nitrogen (SSN) and Peroxide value (PV), Free fatty acid (FFA), Trimethylamine (TMA) as well as Total volatile base nitrogen (TVBN) during frozen storage was studied by Verma and Srikar (1994). Bremner (1977) reported a loss in salt extractable proteins during frozen storage of minced meat obtained from cucumber fish.

2.7. Use of additives in minced meat

2.7.1. Mode of action of cryoprotectant

There are number of theories which have been put forward in order to explain the role of the functional group of protein molecules during freezing.

Matsumoto (1980) has illustrated diagrammatically the prevention of denaturation of protein (Fig) (I). The native conformation of the globular molecules is maintained largely by the intermolecular nonpolar bonds which have resulted from the thermodynamic balance



A schematic model of denaturation (unfolding) of nonhelical (globular) proteins during frozen storage and its prevention by cryoprotectants. $-\oplus$, Cationic side chain; $-\ominus$, Anionic side chain; \cdot , Nonpolar side chain; $\ominus-\ominus$, Water molecule; $\ominus-\ominus$, Dianionic cryoprotectant molecule.

Figure : I. Mode of actions of cryoprotectants in surimi.

between the two system the folded protein water and unfolded protein water.

In the folded protein-water system, the non polar groups on the polypeptide backbone are oriented inwards so as to avoid contact with waterphase. In the unfolded protein - water system, some non polar groups are projected to interface with water. When cryoprotectants are added, some of them may bound to the protein molecules and results in increased hydration of protein molecules and increased resistance against displacement of water even when the system is frozen (Park, 1994).

An entirely different theory has been postulated by scientists to explain the cryoprotective effects of many high molecular weight polyols and glucose polymers. This so - called "cryostabilization" theory is based upon the ability of fish molecular weight solute to raise the glass transition of solution. Cryorstabilization of proteins involves addition of a solute to raise the glass transition to a temperature above the storage temperature, ensuring that the system in the glass state and thereby, effectively minimizing the freeze induced deteriorative process including ice crystal formation, since the water is immobilized in the glass structure. (Lanier and MacDonald, 1992).

2.7.2. Use of Cryoprotectant

Cryoprotectants are compounds that extend the shelf life of frozen foods (MacDonald and Lanier, 1991). These prevent the denaturation of the proteins of the product during freezing, frozen storage and thawing (Pigott and Tucker, 1990 ;Fermema, 1973)

The cryoprotectant act on biochemical material effectively and have a characteristic features eg. (i) low volatility (Nash, 1966) (ii) considerable solubility in water at molecular level and ability to form a multi hydrogen bond (iii) ability to penetrate membranes (Doebbler and Rinfret, 1962) (iv) capacity to dissolve electrolytes. Noguchi (1974) subsequently proposed a list of chemical attributes that seem to be characteristic of cryoprotective substances for proteins. The molecule should possess one essential group, either $-\text{COOH}$, $-\text{OH}$, $-\text{OPO}_3\text{H}_2$ and more than one supplementary group $-\text{COOH}$, $-\text{NH}_2$, $-\text{SH}$, $-\text{SO}_3\text{H}$ and/ or $-\text{OPO}_3\text{H}_2$.

The cryoprotectants not only prevent the denaturation of myofibrillar proteins during frozen storage but also allow proper gel formation during the production of surimi based products (Matsumoto and Noguchi, 1992). Lanier and MacDonald, (1992) concluded that

there are fundamental differences between the mechanisms of "cryoprotection" by low molecular weight sugars and polymers and "cryostabilization" by high molecular weight polymers. Cryoprotectants act by altering the thermodynamics to the system to favour the native state of the protein, while cryostabilizers act to enmesh the protein in a glass where all deteriorative processes are greatly slowed.

A variety of compounds will cryoprotect labile proteins during freeze thawing such as sugars, amino acids, polyols, methyl amines, carbohydrate polymers, synthetic polymers (eg polyethylene glycol, PEG) other proteins (eg bovine serum albumin, BSA) and even inorganic salts (eg: potassium phosphate and ammonium sulfate) (Gekko, 1981; Yamashita, 1996)

In order to confer maximum protection the cryoprotectant should be applied at high concentration but polymers, such as PEG, BSA, and polyvinylpyrrolidone (PVP), are effective at a concentrations of even less than 1% to protect sensitive enzymes such as lactate dehydrogenase or phosphofructokinase. (MacDonald, Lanier and Carvajal, 2000).

The incorporation 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, 1992). The cryoprotectants were originally incorporated into dewatered minced meat by a kneader, Now a days, silent cutters are used as they uniformly distribute the cryoprotectants faster and the temperature rises during chopping is less. Commercial practice for mixing cryoprotectant by a kneader and silent culter are 6 minutes and 2.5 minutes respectively. The temperature of mixing must not exceed 10°C as at greater temperature i. e. > 10°C functionality of protein could be damaged (Park and Morrisey, 2000).

The most commonly used cryoprotectants are sucrose, sorbitol and polyphosphate (Suzuki, 1981). Their action seems to be a combination of water holding to prevent water migration (Matsumoto, 1980) and an increase in surface tension (Arakawa and Timasheff, 1982). The undesirable sweetness and/or browning tendency caused by the incorporation of sugar and/or sugar alcohol to the surimi can be eliminated by the use of poly-dextrose as an alternative cryoprotectant. (Lanier and Akahane, 1984).

Sodium tripolyphosphates, (STPP) monosodium glutamate (MSD) and antioxidant mixtures were used to protect white fish (*Coregonus cupleaformis*) and burbot (*Lota lota*) (Krivchenia and Fennema, 1988).

Commercially, eight percent sucrose was used in the surimi but this made the product sweet and caused a color change during frozen storage. Therefore, the level of sucrose was reduced to 4% and 4% sorbitol (60% as sweet as sucrose) was added to compensate for this reduction (Lee, 1984). In 1992 with commercial surimi processing of Pacific whiting, enzyme inhibitors like, beef, potato extracts was used in conjunction with 8 to 12% cryoprotective ingredients. The gel enhancer property was formulated with sucrose, sorbitol, tetrasodium pyrophosphate or sodium tripolyphosphate. Calcium carries (Calcium lactate, Calcium sulfate, Calcium caseinate) and sodium bicarbonate (Park & Morrisey, 1994 .Matsumoto *et. al.*, 1986, 1987 a, b.)

Lanier and Akahane (1986) discovered and patented the use of poly dextrose, a non sweet and low caloric agent for cryoprotection of muscle proteins. Park *et. al.*, (1987; 1988; 1993) recommended polydextrose as a non sweet cryoprotective ingredient in beef, mullet myofibrils, and Alaska Pollock surimi. Application of polydextrose in muscle food has not been approved by the US food and Drug administration but it is accepted as an additive for surimi in Japan and Korea (Baute, 1993).

The cryoprotectants should be chosen for their effectiveness, low cost, availability, and low tendency to cause Maillard browning (MacDonald *et. al.*, 2000).

2.7.3. Use of Protein additives

Protein additives are globular proteins, where as surimi proteins are fibrillar in nature. They have at least one special property such as functionality, nutrition or economic benefits. (Park, 2000). With respect to functional properties of protein additives, interaction of protein-water, protein-protein and protein-lipid water are important for the formulation of the stable gel network structure (Regenstein, 1984). Protease inhibitors can increase functional properties by making obstruction in proteolytic action, modifying water binding activity (WBA), or forming co gels (Reppond and Babbitt, 1993; Park, 1994).

There are different types of gelling additives used in the sea food industry such as lacto albumin, whey protein concentrate, milk protein isolate, soy protein, egg white, wheat gluten, bovine serum albumin, starches, alginates and carbohydrates (Chung and Lee, 1990). Wheat gluten, soy flour and corn germ protein are some of the commonly used additives in comminute meat products (Gnasambandam and Zayas, 1992).

Whey proteins are composed of β - lactoglobulin (75%) and α - lacto albumin (25%)

and gelation properties of whey protein depends on temperature, pH, ionic strength and functional property depend on nature of cheese (Park, 2000).

Liquid egg white is the most commonly used protein additive in surimi seafood. The level of use is 2.5% (Park, 2000). Soy proteins are usually derived from a defatted flake with protein content of about 50%. Soy protein isolates (SPI) and soy protein concentrates (SPC) are commonly used around the world in the surimi seafood industry (Park, 2000). Cho and Park (1998) evaluated the functionality of SPI and SPC in surimi sea food.

2.8. Characteristics of Surimi

2.8.1. Composition

Japan Surimi Association (JSA) prescribed the quality standard for surimi. According to Suzuki, 1981, moisture content and pH are prime criteria for the judgment of quality of surimi. Moisture content of 79 - 81% and a pH value of 6.8 - 6.9 are considered to be optimum for good quality surimi (Suzuki, 1981; Regenstein and Regenstein, 1991). Japanese Agriculture standard (JAS, 1996) which classified a good quality surimi prevailing on the market should have Breaking Strength (BS) more than 400g.

2.2.8 Functional properties

The functional properties can be defined as an effect induced by an ingredient on either organoleptic properties of a food such as odour, flavour, texture or the mechanical properties of the food during its processing such as extractability, resistance to breakage etc. Functional properties depend on ingredients and processing method (Lanier and Lee, 1992).

The use of salts in the wash water, including calcium and magnesium chlorides, has been proposed and said to improve the functional properties of the resulting surimi (Ofstad et al., 1993).

2.8.2.1. Gel forming ability

The gel forming ability of fish muscle is an important determinate of textural quality. In salt ground marine fish paste, setting process generally occurs at < 40°C (Suzuki, 1981). During this low temperature setting the fish paste sol transforms into gel resulting in a three

dimensional protein network (Niwa, 1992). The mechanism of setting involves a denaturation of myosin molecule and interaction of denatured myosin to form high molecular weight cross linkage (Niwa, 1992). The set gel show changes in rigidity, elasticity and brittleness depending on species variation, protein concentration and heat processing method (Shimizu, et. al., 1981; Shimizu and Kaguri, 1986).

The first step forming gel is turning myofibrillar protein into sol by incorporating 2.5% salt, Swari gel is achieved by heating actomyosin sol at temperature near 50°C. Heating swari gel at temperature around 60°C modari gel is formed and Kamaboko gel is formed by heating modari gel more than 60°C. Two distinct reactions in the gel forming process generally occur where setting starts at around 30-40°C and gel disintegrates at around 60-70°C (Shimizu, 1981). Sarcoplasmic proteins retard gel network formation by interfering with actomyosin crosslinking process (Suzuki, 1981).

The pressure treatment has actually helped to develop the gel character (Chung et. al., 1994) and this is achieved by the addition of ammonium salts (Shoji et. al., 1994). Lin and Park (1996) found that sarcoplasmic proteins in pacific whiting mince were readily soluble in water (0% NaCl) and recover in the initial washing step.

Farr, 1990; Okamoto et. al., 1990; Hoover, 1993 found that high hydrostatic pressure kill microorganisms, affects enzymatic activity and creates rheological changes in several foods.

The gelation phenomenon in tropical freshwater carp does not follow exactly similar pattern which is observed in marine species (Suzuki, 1981). Rohu paste showed good gelling ability with respect to both breaking strength, expressible moisture and sensory evaluation (Nowsad et. al., 1999). The next superior species in the order were Catla > Mrigal > Silver Carp (Nowsad et. al., 1999). Hatae et. al., (1990) found a definite texture and firmness in cooked fish tissue which was species specific. Species variation in the disintegration of the gel was observed by Nowsad et. al., 1999. Klesk et. al., 2000 performed a comparative study between Tilapia and Alaska pollock surimi and suggested that the optimum heat treatment for Tilapia should be at 40°C for 1 hour and then 90°C cooking for 15 minutes.

2.8.3. Microbiological consideration

Freshly caught fish for surimi production, the sites of bacterial contamination are, as usual, the skin, gills and guts. These are removed in the preparation stage through careful filleting, skinning, gutting and heading processes, the bacterial load on the flesh should be minimal. (Hall & Ahmad, 1997). The washing in the surimi process may help to remove contaminant bacteria and the incorporation of salt and cryoprotectants further contribute to their suppression (Lee, 1992).

