DEVELOPMENT OF A READY TO CONSUME SWEETENED FISH POWDER FOR CHILDREN

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"DEVELOPMENT OF A READY TO CONSUME SWEETENED FISH POWDER FOR CHILDREN "** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or society.

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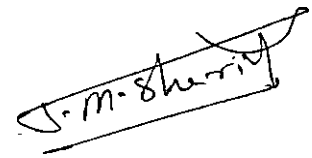

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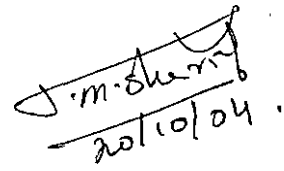

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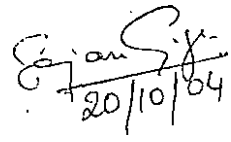

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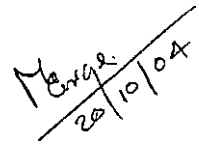

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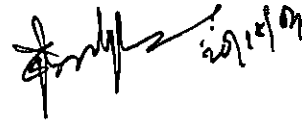

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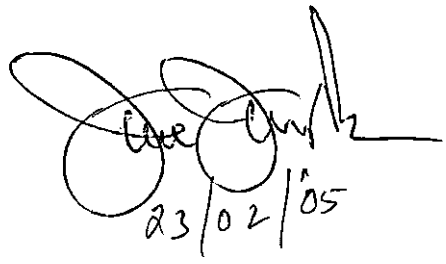

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1. INTRODUCTION

1.1. PROTEINS IN NUTRITION

The word 'protein' was coined by the Dutch chemist Gerardus Mulder in 1838 and from the Greek word 'protos' meaning 'of prime importance'. It is the second major component in animal tissues next to water.

Our bodies constantly assemble, breakdown and use proteins, so we have to count on our diets to compensate this loss. Main protein foods are beef, chicken, fish, milk and plant foods such as beans, peas, grains, nuts, seeds and vegetables.

People living in poverty may suffer from a shortage of both protein and energy in the diet. Then the body breaks down its own tissues such as muscle and makes use of it. This causes 'wasting' of muscle, organs and other tissues. Protein deficiency also increases susceptibility to infection and impairs digestion and absorption of nutrients (Paul *et al.,* 2002).

The major functions of proteins in the body include, providing structural protein (collagen, keratin), motor proteins (eg. muscle contraction, sperm swimming), enzymes, hormones, antibodies, fluid balance (edema), acid-base balance, transport proteins (eg. lipoproteins) and acting as a source of energy. So this diversity makes proteins, 'of prime importance'.

1.2. SEAFOOD - SOURCE OF TOP QUALITY PROTEIN

The fish protein is highly digestible because of very low content of stroma protein and has an excellent spectrum of essential amino acids. Cereal grains are low in lysine and / or the sulphur containing amino acids, whereas fish protein is a rich source of these amino acids. In diets based mainly on cereals, fish as a supplement can therefore raise the biological value significantly (Pigot and Tucker, 1990; Sherief 2003). Protein quality is determined by the amounts of essential amino acids present. The quality of

seafood protein is comparable with that of meat and poultry (Pigot and Tucker, 1990). Nutritive quality and protein efficiency ratio (PER) rank fish protein above casein. Protein from seafood has, protein-digestibility greater than 90% (Leu *et al.*, 1981).

Seafoods in general are low in calories (usually 150 Kcal per 100g), low in fat (normally less than 5%), low in saturated fat, rich source of omega-3 fatty acids, low in cholesterol, high in protein (normally 17 to 25%), important source of B vitamins, minerals and trace elements (Nettleton, 1987).

1.3. PROTEINS AND CHILDHOOD

Childhood is the period that covers the years from age 1 through the beginning of adolescence. Growth in childhood, while continuous, occurs at a significantly slower rate than in infancy. Children can be divided into three groups based on their age and development

Table 1: Age groups of children

Toddlers	age 1-3 years
Preschoolers	age 4-5 years
School children	age 6-10 years.

Since the anabolic activities are considerable during the entire period of childhood, the nutritional requirements in proportion to the body size are much higher than that in the adult years. Moreover, childhood and adolescence are times of considerable physical activities and hence the energy requirements are greater. Because their bodies need to grow, children are more vulnerable than adults to the effects of malnutrition. By the age of four years the brain reaches 80 to 90% of its adult size. Once the critical period of cell division is over, an adequate diet given subsequently, cannot bring about an increase in the brain cell numbers. The Recommended Dietary Allowance (RDA) of protein intake for toddlers is 1.2g protein per kg body weight, for preschoolers 1.1g per

kg and, for school aged children 1.0g per kg (Paul *et al.*,2002) . Corinne and Marilyn (1982) considered that in order to counter the lesser efficiency in a mixed diet the RDAs should be 1.8g per kg, 1.5g per kg and 1.2 g per kg, respectively. While the total energy and protein requirements increase, the protein needed per kg body weight slowly decreases as children move through childhood.

The nutritional requirements of child cannot be satisfied apart from an understanding of the behavioral changes that occur. One of the most dramatic and normal changes taking place during the second year of life is the reduction in appetite, corresponding to the slower rate of growth. Toddlers have a short attention span and are easily distracted from eating. Their response to food is often inconsistent (Corinne and Marilyn, 1982). Among the preschoolers, a common phenomenon is the practice of 'specializing in eating' certain favoured food (Fredrick and Margaret, 1981). The above behavioral pattern of children with regard to their food, is of great concern to the mothers world over, that whether the children are able to meet their required RDA of protein.