Several common types of seafood bacteria can be present and are retained during surimi processing. Fresh Alaska pollock contains a mixed bacterial flora predominated by *Flavobacterium*, *Moraxella* or the *Arthrobacter/Corynebacterium* group (Himelbloom, B.H. & Brown, E.K., 1991). Micro organisms associated with fish skin can inoculate fillets being conveyed in the processing facility by way of residual fish fragments remaining on chain linked metal or plastic belts. (Himelbloom, B.H., 1991). Microbial adherence to equipment and processing surface also results in biofilms, which are reservoirs for cross-contamination. (Himelbloom, B.H., 2000).

The microbiological aspects several fresh water fish species was studied by Lindberg et. al., (1998). It was found that *Enterobacteriaceae* were in high numbers after storage at 7°C in 31% of retailed fish and in 100% of retailed packs of minced meat.

Few studies have investigated microbial growth and survival using inoculated packs of spoilage bacteria and food borne pathogens (Ingham, S.C., 1991)

Human handling, such as manual fillet trimming at some processing plants may add to the microbial load. Movement of equipment by personnel throughout the facility increases the chance of microbial contamination (Himelbloom, 2000). The presence of non protein nitrogen compounds in minced fish hastens the growth of psychrotrophic seafood bacteria (Liston et. al., 1982). The quality of incoming water and recycled water also needs to be controlled to prevent microbial contamination during processing of surimi. Using ultrafiltration reduces aerobic plate counts from $10^3 - 10^5/g$ to less than $10^3/g$. (Lin, Park & Morrsey, 1995).

2.9. Commercial Utilization of Surimi

The surimi is the intermediate raw material for the production of a wide variety of traditional Japanese products such as kamaboko, chikuwa, hampen and satsumage (Wu, 1992), which contribute 90% of marine products and other 10% is composed of sausage, ham, burger etc.

Kamaboko is the most typical surimi based product in Japan. During preparation of kamaboko the raw or frozen surimi is mixed with salt (ka-eu) or without salt (Mu-eu), potato starch, sugar, sodium glutamate and egg white. Kamaboko products can be classified into 3 groups namely broiled, steamed and fried. Kamaboko is called 'STATSUKI' which is mounted on a tinboard, 'CHIKUWA' is broiled which is similar to stem of bamboo. The fried kamabokos are known as 'SATSUME - AGE' and "TEMPURA". There are various type of kamaboko in Japan depending on their shape such as "SUSA" (bamboo leaf shaped), "SOBA" (needle shaped), "DATE - MAKI" (Rolled) and "KEZURI" (chipped) (Flick *et. al.*, 1990). The surimi based products are following categories :-

1. Molded products :-

Molded products are formed by single extrusion or coextrusion (Flick *et. al.*, 1990). Surimi paste which is commonly mixed with prepared fibres is formed in a molding machine or cold extruded in a three dimensional shape such as shrimp - shaped or lobster shaped seafood. (Park, 2000).

2. Composit Molded product :-

The strings of desired length are mixed with or without surimi paste and then extruded through a extruder machine. The strings are manufactured by slicing a block of surimi gel and stripped. (Flick *et. al.*, 1990).

3. Flbrised product :-

These are of mainly 2 types (a) Crab analogue (b) scallop analogue. The former one is produced by extruding the paste into a thin sheet and then it is cut according to required shape by flake machine where as the latter one is produced by extruding the paste into a thick sheet. (Flick *et. al.*, 1990; Wu, 1992).

4. Emulsified products :-

Emulsified products are produced from fat treated surimi basically, less than 10% animal fat or vegetable oil is used. The other ingredients are sugar, starch, MSG & Egg White (Flick *et. al.*, 1990).

2.10. Value added products

The term value added refers to value that is added to a product from the time that it enters the processing plant to the time it leaves. Value added products are ready to serve, ready to cook, hygienically prepared, nutritious and attractively packed products. Value added products can be produced either from mince or surimi. Mince products include sausage, pastes, balls, wafers, loaves, burgers, fish fingers, creamy fish bites. (Bello and Pigott, 1980;1994 Suzuki 1981; Rergrudee, 1992; Meyers, 1994)

During preparation of this type of product needs a sound understanding of the inter-relationship of the substrate, the equipment that will be performing the transformation of the added ingredients and regulatory considerations. The processing of sea food encompasses the transformation of a raw aquatic commodity into an item which is designed to meet a consumer need. Factors such as portion control, taste, intended method of cooking, cost and resource availability certainly play an important roles in this endeavour (Sasiela, 2000)

2.10.1. Battered and breaded products

There are five general categories of coated seafood products such as pre cooked (for oven finishing), raw breaded (for fryer finishing), battered (for fryer finishing), battered (for oven finishing), sauced (for oven finishing). (Sasiela, 2000).

The coating ingredients for battered and breaded product are corn or wheat flour based aqueous cohesive batter, carckmeal and bread crumbs. Commercially available crumbs coatings are generally colored with naturally vegetative extracts to assist increasing an attractive goldenbrown crust appearance. These coloring agents include paprika, annatoo, turmeric or caramel. Synthetic certified food colors can also be used, but are not preferred by consumers (Sasiela, 2000).

2.10.1.1. Fish patties

It is prepared from lean white fish mince. The cooked mince is mixed with cooked potato, mild spices etc. Then they are formed into round shapes. The products are battered, breaded and fried at 175 - 180°C.

A dried product (fish patty) was developed from mince fish flesh, soy protein, starch and salt by Bello and Pigott, (1980). Spices were used in order to improve the palatability of fish patties by adding flavor. (Bello and Pigott, 1980). Spices were used in this study to improve the palatability of fish patties by adding flavor and also to act as antioxidant and antimicrobial agents (Nadamoto *et. al.*, 1992; Joseph *et. al.*, 1992; Hefnawy *et. al.*, 1993; Teinter & Greins 1997).

2.10.1.2. Fish ball

Fish balls are a product quite acceptable to consumers and especially Greek consumers, as their diet includes meat balls at least once a week. (Lazos, 1996). Fish balls are formed from minced or ground fish mixed with bread crumbs. Then they are mixed with whole eggs, salt, herbs and spices which are then made into balls of 3.0 to 3.5 cm. in diameter and consumed after frying at 175-180°C. (Lazos, 1996).

The main problems during fish ball preparation from sea fish were fishy flavor and odour, and sometimes the high fat content (Tidholm & Bengtsson, 1992). Acceptable fresh, chilled and frozen fish balls were achieved after leaching with water or dilute salt solutions or 4% acetic acid (Bigueras *et. al.*, 1985; El-Sahn *et. al.*, 1990; Boonrat *et. al.*, 1991).

Addition of wheat or rice starch significantly increased elasticity and decreased this succulence as well as acceptability of fish balls (El-Sahn *et. al.*, 1990; Boonrat *et. al.*, 1991.). The shelf life of fish balls at -18 to -20°C is 75 days (Shenoy *et. al.*, 1975). The cohesiveness of fish balls from by-catch was acceptable for 10-12 days, while from Tilapia it was satisfactory after 23 days and for common carp after 36 days. So freshwater fish species might be more promising in producing such fish products (Atkinson and Evans, 1980). Pruthiarenun *et. al.*, (1989) compared the storage life in ice of by-catch species and some freshwater species.

2.11. Quality Control in Surimi Production

On December, 18, 1997, the U.S. Food and Drug Administration (FDA) mandated the application of Hazard Analysis Critical Control Point (HACCP) principles to the processing and importing of fish and fishery products. The developments and use of this processing has been discussed by Huss, (1992). This system assures the control at the source and results in defect free products (Jacoben, 1993). Critical control points for the potential hazards in surimi and value added products are pasteurization, cooling and metal fragments. (Himelbloom, 2000).

First CCP is that during preparation of surimi and value added products lots of machinery are involved. So, the chances of cross contamination is high especially, hotspots are conveyer belts, deboners, in the surimi plant even after clean up (Lee, 1992). Second, surimi is composed of flesh from hundred and thousands of individual fish, a single fish containing high bacterial load can contaminate entire batch of surimi.

A distinct health hazard exists from any metal parts (> 0.3mm) in the surimi product. (Himelbloom *et. al.*, 2000). The bacteriological standards for fish and products were established in 1986 and the values still remain acceptable according to ICMSF, given below.

Microbiological standard for fish and Ready to eat product

Microorganisms	m	M
Aerobic plate count	10 ⁵	10 ⁶
<i>Escherichia coli</i>	11	500
<i>Salmonella spp.</i>	0	–
<i>Staphylococcus aureus</i>	10 ³	–
m and M, minimum and maximum bacterial level		

Chapter-III

MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Raw Material

Fresh and live Rohu (*Labeo rohita*) caught off the pond in Kanchrapara, 24 Parganas (N.) were used in the present study. The fishes were processed within three hours of harvesting and the processing was carried out under hygienic condition. During transportation and processing, the temperature of the raw material was kept as low as possible by using crushed ice and chilled water, and the fishes were carried by using insulated boxes. The dewatered minced meat which was derived from fresh raw material was divided into three groups. One group of dewatered minced meat was kept as control. From second group, surimi was prepared mixed with 4% Sorbitol, 4% Sucrose, 0.3% Sodium Tripolyphosphate. And from the third group of minced meat, value added product i. e., fish balls were prepared by mixing with ingredients. All the samples were frozen at - 35°C and stored at - 20°C until further use.

3.1.2. Chemicals used :

All the chemicals used in the analysis were either of 'Analytical or Guaranteed 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. Glass ware

All the glasswares used for the study were made from 'Borosil'.

3.1.5. Equipments

3.1.5.1. Processing equipments and accessories

- **Meat picking machine** : In order to separate meat from skin and bones of dressed fish, Roll type fish meat picking machine made by 'Stadler Corporation' was used.

- **Fish meat mincer** : To mince the meat, meat mincer machine made by 'Stadler Corporation' was used.

- **Silent Cutter** : The mincemeat was mixed with cryoprotectants in silent cutter made by "Stadler Corporation."

- **Hydraulic Screwpress** : In order to remove water from the mince meat hydraulic

screw press made by "Stadler Corporation" was used.

- **Deep freezer** : Horizontal al model deep freezer (- 35°C ± 1°C) made by 'Anonym' was used.

- **Refrigerated Display Unit** : Refrigerated Display Unit made by 'Dev Enterprise' was used.

- **Mixer & Grinder** : In order to grind and mixing the ingredients for value added product Mixer & Grinder made by "Summit Limited" was used.

- **Vessels, utensils & processing tables** : All the vessels, utensils and processing tables were made of stainless steel.

3.1.5.2. Analytical instruments :

- Kjeldahl digestion system (Borosil)
- Kjeldahl distillation unit (Borosil)
- Soxhlet digestion unit (Borosil)
- Muffle furnace (BITA)
- Hot air oven (Instrument India)
- Vacuum dryer. (Anonym)
- IIC, Bacteriological incubator
- REMI, R & C Laboratory centrifuge
- Sterilizer (Instrumentation India)
- pH meter (Systronics)
- Electronic single pan balance (Dhona 100Ds)
- Distillation apparatus (Borosil)
- Water bath
- Vortex mixer.
- Laminar floor (Bitto)

3.1.5.3. Packaging material

Low-density polyethylene (LDPE) bags were used for packing the dewatered mince as well as surimi and value added product (fish ball)

3.2 Methods :

3.2.1. Study of raw inaterial characteristics

Certain physical, chemical, sensory and microbiological characteristics of fresh fish were analyzed.

3.2.1.1 Physical characteristics

Fishes with average length and weight of 49.4 cm. and 1.32kg. respectively were used for the study. 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 Chemical Characteristics of fish are described in the section 3.2.5, 3.2.6 and 3.2.7

3.2.1.3. Sensory Characteristics

Fresh Rohu (*Labeo rohita*) was evaluated for freshness by using descriptive scoring for appearance, odour, gills, eyes, texture, color and total score of raw fish on 10 point hedonic scale (Chouksey, M.K. and Gadre, U.V., 2001). The overall acceptance of the fish storage stability 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 Rohu was assessed according to the standard method recommended by APHA (Speack, 1976). Appropriate dilution of homogenate was made in a physiological saline (0.85%) and plated in duplicate on nutrient agar, by pour plate method. Incubation was carried out at 37°C temperature for 48 hours.