1.4. HELPING CHILDREN TO CHOOSE FISH

The key findings of a research conducted by the UK's Seafish Industry Authority are the following (MPEDA, 2004):

- Children's food orientation is influenced by the attitudes and behavior of their parents
- The majority of children judge food on appearance
- Advertising, packaging, education and media are influential
- The key strengths of seafood identified by the mothers are its taste, light texture and the common understanding that fish is good for health
- Barriers identified include the presence of bones, lack of cooking skills and the perceived taste and smell.

Considering the major role of protein in the growth of children it is important to understand what they think about fish and what can be done to influence their thinking. Making fish good to look at, completely bone free, avoiding the problem of odd taste and smell, and fun to eat, should certainly attract their attention and preference to that food. The barriers of seafood identified by mothers, are well overcome in all the surimi-based products. So naturally, surimi becomes an ideal base material for developing a protein food for the children. Surimi is a wet concentrate of proteins from fish muscles that is mechanically de-boned, water-washed and cryoprotectants added to retain the functional properties during frozen storage (Okada, 1992). The undesirable components of fish mince are removed by water-washing, which makes the surimi odourless, white in colour and bland in taste. So any colour, taste or flavour can be incorporated into surimi to suite to the characteristics of the intended final product, which would be attractive to the children.

1.5. SURIMI - NUTRITIVE ASPECTS

The protein of minced flesh of underutilized species is generally comparable in percentage of protein, amino acid profile, chemical score and PER to fillet protein. Surimi and products made from it supply essentially the same nutrients as the minced flesh from which it is made (Pigot and Tucker 1990). Since the quality of surimi protein is high, the incorporation of it to poor quality proteins present in other foods enhances their values. The fat content of surimi is very low compared to the original fish and the iron content comparable with the original fish. Fish is an important source of niacin and to a lesser extent riboflavin. During the preparation of surimi, the water-soluble vitamins to a greater extent are lost due to water washing of the mince. There is an increase in the sodium content from the sodium tri polyphosphate (STPP) and salt added. The sugar added increases the caloric content of the product without enhancing the nutritional worth (Nettleton, 1985).

1.6. SURIMI - INDUSTRY

Prior to 1960, the Japanese Alaska pollock catch was based on its yield of 'roe', a highly-prized food item in Japan. The fish after extraction of the roe had little value because the flesh loses its functional properties very rapidly during iced, as well as frozen storage. Thus, maintenance of initial quality after catch was the key to its efficient utilization and turned out to be an important project for a group of researchers headed by K.Nishiya of the Hokkaido Fisheries Experimental Station (Okada, 1992). They discovered a unique technique of stabilizing the muscle protein of surimi during frozen storage. By washing out water-soluble components from the minced fish and adding cryoprotectants such as sugar compounds and polyphosphates, the functional properties could be maintained (Nishiya, 1961). This resulted in the dramatic growth in the surimi-based product industry in the mid 1960s. The surimi based product plants could thus, stock-pile their raw material to assure production throughout the year on steady schedules (Okada, 1992).

In the United States, the Alaska pollock fishing started in 1985 for making surimi. By 1989 the US produced more than 20% of the world's surimi. The fall in the catch of king crab from 1982 made the Americans to shift to the imitation crab, which also had a price advantage (Gwinn, 1992). Now the Americans are eating more of the imitation crab rather than the natural crab. In addition to the imitation crab, imitation shrimp, scallop analogues, freeze-fabricated products and now, the third generation products like 'seafood slice' and imitation lox are in the market. Seafood slice is made as a large diameter sausage, using surimi in salmon flavour and sold frozen. Imitation lox is the cold-smoked salmon analogue. Blends of surimi with beef or poultry offers the most intriguing area for new products because the possibilities are endless (Gwinn, 1992). Now the four major surimi producers in the world are US, Korea, Japan and New Zealand.

India has a long coastline of 8129 km with 2.02 million sq. km of EEZ. The export figures of marine products from India for the years 2003-2004 and 2002- 2003 are given below (source: MPEDA – statistics division).

Table 2: Export figures of marine products from India.

		share(%)	2003-2004	2002-2003
Frozen Shrimp	Qty	31.50	129768.00	134815.00
	Value	65.88	4013.07	4608.31
Frozen Fish	Qty	33.50	138023.00	196322.00
	Value	10.19	620.73	841.65
Frozen Surimi	Qty	7.79	32088.00	31268.00
	Value	3.13	190.37	288.90
Crab Stick	Qty	0.65	2688.00	1898.00
	Value	0.22	13.51	10.64

Quantity in tons and value in crores (Rs)

The Table shows that in 2003-04 about, 1,38,000 tons of fish fetched a value of only 621crores(Rs), at the same time a mere 32000 tons of surimi fetched 190 crores (Rs). This simply shows the importance of value addition to fish by converting to surimi and surimi products. It is interesting to note that nearly 67% of the total quantity of fish exported comprises of two species *viz* ribbon fish and croakers. Both these are not the so called Table fish varieties but can very well be converted to surimi. This shows that even with the current level of landings, there is better scope for surimi-based industry in India. There are nearly 12 surimi plants distributed along the coast of India, Veraval (1), Porbander (2), Mumbai (2), Ratnagiri (4), Thana (1), Mangalore (1) and Visakhapatnam (1).

The following Table shows the main markets of surimi exported from India for the years 2003 - 2004 and 2002 -2003 [Source : MPEDA]

Table 3: Main markets of surimi exported from India.