3.2.2. Standardization of washing procedure

In this study the minced meat was subjected to water washing, following the method described by Suzuki (1981). Chilled water (5°C) was used in order to remove blood pigment, fat and components with low molecular weight. The minced meat was washed repeatedly in order to improve colour. In the present study two washings were performed with chilled water (5°C) giving a residence time of 5 minutes for each wash. Water to mince ratio was 2:1. After each wash, meat was gently squeezed in a muslin cloth to remove as much water as possible. At the end the, meat was almost devoid of fishy odour. Water was removed in a screw press by reducing the water content of washed meat to almost equal to the original moisture content of the product which was maintained about 80%. In order to facilitate easy removal of water the last washing was carried out by 0.1% NaCl solution.

3.2.3. Production of surimi

With a view to stabilise, dewatered minced meat was mixed with 4% sorbitol, 4%

sucrose and 0.3% sodium tripolyphosphate in a silent culter for 2 minutes at 15-18°C. The stabilized mince of surimi was packed in low-density polyethylene (LDPE) bags and then frozen and stored at - 20°C temperature. The procedure used for production of surimi is given in the Fig.(II) The dewatered minced meat and surimi were stored at same temperature (- 20°C)

3.2.4. Production of Value Added Product (Fish Ball)

The ingredients & spices required for the preparation of fish ball (Table below) were weighed. The recipe is based on the recipe of fish cake which is recommended by Gopakumar (1997). A few alterations have been done keeping in view regional consumer preference. All the ingredients and spices were mixed in a mixer and fried in oil to give a characteristics flavour and color. The meat both from the dewatered mince and surimi (treated sample) were mixed with mashed potato and fried spices and ingredients and were given the shape of a ball (about 30g. each). At the end, the fish balls were packed in LDPE bags and then frozen stored at (- 20°C) temperature. Among them few balls were dip fried at 180°C in mustered/ refined oil till the golden yellow color developed.

The recipe of Fish ball

Ingredients	%
Minced fish/Surimi	50
Onion	15
Garlic	1.6
Ginger	1.6
Chilli	0.5
Cumin	0.5
Chillipowder	0.5
Salt	2.5
Potato	28
Garam masala	0.5
MSG	0.2
Eggs for Batter	qs.
Breadcrumbs	qs.

Source : Gopakumar. K., Tropical Product, 1997

The quality characteristics of 2 groups were analysed. One group was control (dewatered mince) and the second was treatment (surimi) sample A. Comparison between control and treatment sample was studied depending on changes in quality during frozen storage at (-20°C) in terms of biochemical and microbiological characteristics. Similarly, storage stability of value-added products (fish balls) both in refrigerated display unit (10 ± 2°C) and deep freeze were analysed.

3.2.5. Chemical Composition

3.2.5.1. Moisture

Moisture content was determined by the standard hot air oven method (AOAC, 1995). About 5 g. of skeletal muscle from whole fish, dewatered minced meat and products prepared from control and treatment sample were taken in moisture bottles and dried in a hot air oven, maintained at 105°C ± 2°C for 12 hours. The weight loss was expressed as moisture content percentage of the sample.

3.2.5.2. Crude Protein

The total nitrogen was estimated by Kjeldahl's 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 250ml of digestion tube and 10-12 ml of concentrated sulphuric acid and 0.2g 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 100ml with distilled water. Then 2ml of digested solution was taken for distillation in Kjeldahl's distillation unit with 40% NaOH 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). The pink color 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/100g of sample.

3.2.5.3. Crude fat

Fat content of sample was determined by extracting the fat with petroleum ether by using Soxhlet apparatus (AOAC, 1995).

About 10g of oven-dried moisture free sample was taken in an extraction thimble and it was placed in the extractor with an attached receiving flask. The solvent was poured into the thimble through a glass funnel. The receiver containing ether was heated (40-60°C)

at such a rate that the ether drops from the condenser to the thimble at the rate of 5 to 6 drops per second. When sufficient solvent was transferred to the extracting tube to fill the siphon arm, it was siphoned back into the receiver. This process was continued until the extraction was completed. Then flask was removed and the volatile solvent was evaporated at 60-80°C on a water bath. The residue was dried in an oven and cooled in a dessicator and weighed. The least weight of residue gives the weight of fat in the sample. The fat content of the sample was expressed as g/100g of sample.

3.2.5.4. 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.

3.2.6. Quality changes during storage

3.2.6.1. Total volatile base nitrogen (TVB-N)

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

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

3.2.6.2. Non-protein Nitrogen (NPN)

10g of sample was belended with 10% trichloroacetic acid (TCA) in a mortar and filtered and then the precipitate was washed with TCA. 10ml of TCA extract was digested by microkjeldahl method. The digested sample was used for estimation of NPN by kjeldahl distillation procedure.

3.2.6.3. Salt Soluble Nitrogen (SSN)

The SSN was estimated by the method of Dyer et. al., (1950). 5g of sample was homogenized for 3 minutes using chilled 5% sodium chloride solution, buffered with 0.02 M sodium bicarbonate. pH was adjusted between 7 and 7.5 using 0.1 (N) HCl. The total volume of the homogenate was made up to 100ml. It was centrifuged 4000 rpm for 10

minutes and then nitrogen content of 2ml supernatant was determined by Kjeldahl's method and expressed as percentage of total nitrogen.

3.2.7. Determination of Lipid Quality.

3.2.7.1 Free Fatty Acid (FFA)

The FFA was estimated by the method of Damodaran, N., (1985). 10g of sample was blended thoroughly with twice its weight of anhydrous sodium sulphate in a mortar. The blend was shaken with distilled chloroform for 5-10 minutes and filtered. 10ml of the extract was taken into a clean weighed petridish. Chloroform evaporated off on a water bath and weight of fat determined. Another 10ml of the extract transferred into another conical flask. Chloroform is evaporated off. To this fat, 10ml of neutral alcohol was added. It was titrated against 0.01N. NaOH using phenolphthalein as indicator. % FFA was calculated. Acid number would be twice that of FFA% as oleic acid.

3.2.8. pH

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

3.2.9. Changes in microbiological Quality of dewatered mince and surimi and product (Value added)

Total Plate Count (TPC) : About 10g of the sample was blended with 90ml of physiological saline (0.85%) in a sterile blender or sterile glass mortar or mortar and pestle. Then these were transferred aseptically dilutions beginning with 1ml of 10^{-2} dilution and ending with 10^{-6} dilutions into sterile petriplates. Then 15ml (approximately) of the melted and cooled agar (37-40°C) was introduced into the petridish and mixed by rotating. After setting the petridishes were incubated at 37°C for 48 hours. The colonies were counted in the appropriate dilutions and computed their number per gram.

3.2.10. Changes in Sensory Quality

Sensory attributes like appearance, texture, flavor, fish flavor intensity, over all acceptability of fish ball prepared from both controlled and treated sample on the 0th day, 45th day, 90th day of storage at -20°C were analyzed on 8 point hedonic scale (Keeton,

1983). The mean scores of 8 trained panelists were calculated for each attribute. The fish balls prepared from both controlled and treated sample stored in refrigerated display unit at $10 \pm 2^{\circ}\text{C}$ were analyzed on 0th day, 3rd day, 5th day, 7th day by sensory evaluation method.

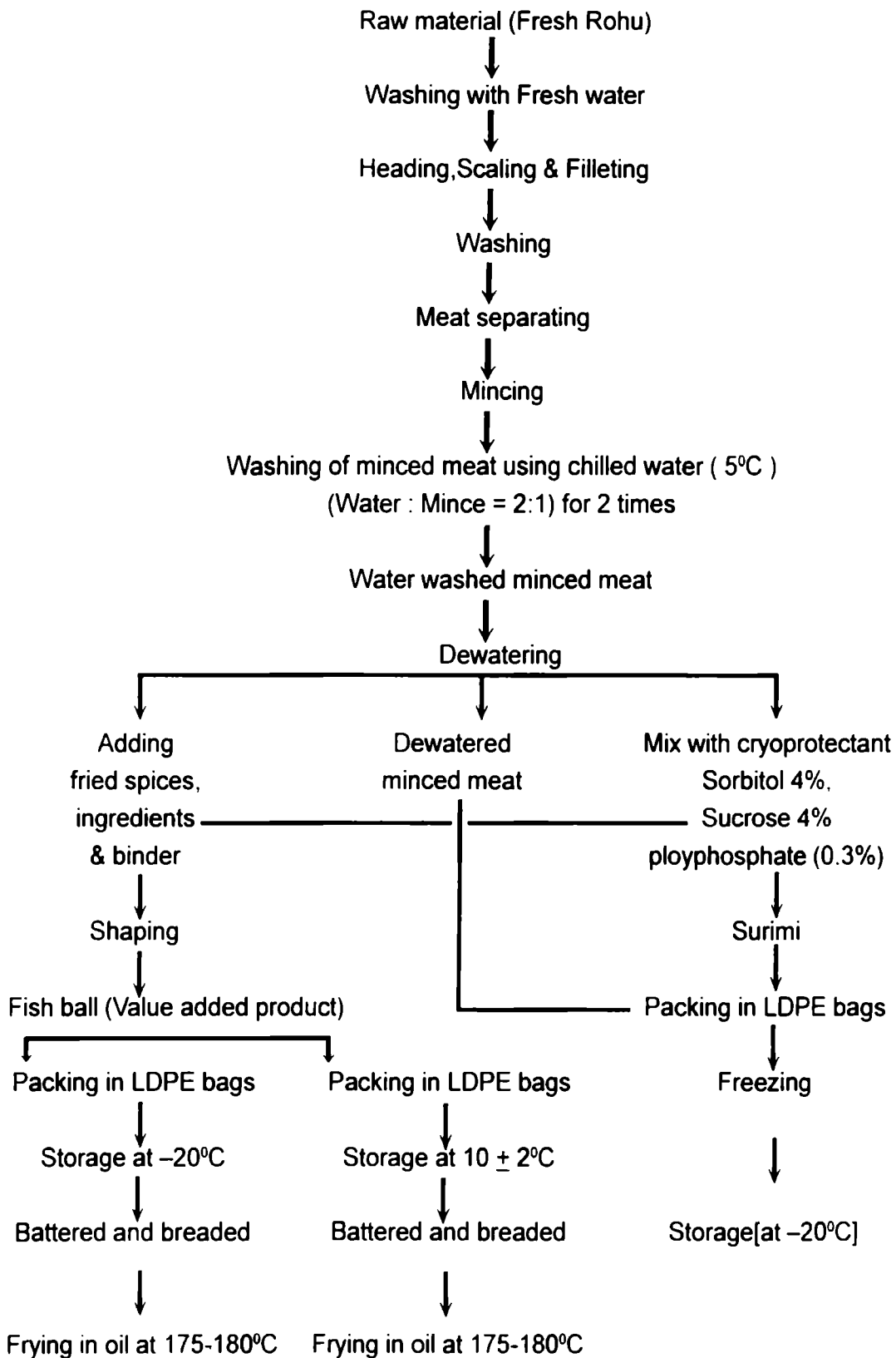
3.2.11 Statistical Analysis

The value of proximate composition of three different samples like fresh raw fish, dewatered minced meat and the product prepared from dewatered minced meat were analyzed by using analysis of variance (ANOVA) (Snedecor and Cochran, 1962). The analysis of variance with different sensory characteristics of raw material was also done. The mean panel scores of product prepared from both controlled and treated sample on the 0th day, 45th day, 90th day were analyzed using two way analysis of variance.). During frozen storage in order find out the relationship between various methods applied to assess the quality, correlation coefficient was calculated. The mean panel scores of product prepared from both controlled and treated sample on 0th day, 3rd day, 5th day and 7th day were analyzed using two way analysis of variance.

3.2.11. Development of HACCP concept in value added product from surimi

The Hazard Analysis Critical Control Point (HACCP) was used for the development of value added product from surimi. The strategy as outlined in ICMSF (1988) was used for the identification of critical control point.

Figure – II



Flow diagram of production of fish ball from surimi of Rohu fish



Plate-1



Plate- 2



Plate- 3



Plate-4



Plate-5

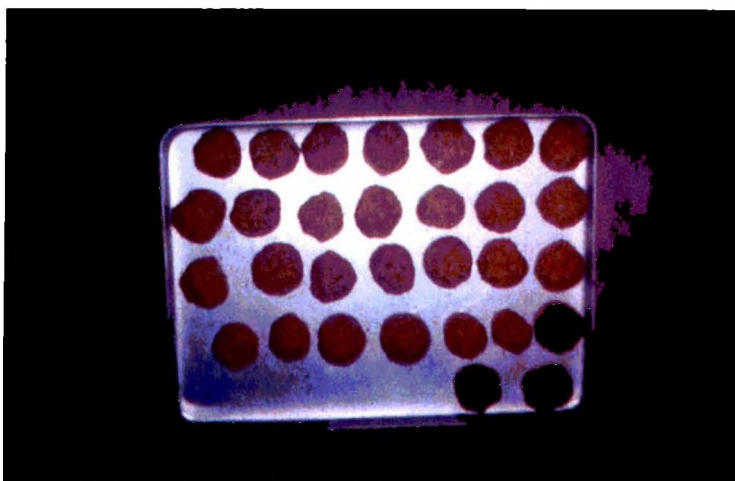


Plate-6

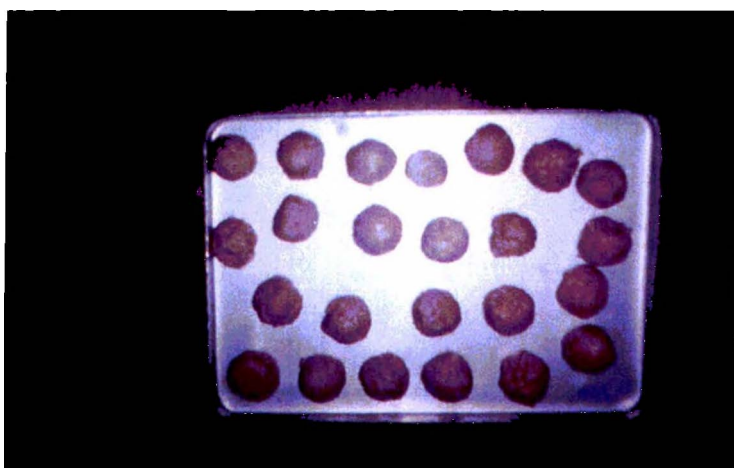


Plate-7



Plate-8

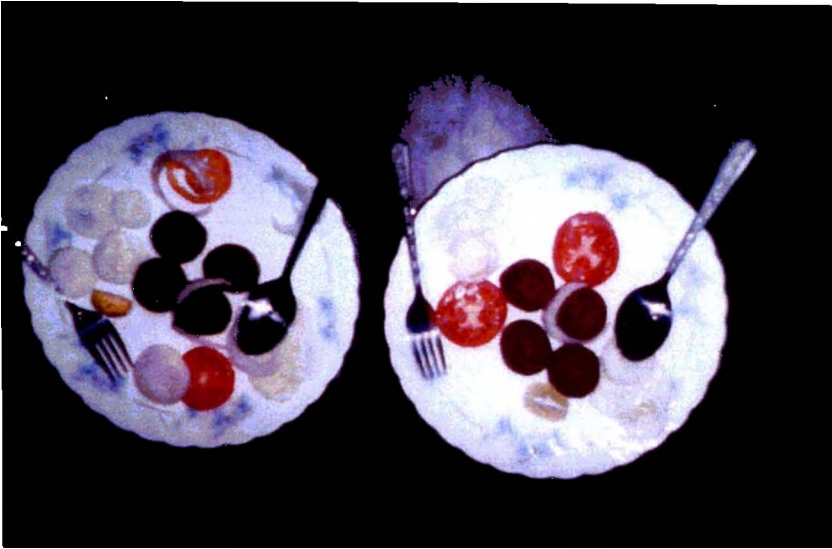


Plate-9



Plate-10

Chapter-IV

RESULTS

4. RESULT

Fresh Rohu (*Labeo rohita*) was used in the present work, and also analyzed for the physical, chemical, microbiological and sensory characteristic. Experimental trials were conducted on the whole fish, dewatered-minced meat, the prepared product to assess the quality changes during the storage period of 90 days. The results of these analysis are giving in the following subsections.

4.1 Raw Material Characteristics

The physical characteristics of Rohu fish are presented in table II. The proximate composition of fresh Rohu fish (skeletal muscle), fresh dewatered minced meat and the product are presented in table III. The other parameters like, salt soluble nitrogen (SSN), non protein nitrogen (NPN), free fatty acids (FFA), total volatile base nitrogen (TVBN) and pH are presented in table IV. The result of microbial analysis of only raw fish (Rohu) are presented in table V.

Sensory characteristics of raw material are presented in table VI. The results showed that all six fishes were acceptable for production of surimi from sensory point of view. The total score of fishes were 54, 52, 49, 48.75, 48.25, and 47.75 respectively. The total score of 54 and 55 belong to excellent grade, 49, 48.75, 48.25, 47.75 belong to very good grade.

4.2. Storage of study of dewatered minced meat with or without additive

4.2.1. Salt Soluble Nitrogen (SSN)

The data on the changes in SSN during frozen storage is presented in table No VII, Fig (IIIA, IIIB). Salt soluble nitrogen label reached a value of 65.38% after storage period of 10 days and gradually it decreased to 45.89% after the storage period of 90 days in case of dewatered minced meat. In case of treated sample, the value was 69.23 after 10 days storage period and reached a value of 54.01 over the same storage period. The SSN value after 40 days storage period were 54% and 63.67% for the product prepared from sample and reached the value of 46.01% and 55.02% respectively after 90 days storage period.

4.2.2. Non-protein Nitrogen (NPN)

The data on the changes in NPN during frozen storage is presented in the table no VIII and figure (IVA, IVB). It has shown an initial decrease followed by again an increase value during the storage period of 90 days incase of control sample and also treated sample. In case of the product prepared from the control sample, the NPN value showed an initial decrease followed by a significant increase on further storage. But incase of, the product prepared from treated sample, it had an initial value of 345.6 mg% and it had a gradually increase, reaching a value of 360.8 mg% at the end of the storage period.

4.2.3. Total Volatile Base Nitrogen (TVBN)

The data on the changes in TVBN during frozen storage is presented in table no IX and figure. (VA, VB)

The value of TVBN has reached 3.69 mg% after 10 days of storage and gradually increased to 13.55mg% after storage period of 90 days in case of dewatered minced meat. But in case of treated sample, i.e., surimi the value was 3.20mg% after 10 days of storage and reached at a level of 12.93mg% over the same period of storage as that of control sample.

The TVBN value after 40 days storage period were 8.61mg% and 8mg% for the value added product prepared from controlled and treated sample and reached the value of 14.16mg% and 13.55mg% respectively after 90 days of storage.

4.2.4. Free Fatty Acid (FFA)

The data on the changes in FFA during frozen storage is given in table No X, Fig (VIA, VIB) the value of free fatty acid was 4.94% as oleic acid after 10 days storage period and it gradually increased to 18.11% after 90 days storage period, in case of dewatered minced meat. In case of treated sample, i.e. surimi the value of FFA was 4.37% of oleic acid after 10 days of storage period and reached a value of 15.75% over 90 days of storage. The FFA value after 40 days storage period were 10.5% and 8.52% for the product prepared from controlled and treated sample and increased to a value of 17.04% respectively after 90 days storage period.

4.3. pH

The pH values of the dewatered minced meat, surimi and product prepared from control and treated sample were measured during frozen storage is depicted in the Table XI and figure (VIIA, VIIB). From the table it is clear that the pH values decreased initially during first 70 days and then there was a slight increase for rest of the frozen stored period in case of DWM and Surimi. The pH value after 40 days storage period were 6.76 and 6.78 for the value added product prepared from controlled and treated sample and reached 6.72 and 6.79 respectively after 90 days storage period.

4.4. (T.P.C.) Total Plate Count

The values of total plate count of the dewatered mince and surimi and the product prepared from control and treated sample is presented in the table XII and fig. (VIII A, VIII B) From the table it is clear that the value of TPC was increasing during frozen storage of three months in case of dewatered mince. The TPC value after 10 days of storage period reached the value of 4.58×10^4 /g of meat and increased to 4.74×10^4 /g in case of dewatered minced meat. But the value of TPC was decreasing in case of treated sample. After 10 days of storage period TPC value reached the value of 4.34×10^4 /g of meat and decreased to 2.72×10^4 /g of meat after 90 days of storage in surimi sample. Similarly in case of sample taken from the control and the product prepared, the TPC. Count was 4.85×10^4 at the end of 40 days storage and then there was marginal increase till 60 days storage period, subsequently it had a decreasing value. The TPC count at the end of 90 day was 4.75×10^4 but in case of product prepared from treated sample, followed the same trend as in case of surimi.

4.5. Sensory Characteristics :

The result of sensory evaluation of both product prepared from controlled and treated sample are presented in table XIII. The average score for each attribute. The values for color and appearance, flavour, texture, fish flavour intensity and over all acceptability were 7.75, 7.5, 7.2, 7.12, 7.37 respectively in case of product prepared from treated sample. On the 0th day, the values had a decreasing trend during the storage period. At the end of the storage period of 90 days, the values for the same parameter were 7.30, 7.2, 7.0, 6.9 and 7 respectively for the same product. In case of product prepared from control sample the values for different sensory quality parameter like color and appearance, flavour, texture, fish flavour intensity and over all acceptability were 7.5, 7.25, 7.125, 7, and 7 respectively and finally, it reached the value of 6, 6.05, 6.5, 6.2 and 5.87 for the same parameters respectively at the

end of storage period.

4.6. Statistical Analysis

The value of proximate composition of three different samples like fresh, raw fish, dewatered mince meat and the product prepared from the dewatered minced meat were analysed by using analysis of variance (ANOVA). (Snedecor and Cochran, 1962) and the result presented in the table(XIV). The correlation co efficient between different analytical method is given in table no (XV). The mean panel score of product prepared from both controlled and treated sample on the 0th day, 45th day, 90th day were analysed using two way analysis of variance and the results obtained are presented in table (XVI).

4.7. H. A. C. C. P.

The critical control point in the production of Fish ball from surimi of Rohu is presented in the table (XX).

4.8. Refrigerated Display Unit (RDU)

The values for FFA TVBN and SSN, of the product prepared from control and treated sample at 0th day 3rd day, 5th day and 7th day of storage in RDU are presented in table No : (XVII). The organoleptic evaluation of fish ball prepared from 2 different samples like control & treated and stored in refrigerated display unit, maintained at $10 \pm 2^{\circ}\text{C}$ is presented in table No(XVIII).

The mean panel score of fish ball prepared from both controlled and treated sample on the 0th day, 3rd day, 5th day, 7th day were analysed using analysis of variance and the results obtained are presented in table (XIX).

4.9. Cost Effective Analysis of Product

The cost effective analysis of product (Fish ball) is given in the table (XXI). The cost per unit of product weighing of 30 g. was found to be Rs. 5.28.