Main markets	2003 - 2004		2002-2003	
	Qty	Value	Qty	Value
Japan	16218	96.50	17979	168.00
South East Asia (Taiwan, Korea, Singapore etc)	13429	79.00	10088	100.00
Spain	381	2.67	1143	8.22
China	359	2.12	93	0.66
USA	68	0.39	133	0.62
Others	1568	9.26	1677	10.30

1.7. SURIMI - THE MAJOR SPECIES USED

The species most commonly used in the manufacture of surimi is Alaska pollock (*Theragra chalcogramma*). The properties such as abundance, accessibility and subtle flavour & odour, make this species the optimal resource for surimi processing. Pollock eat crustaceans and small fishes and are harvested in trawl gear. It is a semi demersal species, begins life inhabiting the upper water column and moves deeper to 150 - 200 fathoms as it ages. It is found through out the north Pacific, north of latitude 30^0N (Holmes, *et al.*, 1992).

The second biggest species for surimi processing is New Zealand Hoki (*Macruronus novaezelandiae*) and the next species is Blue whiting (southern and northern)

Several species are commonly used by shore based surimi plants in Asia (Holmes *et al.*, 1992; Muraleedharan *et al.*, 1997)

These include

- Croaker (*Sciaenidae*)

Croker's myofibrillar proteins are highly stable in frozen storage making it suitable for processing.

- Lizard Fish (*Synodontidae*)

The fresh meat is very white in colour, has a good flavour and a very high gel-forming ability. However freshness and gel-forming ability decrease quickly on storage. Thus freshly caught fish must be processed quickly at low temperatures.

- Greenling (Atka mackerel)

The meat is slightly yellowish gray with relatively high fat content and has a low gel forming ability.

- Thread fin bream (*Nemipterus spp.*)

Its meat is white with very good flavour and a strong gel forming ability. Its myofibrillar proteins are highly stable in frozen storage and it makes a very high quality surimi.

- Big eye snapper (*Priacanthus spp.*)

With a stocky body and a brilliant crimson colour the big eye lives in water from shallow depths down to 200 m. It grows to 30 cm., averaging 10-25 cm. Big eye meat is slightly dark but has a high gel forming ability.

- Ribbon fish (*Trichiurus spp.*)

1.8. SURIMI - FROM DARK FLESHED SPECIES

The fall in the Alaska pollock catch has prompted Japan to search for other cheaper and unconventional resources, such as dark fleshed species. The darker colour is due to the higher contents of red muscle containing haem pigment (primarily myoglobin). Even though the trimethyl amine oxide(TMAO) content is less in dark-fleshed fishes than that in light-fleshed ones, they have a greater fishy odour. This is because of low volatility of trimethyl amine (TMA) at the lower muscle pH of dark fleshed fish. The muscle pH of dark fleshed species decrease rapidly postmortem to about 5.8 versus 6.1 - 6.5 in light fleshed species. This is due to the higher concentration of lactic acid (160 -220 μ mol/g versus 37 μ mol/g) arising from greater glycogen reserves in the muscle. Dark fleshed fishes are

generally pelagic and must maintain high glycogen reserves in order to sustain long periods of swimming (Fujii *et al.*, 1978).

The solubility of myofibrillar protein and the resultant gel-forming ability of these species, are determined by three factors:

1. pH: the pH should be within the range 6-8. Accordingly rapid neutralization of the muscle pH is needed (Shimizu, 1975). This is achieved by employing "alkaline saline leaching" at the first leaching cycle (0.15% NaCl in 0.2% $NaHCO_3$. 1:4 meat : solution for 15 - 20 min.)
2. Sarcoplasmic protein content: the dark fleshed species have higher content of sarcoplasmic protein, interfering with gel formation (Ishikawa and Nakanura, 1982). The solubility of sarcoplasmic proteins of these species is increased in the alkaline saline leaching solution resulting in an enhanced removal. The colour is improved by the removal of haem protein and flavour by the removal of carbonyl compounds (resulting from the lipid oxidation catalyzed by the haem proteins)
3. Proteases: the presence of heat stable proteases, which are active in degrading myosin during heating of the meat sol to form a gel, primarily in the 50-70^{0}C range. This is overcome by proper temperature control.

A process was developed in Japan which employed high pressure water jets (10-20 kg/cm^2) to strip the light meat from fillets without removing the dark muscle or subcutaneous fat. The suspended white muscle pieces are collected by rotary sieve (Horiguchi and Kurihara, 1978). The leaching medium used here is a mixture of sodium pyrophosphate and sodium bicarbonate (0.1% each), for inducing dissociation of actomyosin to myosin and actin (thereby enhancing gel forming ability) and for regulating the pH, respectively (Tokunaga and Nishioka, 1988).

In a commercial production of surimi from dark fleshed species, the following problems still pose difficulties. Seasonally limited supplies of fish, small fish size, high lipid content, fluctuation in gel forming ability, rapid degradation of fish quality even in iced storage and low yields (Shimizu *et al.*,1992).

1.9. SURIMI- DRIED FORMS

Dried proteins offer many advantages in commerce, such as lower shipping costs, more convenient storage and usefulness in dry mix applications. Early attempts to dry fish proteins resulted in protein denaturation and loss of gel forming ability. But recent works showed that cryoprotectant additives used in stabilizing proteins during frozen storage could also be used for stabilizing proteins during drying. Several types of drying processes have been tried but spray drying and freeze drying have the greatest commercial feasibilities (Niki *et al.*, 1992). A shift from surimi production from the frozen to the dried form could thus open many new applications for fish proteins and lead to their wider acceptance as food ingredients.

1.10. OBJECTIVE OF THE STUDY

The objective of this study was to develop a (fish) protein food for children, by considering their behavioral pattern towards food and also their increased nutritional requirements. It should attract their attention and also that the production process should be simple. Moreover, it should be able to be incorporated with other snack foods, as well as consumed directly. Basically it should be a 'convenient food', suitable for storage at ambient temperature, avoiding refrigeration. To achieve this, it must be in the dried form. The product developed in this study was in the above lines.

For this, surimi prepared from locally available white fleshed big eye snapper (*Priacanthus hamrur* Forskal, 1775) was kneaded with 2% salt to solubilize the actomyosin. The protein sol thus obtained was used to prepare dried products containing different levels of sucrose and cocoa powder. These products were then subjected to sensory evaluation to determine the most preferred levels of sucrose and cocoa powder. Finally the product containing the most preferred levels of sucrose and cocoa-powder was evaluated for its shelf-life at room temperature.

Review of Literature

2. REVIEW OF LITERATURE

2.1. FISH MINCE AND MINCE BASED DRIED PRODUCTS

The development of meat-bone separator (fish-deboner) has revolutionized the fish processing industry by the maximal utilization of both the smaller and underutilized species. The range of recovery of minced flesh (37 to 63.8%) is considerably above that of the filleted portion, which commonly ranges from 20 to 35%. Furthermore many small fish cannot be economically filleted but can be processed to recover all the flesh as mince (Pigot and Tucker, 1990; Gopakumar, 2002). Fish mince can be used in formulation and production of several value added products that are already popular in the export market and are becoming popular in the domestic market.

Fish mince is the flesh separated in a comminuted form free from scales, skin, bones and fins of fish. In principle, meat can be separated from any species of fish in this style, but it becomes significant when applied to low value fish which otherwise face difficulty in marketing as well as in utilization. Significant value addition will occur to such fish by the application of this technology because of the use of mince in processing a variety of high value products (Devadasan, 2003).

2.1.1. Drum dried fish mince

Paul(1979) described a method to preserve fish mince (with oil contents upto 7%)by drum drying. Starchy materials (corn, wheat, rice etc), proteinaceous vegetables (lentils, chickpeas, etc.) and skimmed milk powder are mixed with mince together with antioxidants and spices (to overcome rancidity), and drum dried.

2.1.2. Salted-dried fish mince

A better alternative to traditional salting of whole fish was reported by Sudhakaran and Sudhakara (1985) by salting minced meat. They used thread fin bream and oil sardine with different salt mince ratios and drying conditions

to get an acceptable product. Grantham (1981) described the production of salted dried mince, that retains sufficient functionality on rehydration. Salt is incorporated at the deboning stage. Salt reduces the protein -water binding capacity and thereby increases the rate of drying. Drying to water activity (a_w) values less than 0.6 gave total stability. The water activity value of 0.6 was achieved by adding 20% salt and drying the product to 15% moisture level. Before consumption of the product, the desalting was done by several changes of the rehydrating water. According to Pigot and Tucker (1990) the optimal way of increased utilization of fishery raw materials, is by the maximum recovery of fish flesh (ie. by fish deboners) and the subsequent salted drying of the mince obtained . They believed that salted dried mince seafood products are a practical solution to the nutritional problems faced in many parts of the world.

2.1.3. Dried Fish Patties

A low cost product called dried fish patties, consisting of fish flesh combined with soy protein, starch and salt was reported by Pigot and Tucker (1990). When shaped into patties and dried (at 70-80^{0}C to 5% moisture content), the packaged product had a long term shelf life at room temperature. When soaked in water for 15 min prior to cooking, patties is a highly acceptable product that would be prepared as any dried and rehydrated fish.

2.1.4. Dried Fish Cake

Basu *et al.,* (1996) developed a dried fish cake, in which they found that incorporation of 5% tapioca starch and 3% texturized soybean protein improved the texture and juiciness of the rehydrated product. It was also found that mixing the ingredients at slow speed (less 100rpm) in a dough mixer gave the best texture. The dehydrated product (19-20% moisture) had a shelf life of 6 months at ambient temperature.

2.1.5. Spice Minced Fish

Spice minced fish is a traditional Malaysian product from tilapia, as reported by Zain (1980). The separated flesh of cooked fish is mixed with 5% salt and 10% other ingredients (spices etc), pressed to remove the liquid, sun dried, ground and packed . Rancidity was controlled and masked by the spicy flavour and could be kept for a few months.

2.1.6. Fish Satay

Fish satay is also a traditional product from Malaysia and other south east Asian countries, primarily prepared from yellow goat fish which is an unconventional species having some technological difficulties in the separation of flesh. Cleaned boneless butterfly fillets are dried at 45-55^{0}C, pressed by rollers (for better penetration of ingredients to flesh), dipped in sauce (sugar, ginger, chilly powder etc) drained and dried , to a moisture level of 3-7%. This simple method offers efficient utilization of the small sized fish and increases the protein consumption of people through such snack products. (Rahimah,1983; Gopakumar,1997).

2.1.7. Dehydrated Curried Fish Mince

Chakrabarty *et al.*, (1972) described the development of a dehydrated curried fish mince, offering convenience with regard to bulk and weight, handling, transport, ease of preparation and consumption. Cooked fish meat was minced and dried at 65^{0}C to 5% moisture level, then mixed with dehydrated curry - ingredients and hydrogenated oil and packed. Product was acceptable up to one year at ambient conditions. Fish curry was reconstituted by adding five times the boiling water and simmering for 7-8 min.

2.1.8. Edible Fish Powder

A simple process was developed to convert low value small bony fish (eg. silver bellies) to nutritionally rich edible fish powder without deboning the fish . The fish was cooked under pressure (to soften the bones), dried to less

than 6% moisture content, ground and packed, with a storage life of up to 5 months (Chattopadhyay *et al*., 2004).