TABLE II

Physical Characteristics of raw material
(20 number of fishes were taken as raw material)

Sl. No.	Characteristics	Mean
1.	Average total length (cm)	49.4
2.	Average standard length (cm)	44.6
3.	Average weight (kg)	1.32
4.	Yield of dressed fish (%)	77.35
5.	Yield of picked meat (%) [Based on whole fish]	44.67
6.	Yield of picked meat (%) [Based on dressed fish]	57.75
7.	Yield of dewatered minced meat (%) [Based on picked meat]	82.6

TABLE : III

Proximate composition on fresh Rohu, DWM and product
(% of meat)

Proximate composition	Whole fish	DWM	product
Moisture	79.7	82.1	73.84
Protein	17.1	16.50	16.25
Crudefat	1.9	0.6	1.2
Ash	1	0.5	0.9

TABLE IV

Chemical characteristics of raw material

Sl. No	Characteristics	Mean
1.	SSN (g %)	73.52
2.	NPN (mg%)	363.0
3.	FFA (% of Oleic acid)	2.62
4.	TVB -N (mg%)	3.08
5.	pH	6.9

TABLE V

Microbiological characteristics of raw material

Characteristics count/g of meat	Whole fish
Total Plate Count (TPC)	2.5×10^5

TABLE VI

Sensory characteristics of raw materials

Attributes \ Sample No	I	II	III	IV	V	VI
Appearance	9.25	9	8.25	8.25	8.25	8.75
Gill	8.75	8.75	7.75	8	8	7.75
Odour	9	8.75	7.75	8.25	8.25	7.75
Texture	9	8.75	8.5	7.75	7.75	7.75
Color	9	8.25	8.5	8.75	8	8.25
Eye	9	8.5	8.25	7.75	8	7.5
Total Score	54	52	49	48.75	48.25	47.75

TABLE VII

Change in Salt soluble nitrogen (SSN) (g% of total protein) in Dewatered minced meat, Surimi and Value added product during frozen storage At -20⁰c

Sample Storage Days	Dewatered minced meat (C)	Surimi (T)	Value Added product	
			C ₁	T ₁
10	65.38	69.23	---	---
20	60.69	67.6	---	---
30	56.0	65.92	---	---
40	53.96	63.28	54.00	63.67
50	51.92	61.64	52.00	61.34
60	49.88	58.00	50.00	59.01
70	48.55	56.67	48.67	57.68
80	47.22	55.34	47.34	56.35
90	45.89	54.01	46.01	55.02

(-) No data or data not collected.

FIG : III A

Change in Salt Soluble Nitrogen (SSN) (g% of total protein) in Dewatered minced meat and Surimi and Value added product during frozen storage

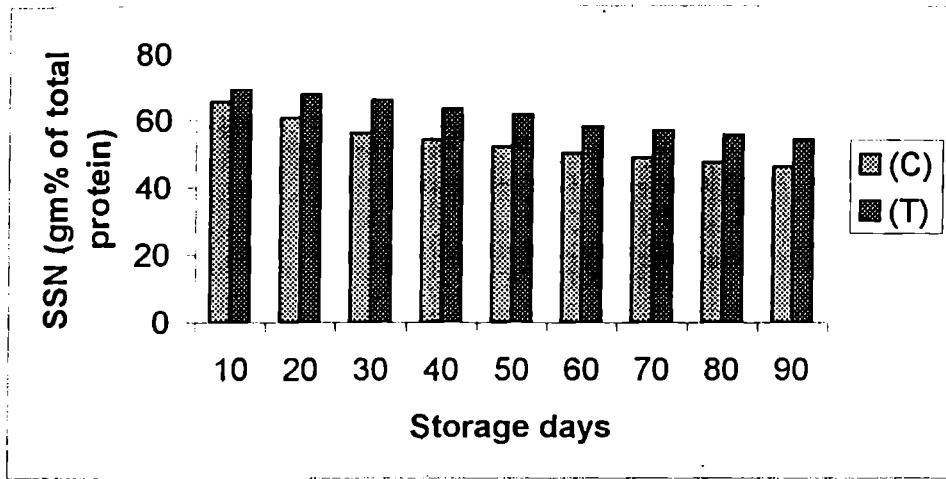


FIG : III B

Change in Salt Soluble Nitrogen (SSN) (g% of total protein) in value added product prepared from controlled and treated sample during frozen storage

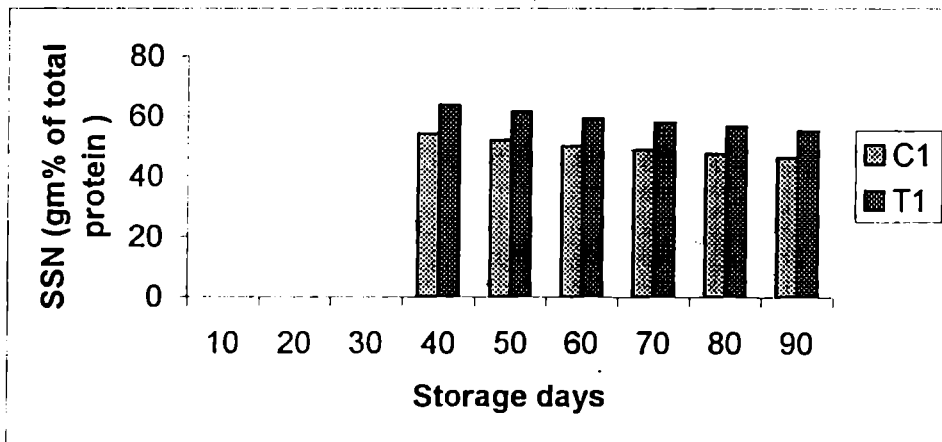


TABLE VIII

Change in Non-protein Nitrogen (NPN) (mg/100g of meat) in DWM, Surimi and Value added product during frozen storage.

Sample/ Storage Days	C	T	C ₁	T ₁
10	362	361.0	---	---
20	359.5	352.0	---	---
30	357.0	343.0	---	---
40	356.3	344.6	357.3	345.6
50	355.6	346.3	356.6	347.2
60	355.0	347.9	356.0	348.8
70	356.3	352.6	357.3	352.9
80	357.6	357.3	358.6	356.9
90	358.9	362.0	359.9	360.8

(-) No data

FIG : IV A

Change in Non-protein Nitrogen (NPN) (mg/100g of meat) in DWM, Surimi and during frozen storage.

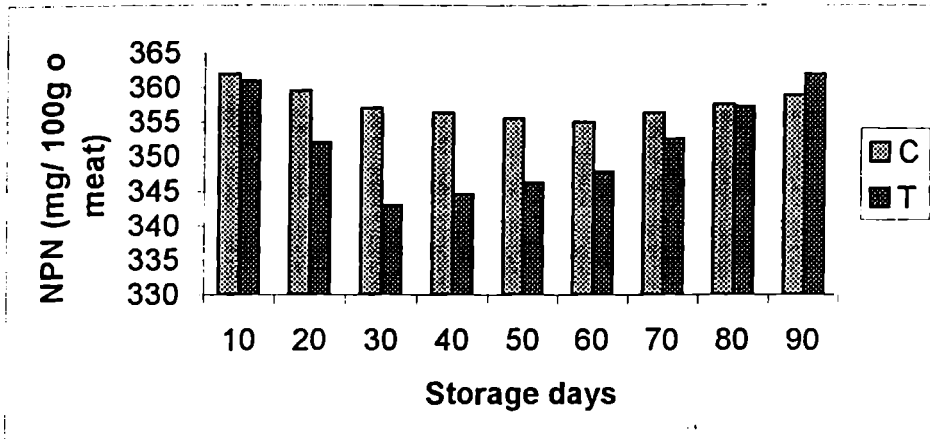


FIG : IV B

Change in Non-protein Nitrogen (NPN) (mg/100g of meat) in value added product prepared from controlled and treated sample during frozen storage.

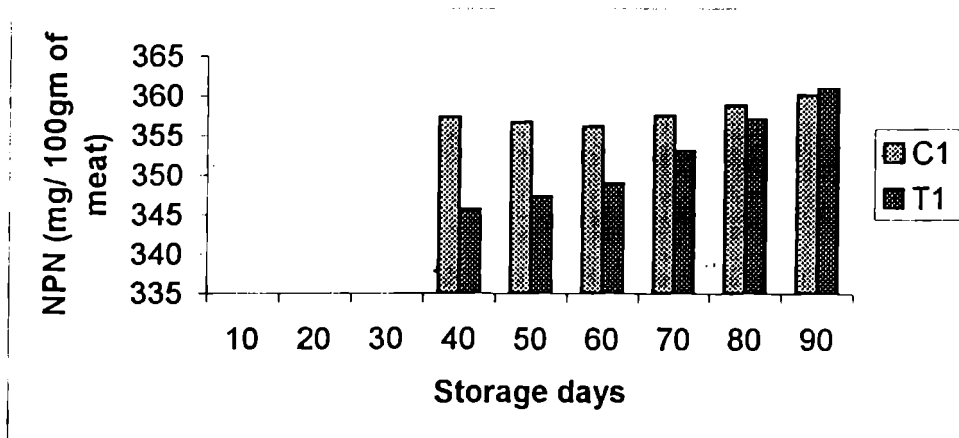


TABLE IX

Change in Total Volatile Base Nitrogen (TVBN) (mg /100g of sample) in Dewatered minced meat and Surimi and Value added product during frozen storage.

Sample Storage Days	C	T	C1	T1
10	3.69	3.20	---	---
20	4.92	4.31	---	---
30	6.16	5.54	---	---
40	7.39	6.77	8.61	8.00
50	8.62	8.00	9.24	8.62
60	9.85	9.24	11.08	9.85
70	11.08	10.47	12.32	10.47
80	12.32	11.70	12.93	12.32
90	13.55	12.93	14.16	13.55

(-) Nodata

FIG : V A

Change in Total Volatile base nitrogen (TVBN) (mg /100g of sample) in Dewatered minced meat and Surimi during frozen storage.

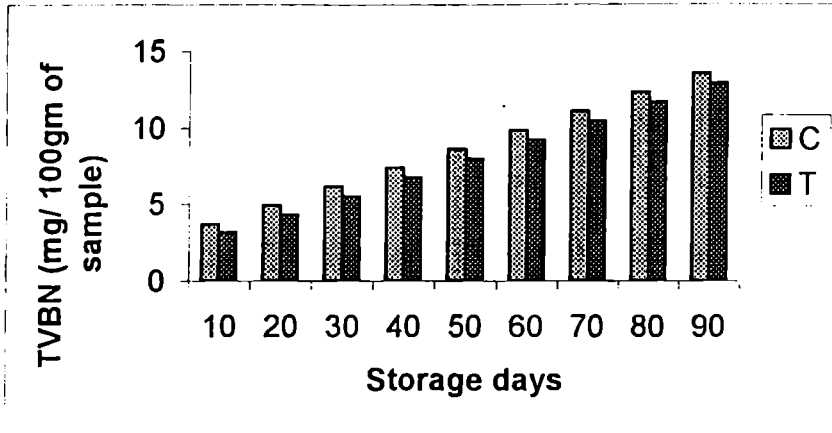


FIG : V B

Change in Total Volatile base nitrogen (TVBN) (mg /100g of sample) in value added product prepared from controlled and treated sample during frozen storage.

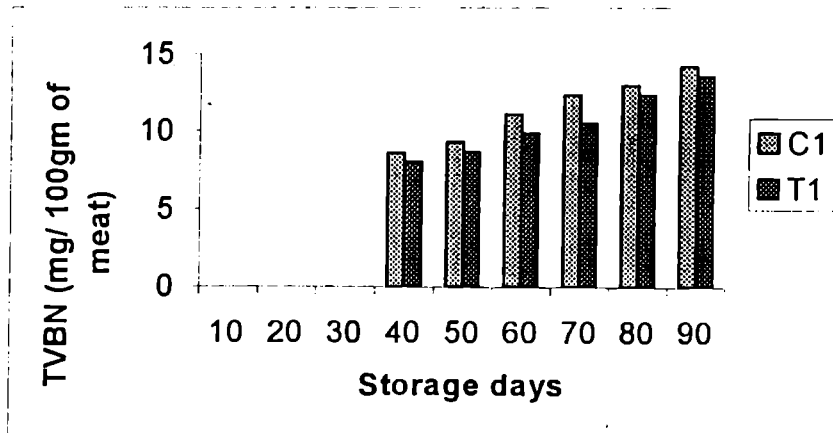


TABLE X

Change in Free Fatty Acid (FFA) (% of Oleic Acid) in Dewatered minced meat and Surimi and Value added product during frozen storage.