2.1.9. Fish Soup Powder

Another way of efficient utilization of miscellaneous fish, as reported by Gopakumar (1997) is the production of fish soup powder. Cooked fish ground with onion and other ingredients, dried on trays at 60-70^{0}C (to below 10% moisture level), mixed with starch and milk powder and packed. The cook-drip after skimming off the lipids, was used during grinding of the meat. The presence of water soluble proteins in the cook drip enhances the dispersion of finished product in the water (5g soup powder for one cup of boiling water). It had a shelf life of two years.

2.1.10. Fish Protein Hydrolysate

Following the slow death of the Fish Protein Concentrate (FPC) programme, efforts were made to develop fish protein hydrolysate (FPH) techniques for producing solubilized proteins for human consumption. Despite being slightly deficient in tryptophan, FPH can be used as a substitute for milk protein (solubility up to 70% in distilled water). Minced meat was directly blended with enzyme (bromolein or papain) in the method described by Gopakumar (2002), but Warrier *et al.,* (1996) used washed and filtered minced meat (papain was used for hydrolysis). In the former method an incubation period of 15 to 30 min (at 55^{0}C) was given, then heated to 80^{0}C for 12 min to inactivate enzyme, cooled, filtered and spray dried. An incubation period of 2 hrs (at 55^{0}C) was given by Warrier *et al.,* (1996), then heated to 95^{0}C for 5 min, cooled, treated with 0.01% sodium tri poly phosphate (STPP) and 0.03% H_2O_2 (for better retention of colour - creamy white - during storage), freeze dried, ground to fine powder and packed. Pigot and Tucker (1990) have reported another method in which, deboned fish was hydrolysed with papain for 4 hrs at 48^{0}C, centrifuged (to remove sludge and oil), ion exchanged (to neutralize and remove digestion byproducts) and spray dried to 8.2% moisture

level. Sathivel *et al.,* (2003) reported the functional, nutritional and anti oxidative properties of freeze dried FPH from herring byproducts. They reported that it had 77 to 87% protein content and had desirable essential amino acid profiles and mineral contents.

The effects of 5% FPH (from fish scrap) on the state of water and the denaturation of lizard fish myofibrils were evaluated by Khan *et al.,* (2003) and reported that the myofibrils with FPH had higher amounts of unfrozen water than the control. They suggested that the FPH suppressed 'dehydration - induced denaturation', which seems to be attributable to the stabilization of the water surrounding the myofibrils.

The formation of bitter peptides render a mild bitter taste to the FPH powder which can be reduced by controlling the degree of hydrolysis and by incorporating to conventional foods like soups etc. To overcome this problem Gopakumar (2002) described a method which was developed in CIFT, by adding malt, sugar, cocoa and milk powder and then spray dried, which could be used as a drink. Liaset *et al.,* (2000) reported the utilization of by-products from fish filleting industry by proteolysing with industrial enzymes neutrase, alcalase or pepsin and freeze drying. To minimize the bitterness, prehydrolysis was done with alcalase® (150 min) followed by treatment with kojizyme® (510 min)

2.1.11. Fish Floss

A granulated snack food known as 'floss' is prepared from snapper or thread fin bream in Malaysia, Thailand and Vietnam. The ingredients used were generally soya sauce, salt and sugar. The process involved cutting the fish and soaking it in 2% brine for 10-15 min. The mince was then separated from the bones and skin. The fat was also removed from the meat. The excess water was removed by pressing the washed meat through a screw press. The mince was heated with ingredients before drying. The powdered product was

usually kept in glass bottles or polythene bags. Shelf life of the fish floss was reported to be 3-6 months at room temperature (Chng *et al.*, 1996).

The heat treatment given to dried minced products inactivates lipolytic enzymes and thus protects the mince against FFA formation (Grantham, 1981).

2.2. SURIMI

Surimi is mechanically deboned fish mince from white fleshed fish that has been washed and mixed with cryoprotectants for good frozen shelf life. Washing removes fat, blood, pigments, soluble proteins and odoriferous materials and increases the concentration of myofibrillar proteins, which improves the gel strength and elasticity of the product. Because of its gel strength surimi is used as an intermediate in the processing of several fabricated products with simulated texture, flavour and appearance such as shrimp, lobster tail, scallop meat and crab legs (Devadasan, 2003).

2.2.1. Surimi Manufacturing Process

2.2.1.1. Preprocess Handling

High quality surimi can be made from fish whose proteins have not been denatured by heat or during storage (Matsumoto,1979). Freshness is the most important requirement for the raw material, regardless of the species. As a general rule factory trawlers have access to the freshest fish (less than 24 hrs post harvest) and are able to produce higher quality surimi than factory mother ships or land based plants (Toyoda *et al.*, 1992). The fish should be kept at a temperature below 5^0C. If the catch is stored at a temperature below freezing point of the fish, the surimi quality can be adversely affected. Gel strengths remained significantly higher upto 2 days but dropped markedly (63%) after three days in chilled sea water (CSW) compared to fish stored in ice (46%) (Lee, 1986a).Pigot and Tucker (1990) reported that fish should be processed as soon as possible after it has gone through rigor. Prior to that it was found to be

difficult to remove the fishy odour and various membranes. Fish caught during active feeding season yielded surimi of highest quality (Gopakumar,1997).

2.2.1.2. Deboning

Filleting of fish influence both quality and quantity of mince gained from subsequent deboning, with quality usually being gained at the expense of product yield. Merely heading and gutting fish (without filleting) will increase yields but lower the product quality (Toyoda *et al.*, 1992). If the position of the head cut during filleting is too far to the rear, the yield will be decreased.

Toyoda *et al.*, (1992) elaborated the importance of using chain belted conveyors for fillets, to prevent accumulation of high levels of exudates and surface slime which contaminate the fillets. Additionally, spraying water at oblique angles to the conveyor is also done.

Normally a belt-drum type meat separator is used for deboning , in which the fish pass between a belt and a perforated drum, the belt held tight to the drum by a pressure roller. Yield can be increased by increasing the tension of the belt but the amounts of bones, fragments of skin also may increase (Keay, 1979). The diameter of the holes chosen for the roller (ranges from 4-7mm) greatly influence the subsequent leaching and dewatering process as well as the yield and quality of surimi . Toyoda *et al.*, (1992) reported that the diameter of the holes is chosen in accordance with the size and freshness of the fish. To improve the processing capacity and maximize yield, the total hole area of the drum should be as large as possible (Pigot and Tucker,1990).

2.2.1.3. Leaching

Sarcoplasmic proteins contain many kinds of water soluble proteins called myogen. The content of sarcoplasmic protein is higher in pelagic fishes (eg. Mackerel 10-14%) and lower in demersal fishes (eg. seabass 6-7%). The heat coagulative sarcoplasmic protein adheres to the myofibrillar protein when fish meat is heated and impedes the formation of gel (Suzuki,1981).

Myofibrillar protein contains myosin, actin and regulating proteins such as tropomyosin, troponin and actinin. It covers 66-77% of the total protein in fish meat and plays the role in gel formation. The gel properties of surimi are dependant on the actomyosin that is present in its native state (Pigot and Tucker,1990).

Problems in development of value added products from underutilized fish arise due to several inherent characteristics of fish muscle structural proteins. The proteins are sparingly soluble in water and if solubilized, the solutions become viscous. Further the proteins are also sensitive to rapid denaturation even under mild heating conditions. Other problems include sensitivity of fish lipids to oxidation and resulting flavour changes, interaction of oxidized lipids with proteins, lead to loss in textural quality and flavour reversion (Venugopal *et al.*, 1998).

The purpose of leaching is the removal of water soluble matter (includes sarcoplasmic proteins, digestive enzymes, inorganic salts, low molecular organic substances such as TMAO, TMA , formaldehyde), lipids, pigments, bacteria and blood from the minced meat. This increases the concentration of myofibrillar proteins, which is primarily responsible for gel formation (Toyoda *et al.*, 1992). The non proteinaceous substances are known to accelerate the denaturation of muscle proteins during frozen storage (Noguchi,1977). The undesirable effects of leaching are the loss of protein upto 25%, loss of vitamins, minerals (Na,P,K), free fatty acids and the secondary problem of effluent disposal (Grantham,1981). While just 77% of the protein was retained, there was no change in amino acid composition (Pigot and Tucker,1990). The rate at which these undesirable solubles are leached from the fish mince, is a function of several factors, including the water temperature, the degree of agitation and contact time between water and meat particles (Green, 1989).

Wash Cycles: The number of washing cycles required depends on the type, composition and freshness of fish to be processed (Toyoda *et al.*,1992). Extraction of water soluble components from the mince at a given washing cycle appears to be a function of agitation time, independent of the meat to water ratio (Lee, 1986b). According to him a 9-12 min agitation is adequate ie. 5min agitation in each cycle for the two washing cycles. If the residence time is prolonged, the fish meat will absorb an excessive amount of water and subsequent dewatering becomes difficult (Toyoda *et al.*,1992). Lee (1986a) suggested a two washing cycle at a 3:1 water to meat ratio from the stand point of gel strength, which may not remove the fish odour completely.

Based on the studies conducted in the washed mince of tilapia, Ninan *et al.*, (2004) suggested that, for washing the mince a ratio of 1:2 (w/v) mince: water and two successive wash cycles of 5min each as optimum. A wash ratio of 1:3 (w/v) resulted in 45% loss of protein (as against 22% for 1:2 ratio) after 2 wash cycles and a third washing resulted in excessive hydration of the mince. According to Venu (2002), three washings with 1:3 mince - water proportion with a washing time of 25min for the first cycle and 15min each for the subsequent cycles, gave maximum gel strength for big eye snapper. Gopakumar (1997) suggested that a 2 washing cycle each of 2 min duration using a mince: water ratio 1:2 (w/v) was optimal for tropical fishes.

Ionic Strength of Wash Water: The greater the ionic strength of leaching water, the easier it is to remove the water from the meat. It is therefore hard to drain the water from swollen meat at low ionic strength, resulting in surimi with high water content. So it is good to add a small amount of salt (between 0.03-0.60%) in the final washing cycle in order to increase the ionic strength. (Okada and Tamoto, 1986). Venu (2002) obtained best results when 0.1% salt was added in the final washing for 15min. However too much salt may cause solubilization of myofibrillar protein, resulting in premature setting of the protein sol (Pigot and Tucker,1990). The increased Ca^{2+} level in the wash

water (hard water)adversely affects the gel forming ability of surimi, by causing denaturation of actomyosin during frozen storage (Tamoto,1971).

pH of Wash Water: The pH of wash water affects water retention during the leaching process, water binding properties and subsequent gel forming ability. It is recommended that the pH of the wash water be adjusted to that of the fish meat (6.5-7.0) to ensure maximum functional performance of fish protein. In the case of red -meat fish, the drop in pH of fish meat (5.7-6.0)is adjusted by alkaline agents such as sodium bicarbonate added to the leaching water (Lee,1986b).

Temperature of Wash Water: Warm water is more effective for water removal from leached mince -the amount of water separated from the meat and the separation speed increases with water temperature. But to prevent the heat- induced protein denaturation as well as to reduce microbial proliferation, the temperature of leaching water should be in the range of 3-10^{0}C (Douglas-Schwarz and Lee, 1988).