Sample Storage Days	C	T	C₁	T₁
10	4.94	4.37	---	---
20	6.58	5.25	---	---
30	8.23	6.12	---	---
40	9.88	7.87	10.5	8.52
50	11.52	10.5	11.66	10.95
60	13.17	12.25	14.00	13.39
70	14.82	13.12	15.16	14.6
80	16.47	14.0	16.33	15.82
90	18.11	15.75	18.66	17.04

(-) Nodata

FIG : VI A

Change in Free Fatty Acid (FFA) (% of Oleic Acid) in Dewatered minced meat and Surimi during frozen storage.

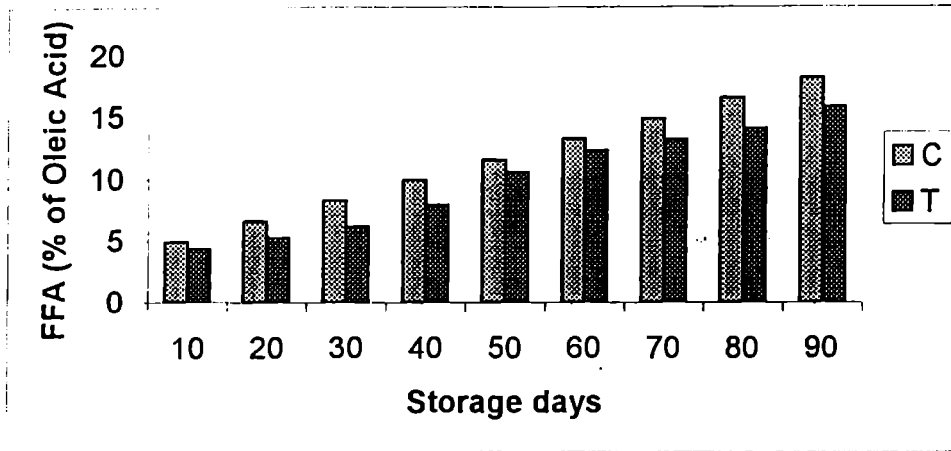


FIG : VI B

Change in Free Fatty Acid (FFA) (% of Oleic Acid) in value added product prepared from controlled and treated sample during frozen storage.

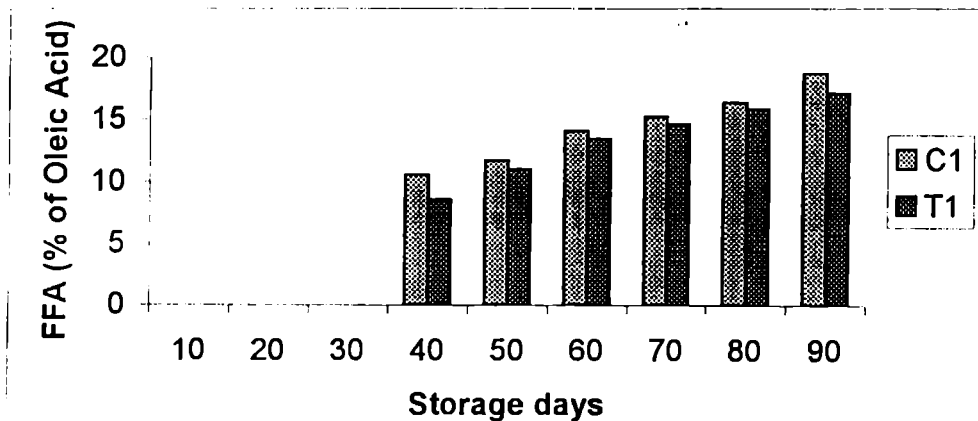


TABLE XI

Change in pH in Dewatered minced meat (DWM) and Surimi (S) and Value added product during frozen storage.

Sample/ Storage Days	C	T	C ₁	T ₁
10	6.79	6.81	--	--
20	6.78	6.79	--	--
30	6.77	6.78	--	--
40	6.76	6.78	6.76	6.78
50	6.75	6.78	6.76	6.77
60	6.74	6.78	6.75	6.77
70	6.69	6.77	6.70	6.76
80	6.70	6.78	6.71	6.78
90	6.71	6.79	6.72	6.79

(-) Nodata

FIG : VII A

Change in pH in Dewatered minced meat (DWM) and Surimi (S) during frozen storage

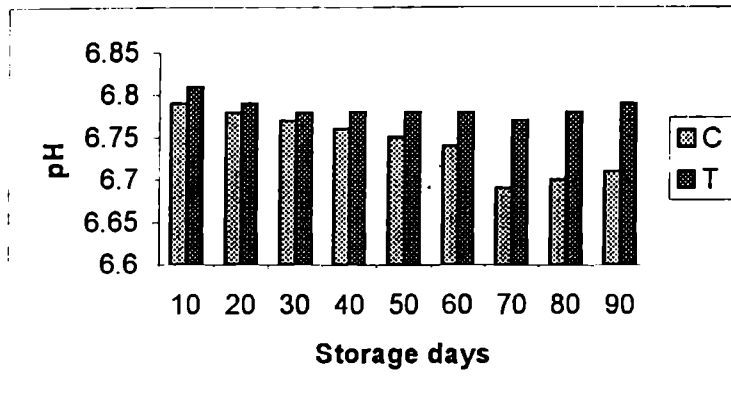


FIG : VII B

Change in pH in value added product prepared from controlled and treated sample during frozen storage

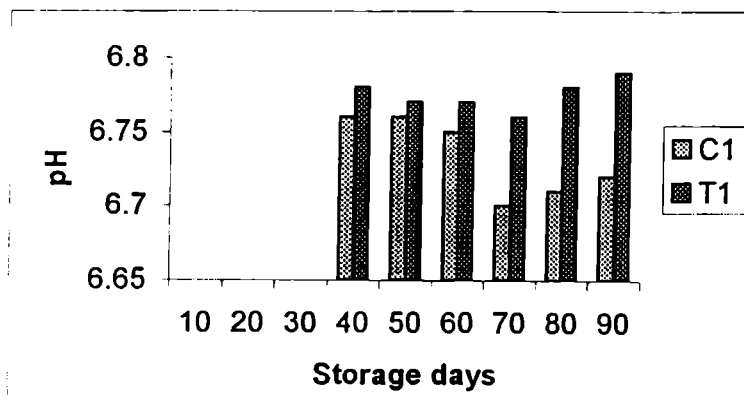


TABLE XII

Change in Total plate count (TPC)/g of meat in Dewatered minced meat and Surimi and Value added product

Sample/ Storage Days	C	T	C ₁	T ₁
10	4.58 x 10 ⁴	4.34 x 10 ⁴	---	---
30	4.66 x 10 ⁴	4.18 x 10 ⁴	---	---
30	4.74 x 10 ⁴	4.02 x 10 ⁴	---	---
40	4.75 x 10 ⁴	3.77 x 10 ⁴	4.85 x 10 ⁴	4 x 10 ⁴
50	4.77 x 10 ⁴	3.52 x 10 ⁴	5 x 10 ⁴	3.7 x 10 ⁴
60	4.78 x 10 ⁴	3.27 x 10 ⁴	4.9 x 10 ⁴	3.6 x 10 ⁴
70	4.77 x 10 ⁴	3.04 x 10 ⁴	4.8 x 10 ⁴	3.3 x 10 ⁴
80	4.75 x 10 ⁴	2.88 x 10 ⁴	4.77 x 10 ⁴	3.0 x 10 ⁴
90	4.74 x 10 ⁴	2.72 x 10 ⁴	4.75 x 10 ⁴	2.7 x 10 ⁴

(-) Nodata

FIG : VIII A

Change in Total plate count (TPC) /g of meat in Dewatered minced meat and Surimi during frozen storage

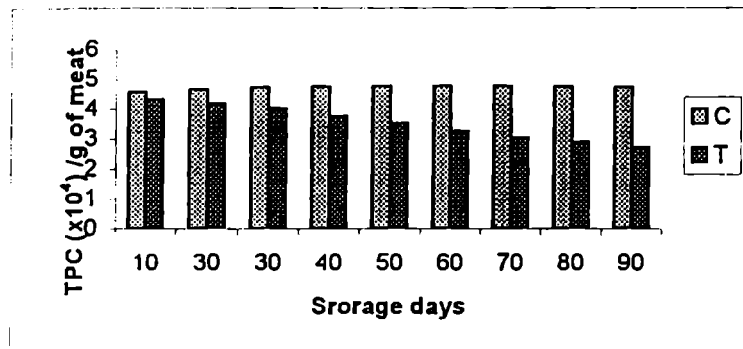


FIG : VIII B

Change in Total plate count (TPC)/g of meat in value added product prepared from control and treated sample

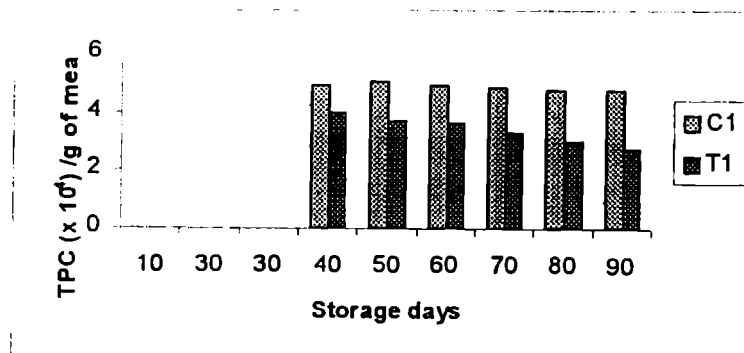


TABLE – XIII

Mean sensory score of Fish balls prepared from Dewatered minced meat (C) and Surimi (T) on 0th, 45 th and 90th days of frozen storage

Days	Sample	Color & Appearance	Flavor	Texture	Fish Flavor Intensity	Over all acceptability
0	C	7.5	7.25	7.125	7	7
	T	7.75	7.5	7.2	7.125	7.375
45	C	6.625	6.625	6.5	6.125	6.375
	T	7.45	7.3	7.2	7.0	7.2
90	C	6	6.05	6	5.62	5.87
	T	7.3	7.2	7.0	6.9	7.0

TABLE – XIV

Analysis of variance (ANOVA) of proximate among Dewatered minced meat (DWM), Surimi (S) and Value added product (VP)

	Source variation	d.f.	Sum of Squares	Mean Square	F Value	CD (%)
Moisture	Between group	2	144.436	72.218	3428.069**	0.32
	Within error	9	0.190	0.021		
	Total	11	144.626			
Protein	Between group	2	1.527	0.763	59.739**	0.28
	Within error	9	0.115	0.013		
	Total	11	1.642			
Crudefat	Between group	2	3.387	1.693	254.000**	0.18
	Within error	9	0.060	0.007		
	Total	11	3.447			
Ash	Between group	2	0.560	0.280	25.200**	0.23
	Within error	9	0.100	0.011		
	Total	11	0.660			

* P<0.05

** P<0.01

TABLE – XV

Correlation between the analytical methods.