In the shore surimi plants, the total amount of water needed in leaching depends up on the freshness of fish and may run from 10-20 times the amount of starting minced meat. Aboard the factory ship where very fresh fish is processed, the leaching water volume can be as low as 6 times the amount of minced meat (Toyoda *et al.*, 1992).

2.2.1.4. Intermediate Dewatering and Refining

Fine particles lost through rotary screens during intermediate dewatering, account for about 8% of starting mince weight. However by settling in conical shaped tanks or by employing centrifugation, a significant portion can be recovered (Lee,1986b).

Prior to the final dewatering, the partially dewatered (90%moisture level) leached mince is refined to remove any connective tissues, skin, scales or other

undesirable materials by a refiner. The higher moisture content and installation of a cooling unit prevents protein denaturation (Toyoda *et al.*, 1992)

Lanier *et al.*, (1992) suggested that use of small muscle particle size, by employing a small drum hole size (1-2 mm) during mechanical deboning, can reduce the need for subsequent refining, since a great deal of refining would occur at the deboning step.

2.2.1.5. Final Dewatering

In the final dewatering the moisture content is reduced to about 80% by the screw press. Generally a compression ratio between 2.5 and 3.5 and hole diameter from 0.5-1.0 mm at the inlet and from 1.0-2.0 mm at the outlet are used in the screw press (Toyoda *et al.*, 1992). The final moisture content of the product is affected by the mechanical pressure applied in the screw press and the water retention properties of the fish meat. Water retentivity varies with fishing season (eg. rising during spawning season) and freshness (increases with decreasing freshness). Water retentivity increases with vigorous agitation, higher water: meat ratios, smaller meat particles and longer leaching time (Toyoda *et al.*,1992).

Fish of very firm texture (extremely fresh and very large fish) generally produce a mince of tight particle structure which does not leach easily, requiring higher meat : water ratio and more vigorous agitation. But large meat particles which are softer in texture such as, that obtained from less fresh or spawned fish must receive a gentler treatment to avoid excessive hydration.

Leaching of small sized meat particles despite the advantages, will also result in a substantial loss of protein during dewatering. Swafford *et al.*, (1985) evaluated the use of a decanter centrifuge for intermediate dewatering, followed by screw press to achieve the final dewatering. They reported enhanced gel forming properties in the surimi produced by this method and

cited other advantages such as reduced water requirement and more precise control.

Shipboard surimi manufacturing incurs higher labour costs, higher initial capital outlays and expensive freshwater production. But minced meat loses its functional properties on frozen storage. MacDonald *et al.,* (1990) developed a means of storing fish meat without denaturation by a combination of low temperature storage and addition of cryoprotectants (12% sucrose and 0.2% polyphosphates, stored at -20 or -50 ^{0}C for upto 6 months). They found that there was no significant difference in gel forming properties between freshly prepared surimi and surimi prepared from stabilized mince. Grantham (1981) also reported the effects of polyphosphates on protein functionality and also as an antioxidant (by sequestering the metal ions), thus stabilizing the frozen mince.

2.2.1.6. Mixing of Additives

In order to stabilize the fish proteins from freeze -denaturation during frozen storage, generally 4% sugar is used along with 4% sorbitol and 0.2-0.3% polyphosphates as cryoprotective additives. Pigot and Tucker (1990) reported that when tri sodium pyrophosphate and sodium tri polyphosphate were used in combination, was more effective than using STPP alone. Japanese processors are now using a mixture of sorbitol and polyphosphates supplemented with a glycerin ester of fatty acids. The glycerin ester is claimed to whiten the surimi products through its light scattering effect (Kaneyama and Tomiyasu, 1979). These must be uniformly dispersed and dissolved before the meat is frozen. Silent cutter type blender is ordinarily used or a continuous type blender in which uniform blending is achieved in less than one minute (Toyoda *et al.,*1992).

2.2.1.7. Packaging and Freezing

The filling and packaging machine extrudes 10kg blocks of fish paste, which are automatically packed and placed onto freezing pans of contact plate

freezer. The blocks are frozen to a core temperature of -25^0C as rapidly as possible, packed in corrugated cartons of 20kg and stored at -25^0C or below (Toyoda *et al.*, 1992). Lanier *et al.*, (1988) proposed an alternate system of freezing- a drum type freezer producing free flowing particulate form of surimi. This would minimize the floor space, transform freezing operation into a continuous process and obviate the present requirement of initial thawing (tempering) and /or shredding of frozen surimi blocks by the user. Nielsen and Borresen (1985) obtained a more functional fish protein than that resulted from the plate freezing, by adopting this method.

2.3. CRYOSTABILIZATION OF SURIMI

Noguchi *et al.*, (1976) reported that hexoses (glucose and fructose) and disaccharides (sucrose and lactose) were the most effective cryoprotectants among sugars. Pentoses (xylose and ribose) were less effective. They also observed that the content of free aldehyde groups was higher for pentoses than for hexoses and that the aldehyde groups brought about the amino - carbonyl reaction. Therefore chemically reactive substances are not suitable as cryoprotectants. The cyoprotective effects of sugar -alcohols were also studied and among these sorbitol is widely used in commercial surimi processing because of its excellent cryoprotective properties, relatively low cost and low sweetness. Initially high levels of sucrose (8%) was used but this made the surimi too sweet and caused a brown colour change during frozen storage (Suzuki,1981). Pigot and Tucker (1990) reported that sorbitol was used to reduce the level of sucrose even though a little less protective than sucrose, is not as sweet and does not cause discolouration. But sorbitol alone gives a harder texture than that containing sucrose. According to them surimi from high quality raw material needs less sugar.