Samples	SSN X FFA	SSN X TVBN	SSN X p ^H	NPN X TPC	TVBN X p ^H
DWM (C)	- 0.967**	- 0.967**	0.900**	- 0.931**	- 0.937**
SURIMI (T)	-0.994**	- 0.994**	0.549	- 0.291	- 0.518

if $> (t_{0.05,7} = 0.666) = *$

$> (t_{0.01,7} = 0.798) = **$

TABLE NO. – XVI

ANOVA of fish balls prepared from controlled and treated sample on
0th day, 45th day and 90th day of storage at - 20°C

ANOVA	FLAVOR	FISH BALL			
Source of Variation	SS	df	MS	F	Fcrit
DUE TO DAYS	0.564375	2	0.282187	2.784173	19.00003
DUE TO TREATMENTS	0.717604	1	0.717604	7.080164	18.51276
Error	0.202708	2	0.101354		
Total	1.484687	5			

ANOVA	texture	fish ball			
Source of Variation	SS	df	MS	F	Fcrit
DUE TO DAYS	0.439375	2	0.219687	1.972872	19.00003
DUE TO TREATMENTS	0.525104	1	0.525104	4.715622	18.51276
Error	0.222708	2	0.111354		
Total	1.187188	5			

* $P < 0.05$

** $P < 0.01$

TABLE – XVII

Changes of biochemical parameters of fish ball prepared from de-watered minced meat (C) and Surimi (T) during refrigerated storage study at 10 ± 2°C

Days	Samples	SSN (g% total protein)	FFA (% of Oleic Acid)	TVBN (mg %)
0	C	70.00	4.66	3.50
	T	70.76	3.65	3.38
3	C	56.00	11.66	9.24
	T	61.34	10.95	8.62
5	C	49.66	18.67	14.16
	T	55.02	17.04	13.55
7	C	43.32	24.69	20.34
	T	48.68	23.04	20.15

TABLE – XVIII

Mean sensory scores of fish ball prepared from de-watered minced meat (DWM) (C) and Surimi (T) during refrigerated storage at 10 ± 2°C

Days	Sample	Color & Appearance	Flavor	Texture	Fish Flavor Intensity	Overall acceptability
0	C	7.5	7.25	7.12	7.0	7.0
	T	7.75	7.5	7.2	7.12	7.37
3	C	7.0	7.1	7.2	7.0	6.5
	T	7.0	7.2	7.2	7.2	6.9
5	C	6.3	6.4	6.2	6.6	6.2
	T	6.8	7.0	7.0	7.0	6.9
7	C	6.0	5.8	5.8	6.0	6.0
	T	6.8	6.0	6.2	6.3	6.5

TABLE NO. - XIX

ANOVA of fish ball prepared from controlled and treated sample on 0th day, 3rd day and 5th day 7th day of refrigerated storage at $10 \pm 2^{\circ}\text{C}$

ANOVA	Flavor	rdu			
Source of Variation	SS	df	MS	F	Fcrit
DUE TO DAYS	2.543437	3	0.847812	35.85463*	9.276619
DUE TO TREATMENTS	0.165313	1	0.165313	6.991189	10.12796
Error	0.070938	3	0.023646		
Total	2.779688	7			

ANOVA	texture	rdu			
Source of Variation	SS	df	MS	F	Fcrit
DUE TO DAYS	1.9104	3	0.6368	9.629032*	9.276619
DUE TO TREATMENTS	0.2048	1	0.2048	3.096774	10.12796
Error	0.0984	3	0.066133		
Total	2.3136	7			

* $P < 0.05$

** $P < 0.01$

TABLE - XX**HACCP OF VALUE ADDED PRODUCT FROM SURIMI**

<u>Product flow</u>	<u>Hazard</u>	<u>Preventive Measures</u>	<u>CCP</u>
Arrival of raw material at laboratory	Substandard quality	Ensure reliable source, sensory evaluation.	CCP
Storage of raw material	Growth of bacteria	Time temperature Control	
Washing	Contamination	Ensure pure potable water supply.	
Beheading descaling and degutting	Contamination from knife/handling	Ensure surface disinfection & personnel hygiene	
Meat picking	Contamination from meat picking machine /presence of metal fragments.	Use properly clean and hygeinic meat picker and metal detector	
Minicing	Do	Do	
Washing	Contamination	Ensure portable water supply	CCP
Pressing in screw press	Contamination from screw press and presence or metal fragment, presence of iron.	Use properly clean screw press and metal detector	
Mixing with cryoprotectants	Contamination from silent cutter	Use proper food grade quality cryoprotectants & use clean silent cutter.	
Adding ingredients and spices for the preparation of ready to eat product	Contamination	Use food grade quality spices or Sterilised fried spices.	
Shaping	Contamination from handling	Less handling	

Contd.....

Packaging	Spoilage (Oxidation).	Suitable packaging material/Vacuum	
Freezing	Chemical/autolytic spoilage	Time and Temperature control.	CCP
Frying	Charring, over Cooking.	Temperature control	

TABLE - XXI

THE PRODUCTION COST OF PERUNIT OF FISH BALLS FROM SURIMI

Item	Quantity	Price/Rs. (Approx)
Rohu fish	2kg.	120.00
Picked meat	1 kg.	
Dewatered Minced meat	750g.	
Onion	150g.	1.50
Garlic	12g. (50g.)	2.00
Ginger	12g. (50g.)	1.00
Chilli	3.75g.	2.00
Cumin	3.75g.	1.00
Chilli powder	3.75g.	1.00
Garam masala	3.75g.	1.00
Salt	18g.	.25
Potato	210g.	1.50
MSG	1.5g.	.50
Egg	4 No	6.00
Bread crumbs	1 packet	6.00
Oil (Mastered Oil)	200ml	12.00
Gas (L.P.G.)	-	4.58
Freeze (Meter charge)	-	6.00
Labour Charge		10.00
Profit (20%)		35.26
Total		211.59

Hence, No of fish balls are 40
(30g.)

$$\text{Approximate cost of each unit} = \frac{211.59}{40} = 5.28$$

(Excluding, packing cost + distribution cost + advertisement cost)

Chapter-V

DISCUSSION

5. DISCUSSION

5.1 Raw material characteristics

5.1.1. Physical characteristics

The average total length and weight of 20 no of Rohu fishes used in the present study were 49.4 cm and 1.32 kg respectively. Dressing yield of Rohu was 77.35%. Bhat, (1983) has reported a dressing yield of 68.04% from medium sized Mrigal.

The yield of picked meat was 44.67% based on whole fish. However, picked meat yield of 26.1% was observed in Tilapia (Finne et. al., 1980). Generally, the picked meat yield relates directly to the size of fish. The fairly high yield obtained in the present study may be due to the bigger size of fish used. The yield of dewatered-minced meat based on picked meat weight was 82.6%.

5.1.1.2. Chemical Characteristics

The proximate composition of raw fish samples is given in table (III). Sankar, T.V. et. al. (2001) and Nowsad et. al., (1999) have reported the proximate composition of raw fish Rohu. The proximate composition of fish depends on the diet, size, sex, physiological state of fish and also the ecological conditions. In the present study, the fat content (1.9%) appeared to be low, but however, a low fat content is very important to get good quality gelled or emulsion type and analogue products. (Suzuki, 1981; Flick et. al., 1990). The results obtained in the present study are in concurrence with above reports within reasonable limits. Some of the chemical characteristics like TVBN, FFA, NPN, SSN and pH were analysed to assess the quality of fish meat. (Table No IV). Connell, (1980), recommended TVBN value of 20mg% as the safe level for 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 chemical parameters such as TVBN and SSN were within the limit of acceptance 3.08 mg%, 73.52% respectively indicating that the raw material was of high quality. The NPN value was 363.0mg% which coincides with that of Silver Carp where the result for NPN was 361.0mg% (Siddaiah et. al., 1999).

The organoleptic analysis supported by TVBN values and SSN obtained for Rohu fish indicate that the raw material was quite fresh and suitable for the preparation of minced meat.

Shenoy and James, (1972) observed a FFA content of 1.3 (as% of Oleic Acid) in case of *Tilapia mossambica* which almost coincide with the value obtained in the present

study. In the present study, the value for pH was 6.9 coinciding with such similar result of Nowsad *et. al.*, 1999.

5.1.1.3. Microbiological Characteristics

The TPC/g of whole fish observed in the present side table (V) was found to be in conformity with the result as described earlier. (Siddaiah *et. al.*, 1999)

5.2. Standardization and Preparation of Surimi

The fresh Rohu was processed within three hours of harvesting without further delay. The whole fishes were dressed. During evisceration special care was taken in order to keep the fish in chilled condition. The dressed fishes were subjected to mechanical separation of fish flesh. The separated Rohu mince was reddish in color. Then the meat was subjected to repeated washing with chilled water (5°C). Nowsad *et. al.*, (1999) reported two washing cycles for the mince prepared from Rohu fish with meat to water ratio of 1:4.

They also recommended 0.1% NaCl solution for final washing for tropical major carp minced meat, In the present study two times washing was performed with chilled water (5°C) giving a suspension time of 5 minutes. 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 given due consideration.

During washing of minced meat sarcoplasmic protein which inhibits gelation is removed (Roussel and Cheftel, 1988; Shamsundar *et. al.*, 1988). Through increasing the number of washing cycle it is possible to remove fat, colour pigments, odour bearing components and myofibrillar protein concentration is increased. The major disadvantage in surimi production is the need of large amount of water. Among many alternative, a 3:1 proportion of water to picked meat was found satisfactory according to Babbitt (1986). Suzuki (1981) has recommended a ratio of 1:2 to 1:8 (minced : water) depending on the species.

In the present study, the moisture content of minced meat was increased from 79.7% to 82.1% after 2 washing cycles. Hydrophilic nature of myofibrillar protein is responsible for such an increase in moisture content. Dewatering is an essential step in maintaining the moisture content of surimi around 80%. Good quality surimi usually contains less than 85% moisture (Lee, 1985). After washing the fat content decreased from 1.9% to 0.6%. Lin and Morrissey (1995) reported a 39% reduction of lipid on fresh water squaw fish mince after third wash.

Washing resulted on the decrease in protein content due to removal of sarcoplasmic protein (Lin & park, 1996). The dewatered sample contained 16.5% 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; Grantam, 1981; Roussel and Cheftel, 1988).

The unwashed mince was pinkish in color having fishy odour. Washing resulted in blunt color and notable reduction in odour of the mince due to removal of odour producing compounds and color. Similar result was observed by Nowsad *et. al.*, (1999) in tropical major carp mince.

Surimi is used as frozen material and the protein denaturation results in loss of solubility, water holding capacity, gelling ability and lipid emulsifying capacity. ^(Grabowska *et. al.*, 1974, 1975) This problem necessitates a step of stabilazation against the freeze denaturation, which is an irreversible change in the myofibrillar protein namely actomyosin. This change is occurreds due to intra molecular 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. Hence, in the present study sucrose (4%), sorbitol (4%) and sodium tri polyphosphate (0.3%) were mixed with the dewatered minced meat in order to protect the deteriorative changes during the frozen storage period.

5.3 Quality Changes During Storage

5.3.1. Nitrogenous Compounds

5.3.1.1. Salt Soluble Nitrogen (SSN)

During freezing and subsequent storage, a number of microstructural and chemical alterations occur resulting in drip from third muscle and a decrease in salt soluble protein, loss of succulence, and development of off flavours. There was a gradual fall in SSN throughout the period of storage for all the samples (TableVII). The decrease was more in dewatered minced meat compared to the treated sample. This decrease in SSN content can be attributed to the aggregation leading to the insolublization of myofibrillar protein fractions. It has been pointed out by Dyer and Fraser, (1959) that lean fish are more prone to denaturation than any fatty fish when held under similar condition of storage. Since Rohu may be grouped under lean variety due to its low lipid content, the above reason may hold good for the loss of SSN from the meat of this species during frozen storage.

5.3.1.2. Non-protein Nitrogen (NPN)

The data on the changes in NPN during frozen storage showed an initial decrease followed by significant increase on further storage. (Table No VIII). Siddaiah et. al., (1999) have reported the NPN content of meat separated from silver carp. The results obtained in the present study are in concurrence with the above reports within reasonable limits. NPN may be attributed to the enzymes both muscles and microbial nature, through which considerable amount of free amino acids are released. (Reddy, V.S. et. al., 1991). In spite of its continuous production, the low NPN content can be attributed to its utilization by the microorganisms and lost through the thaw drip.

5.3.1.3. Total Volatile Base Nitrogen (TVBN)

Total volatile base nitrogen indicates the production of ammonia, mono-dimethylamine nitrogen and are found in the common pattern of spoilage. Kimura and Kiamakura, (1934) recommended a level of 10mg% or less for fresh fish, 20-30mg% at the beginning of spoilage and over 30mg% for spoiled fish. In the present study, the TVBN of fresh fish 3.08mg% is in concurrence with above mentioned recommendation indicating that the raw material was of high quality. During 3 months storage period, the TVBN content of all samples increased gradually (Table No IX). Siddaiah et. al., (1999) observed increasing trend in case of mince prepared from Silver Carp both for controlled and the sample treated with sugar, sorbitol and sodium tri polyphosphate. However, the values obtained for TVBN in the present study indicate that all the samples are in acceptable condition during the storage period of 90 days.

5.3.2. Free Fatty Acids (FFA)

There was an increase in FFA of minced meat in all the samples stored at -20°C. (Table No X) Increase in FFA indicates lipolysis. Lipases and phospholipases which are present in the animal tissue are known to be responsible for the liberation of FFA. Koning et. al., (1985) found an increase in FFA of frozen hake mince from 17.62 to 21.5% at the end of 73 days.

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 sample (Botta and Richard, 1973). The pH values 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 tripolyphosphate. (Siddaiah *et. al.*, 1999). The increase in pH was probably due to the production of basic volatiles. Verma (1992) has recorded a low pH of 5.2 in surimi samples treated with ascorbic acids. Generally, pH does not vary during frozen storage.

5.5. Microbiological Characteristics

Exposure to low temperature causes an initial decrease in the number of living bacteria followed by a lag period before the development of survivors. The extent of initial decrease and the lag period depend on temperature (Stewart, 1984). Nickelson *et. al.*, (1980) observed a similar decrease in the frozen mince of black drum, sand trout and tilapia upto 30 days in the first 2 species and 15 days storage in tilapia followed by an increase latter. On the contrary the mullet mince showed a continuous decrease during the frozen storage of 6 months. The minced meat procured from Rohu fish can be successfully utilized for preparation of value added product like fish ball and the incorporation of cryoprotectants like sucrose (4%), sorbitol (4%), and sodium tri-polyphosphate are found to be most beneficial in preventing the protein deterioration during frozen storage of minced meat.

5.6. Sensory Characteristics

The organoleptic analysis of the product prepared from the minced meat treated with different cryoprotective agents like. Sucrose, Sorbitol, and STPP were acceptable even after 90 days storage period, (Table No. XIII) where as, the product prepared from the control after the storage period of 90 days were found to be of the average grade, if not rejected.

Similar observation has been reported by Siddaiah *et. al.*, (1999) for Silver Carp mince treated with sugar, sorbitol and sodium tripolyphosphate.

5.7. Statistical Analysis

The value of proximate composition of three different. Samples like, fresh raw fish, dewatered minced meat and the product prepared from dewatered minced meat were analysed by using analysis of variance (ANOVA) in one way method. From the result (Table No. XIV) it can be concluded that a significant difference at 1% level ($P < 0.01$) is present among the three samples in case of moisture with CD value of 0.32. Similarly, in case of protein a significant difference at 1% level among fresh raw fish, DWM and product, the CD value was 0.28. Besides, in case of other two parameters, i.e., Crudefat and ash, we found a significant difference at 1% level with CD value of 0.18 and 0.23 respectively.

From the table (XV), we can draw inference that negative correlation exist between SSN and other chemical parameters such as FFA and TVBN. SSN is significantly and negatively correlated with FFA at 1% significant level. SSN is negative correlated with TVBN at 1% level. Verma and Srikar (1994) suggested a significant inverse correlation between salt soluble nitrogen (SSN), free fatty acid (FFA), trimethylamine (TMA) as well as total volatile base nitrogen (TVBN). NPN is negatively correlated at 1% significant level with TPC. This was confirmed by Siddaiah *et. al.*, (1999). According to their study, the NPN content can be attributed to its utilization by microorganisms. From the correlation coefficient, it can be concluded that it is necessary to use different methods to assess the quality of surimi and it is not safe to depend on single quality assessment test.

The results (ANOVA) (Table No. XVI) for flavour showed an insignificant variation ($P>0.05$) between the batches due to days and treatments. As far as texture is concerned, there exist an in significant variation between the days batches, as well as between treatments batches. Therefore, from statistical analysis it may be concluded that use of cryoprotectants have no definite advantage as far as flavour and texture are concerned. This may be due to an uniform reduction in rheological properties for both the treatments till 90 days of storage at -20°C . However, it may be concluded that for long term storage, beyond 90 days, the products prepared from the minced meat treated with cryoprotective agents may have an edge over the products prepared from the minced meat without any cryoprotectant treatment.

5.8. Use of HACCP Concept in Surimi Production

HACCP is becoming popular as a tool for quality assurance of fish and fishery product. The hazards of each processing step of fish balls production form surimi of Rohu have been suggested (Table No. XX). The raw material quality, washing, addition of cryoprotectants, addition of ingredients and spices and frying are the steps that have been identified as critical control points. The storage temperature and suitable packaging materials are important factors in determining the quality of product. Sufficient care should be taken in water quality monitoring, less handling, time & temp control, sanitation of plant, personnel hygiene and use of proper food grade quality spices and ingredients render the safety of the product.

5.9. Refrigerated Display Unit (RDU)

As seen in table no (XVII) the value for SSN, FFA and TVBN during the storage period of 7 days in the RDU maintained at temp. $10 \pm 2^{\circ}\text{C}$ were well within the acceptable limit. However the values obtained for the product prepared from the controlled sample were on the higher side.

The organoleptic attributes of the samples showed higher mean values in the beginning of experiment and scored reduced mean values as the storage period progressed (Table No. XVIII). But, the trend increase of organoleptic mean panel scores in both the samples was similar as that of chemical parameters present in table No. (XVII). Such results have been reported for fish sausage (Chandrasekhar and Cross, 1986; Hegde and Ganapati 1991).

The results of (ANOVA) (Table No. XIX) analysis of variance for flavour showed a significant variation ($P < 0.05$) due to days between batches and showed an insignificant variation ($P > 0.05$) due to treatments. As far as texture is concerned a significant variation ($P < 0.05$) is found due to days between batches and insignificant variation ($P > 0.05$) is found due to treatments.

5.10. The Cost Effective Analysis of Fish Ball

The cost effective analysis of the product was done in small scale and it was found that the cost for each unit of the product (30 g.) was Rs. 5.28 (approx) (Table No. XXI) excluding the expenditure involved in packing, carrying and advertisement which may vary and determined by the entrepreneur. It is expected that this product may have good consumer acceptability due to its reasonable cost.

Chapter-VI

SUMMARY

6. SUMMARY

In spite of the fact that the use of fish for human consumption as an alternative or substitute for red meat has increased recently, there are many freshwater fish species that have not yet been utilised effectively for various reasons, including bony flesh, characteristic flavor, odour, small size, unacceptable textural properties, and other factors. However, the utilization of such fish species would be desirable because they are excellent sources of high quality, well balanced essential amino acids and highly digestible proteins.

Fish ball is a popular fish based processed product, commonly consumed with noodles in the South East Asian region. Fish balls are also popular in many South East Asian countries. The production of fish balls used to be usually a small, family based enterprise, but in recent years many factories have invested in modern machinery to increase the production of fish balls.

The present study was undertaken to utilize the Rohu fish (*Labeo rohita*) for the production of value added product like fish ball and study its acceptability during the storage period of 3 months at (-20°C). A brief summary of the present investigation is as follows :-

- Fresh Rohu fish of average weight of 1.32 kg and average length of 49.4 cm. was used in the present study.
- Meat was separated using meat picking machine. The different physical chemical, microbiological and sensory characteristics of raw material. (minced meat) were analysed.
- Attempts were made in the present study to improve the stability of minced meat using some cryoprotective agents like sucrose (4%), sorbitol (4%) and sodium tripolyphosphate (0.3%) and another lot was kept as control without any additive and frozen stored at - 20°C. At regular interval samples were analysed for minced meat and fish ball for their quality.
- While there was an increase in the value of FFA during the entire storage period for both the minced meat and also the prepared product, there was decrease in the value of SSN in both the samples. There was an increase in pH during the frozen storage in case of all the samples. So also, the TVBN showed an increasing trend during the storage period. The Total plate count (TPC) of bacteria decreased initially and there after a rise was observed. The data on the changes of NPN during frozen storage showed an initial decrease followed by increase value on further storage. The acceptability of product during frozen

storage decreased with the decrease in the organoleptic scores. However, the shelf life of the dewatered minced meat treated with cryoprotective agent was found to be better over the control sample and also the product (fish ball) prepared from the treated sample was superior over the product prepared from control sample during the period of storage.

An attempt was made to find out the storage life of the product prepared from the control and treated sample in the refrigerated storage maintained at temperature of $10\pm 2^{\circ}\text{C}$ during the storage period of 7 days. It was observed that both the samples were acceptable at the end of 7 days storage period both chemically and organoleptically. However, the product prepared from the sample treated with cryoprotective agents had a better acceptability over the control sample.

The critical control points for the preparation of value-added product from surimi are in different stages like arrival of raw material at processing centre in a good condition, washing of the minced meat, Freezing and storage at suitable temperature which need to be given due consideration.

The cost effective analysis of the product was done in small scale and it was found that the cost for each unit of the product (30g.) was Rs. 5.28 excluding the expenditure involved in packing, carrying and advertisement cost which may vary and determined by the individual entrepreneur depending on the local conditions, demand and consumer acceptability. It is expected that this product may have a good consumer acceptability because of its reasonable cost as found out in our study.

Chapter-VII

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7. REFERENCES

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Chapter-VIII

APPENDIX

APPENDIX - I

Physical and organoleptic characteristics of fish : The samples are assessed organoleptically based on the general appearance, flavour, odour, eyes and texture etc. using the following 10 points scale :

ORGANOLEPTIC SCORE CARD FOR FRESH FISH

Individual rating	Excellent	Very good	Good	Fairly good	Fair	Below average	Poor	Very poor	Bad	
	10	9	8	7	6	5	4	3	2	
	Grade I			Grade II			Grade III			
Gills	Bright red gills, no mucous	Less bright red gills, white mucous	No odour, neutral odour	Faint pink gills, pinkish white mucous	Reddish brown gills with reddish brown mucous	Grassy, slightly sweet	Dark brownish gills and brownish mucous or dry			
Odour	Fresh seaweedy	No odour, neutral odour	No bruises, bright with lustrous sheen, but colours not distinct	Bready, malty, yeasty	slight bruises, dull	Ammonical, putrid				
Appearance	No bruises very bright distinct colours, lustrous sheen	Firm, elastic, and leaves no thumb impression when pressed	Very slightly soft and leaves thumb impression when pressed	Very soft muscle, falling apart.						
Texture	Shiny cornea, black bright pupil, convex	Bright lustrous sheen, but varied colours not very distinct	Cloudy cornea, black pupil, blood spots visible	Cloudy cornea, faded pupil sunken, very bloody						
Eyes	Very bright, lustrous sheen, with distinct varied colours	46 44 42 40 38	Tarnished with colours fading	Dull						
Colour	58 56 54 52 50	22 20 18 16 14	34 32 30 28 26	22 20 18 16 14	10 8 6 4 2					
Total score	48			36			24			12

APPENDIX – II
SENSORY EVALUATION OF FISH PRODUCT

Name _____

Fish sample **FISH BALL**

Attribute	Score	8	7	6	5	4	3	2	1
Color & Appearance		Extremely Bright	Bright	Moderately Bright	Slightly Bright	Slightly Dull	Moderately Dull	Dull	Extremely Dull
Flavor		Extremely Desirable	Desirable	Not Detectable	Not easily Detectable off Flavor	Slightly Detectable off Flavor	Moderately Detectable off Flavor	Detectable off Flavor	Putrid Flavor
Texture		Extremely Desirable	Desirable	Not Detectable	Not easily Detectable Poor Texture	Slightly Detectable Poor Texture	Moderately Detectable Poor Texture	Detectable Poor Texture	Poor Texture
Fish Flavor Intensity		Like Extremely	Like	Like Moderately	Like Slightly	Dislike Slightly	Dislike Moderately	Dislike	Dislike Extremely
Overall Acceptability		Like Extremely	Like	Like Moderately	Like Slightly	Dislike Slightly	Dislike Moderately	Dislike	Dislike Extremely

Sample
1.
2.
3.
4.
5.
6.

Color & Appearance

Flavor/Texture

Fish Flavor Intensity

Overall Acceptability

Remarks :

Signature