Pyrophosphate and tri polyphosphate are widely used at present in surimi manufacture. The oxygen bonds of the phosphate ion are electrovalent rather than covalent , giving them the properties of highly charged anions. This

anionic charge is critical to the ability of phosphate to interact with long chain polyelectrolytes such as proteins, orienting along the charged sites of the proteins and adding to their water binding capacity. The phosphates are also able to sequester Ca^{2+}, Mg^{2+}, Fe^{2+} and other cations,again increasing the number of active available polar groups. The protein's ability to reabsorb liquids during thawing is due to the provision of polar sites on the protein surface. The sugar act as an antifreeze, preventing the formation of large crystals, while the phosphates bind to the active sites of actomyosin preventing them from either irreversibly binding to each other (aggregation) or denaturing (unwinding). Upon thawing the water may again be held by the charged sites on the still soluble proteins (Pigot and Tucker, 1990; Weilmeier,1998). Addition of sucrose and phosphates was found to retard the development of dimethyl amine (DMA) and formaldehyde from TMAO, besides serving as a cryoprotectant of protein (Toyoda *et al.*,1992).

But the role of polyphosphates as a cryoprotectant has long been questioned, whereas the role of sugars has been well accepted. Noguchi (1971) and Park *et al.,* (1988) have suggested that the polyphosphates act mainly to enhance the cryoprotective effect of sugars, perhaps by the buffering effect of polyphosphates on muscle pH and /or the chelation of metal ions rather than imparting any direct cryoprotective effect of their own. According to Noguchi (1984) and Park *et al.,* (1988) Alaska pollock surimi did not benefit significantly from the presence of polyphosphates in frozen storage. Thus a phosphate free surimi could be manufactured which would obviate the problems of nutritional balance of calcium and phosphorus.

Matsumoto and Noguchi (1992) believed that there was a correlation between the molecular structure of the compounds and their cryoprotective effectiveness, for which the molecules should satisfy the following conditions:-

- The molecule has to possess one essential group, either COOH or OH and more than one supplementary groups (COOH,OH,SH,NH_2, SO_3H, OPO_3H_2)
- The functional groups (both essential and supplementary) must be suitably spaced and oriented relative to each other.
- The molecules must be comparatively small (this is violated in the case of materials like polydextrose ®)

The cryoprotectant molecules interact and bond with the protein molecules via functional groups on the surfaces. Water molecules are hydrated on to the other remaining functional groups of the cryoprotectants. Thus each protein molecule is covered by hydrated cryoprotectant molecules. In this manner the frequency of mutual contact between protein molecules is lowered resulting in increased hydration and decreased aggregation of the proteins (Matsumoto, 1980). The increased hydration of protein molecules slower ice crystal growth due to increased resistance to the displacement of water from protein surfaces, and an incomplete freezing of water due to increased amounts of bound water. These lessen the degree of protein unfolding or denaturation (Matsumoto,1980).

2.4. SURIMI BASED DRIED PRODUCTS

2.4.1. Solubilization of Surimi

Conversion of the frozen surimi into a protein sol, is the pre-requisite for the manufacture of products from surimi. The actomyosin in surimi is solubilized by salt during the chopping and kneading process. Elasticity and resilience of surimi gel increases with increase in the concentration of actomyosin.

Before using, frozen surimi blocks must be tempered to prevent damage to the knives of the comminution equipments. Normally a silent cutter is used for comminution. Salt is added after the initial chopping of surimi. If salt is

added at an early stage the surimi temperature will decrease and ice crystals may form which will disturb the extraction of the protein by the salt (Suzuki,1981; Wu, 1992). Solubilization of the functional myofibrillar proteins generally increases with mixing time. However over comminution will cause the temperature of the paste to rise too high, resulting in protein denaturation (Wu,1992). Wu (1992) described the advantages of using vacuum chopping. It reduced protein oxidation and denaturation. Less air is incorporated in the protein sol. Vacuum choppers usually completes blending within 10-15 min due to their higher blade speeds (as compared to silent cutter which takes 25 min).

The raw surimi becomes a viscous sol or paste upon grinding or comminution with salt. Such a paste cannot be obtained in the absence of salt even if comminution is carried out for many hours. This is because of the dissolution of myofibrillar proteins in water with the aid of salt. Dissolved myosin combines with actin filaments to yield actomyosin (Niwa,1992). Salt levels of 2.5-3.0% is reported as optimum. Higher concentration of salt will reduce heat- stability of fish proteins (Pigot and Tucker, 1990).

More than half of the amino acids that constitute myosin are hydrophilic. At the post rigor pH of the fish flesh or surimi, the carboxyl groups (eg. of glutamic acid and aspartic acid residues) are negatively charged while the amino groups (eg. of lysine and arginine residues) are positively charged. Therefore an intermolecular linkage will be formed between these groups and the myofibrillar protein is insoluble in water. When salt is introduced the salt ions, individually hydrated with water, will bind to the oppositely charged groups exposed on the protein surface and are dissolved in water because of their increased affinity for water (Niwa,1992).

$$\text{Protein-}COO^- \ldots\ldots\ldots {}^+H_3N\text{-Protein} \qquad \text{(Insoluble)}$$

$$\text{Protein-}COO^- \ldots Na^+ + Cl^- \ldots\ldots {}^+H_3N\text{-Protein} \qquad \text{(Soluble)}$$

Pigot and Tucker (1990) suggested that the use of phosphates (along with salt) will sequester the Ca^{2+}, Mg^{2+} and Zn^{2+} again increasing the number of active polar sites. According to Lee *et al.*, (1992) phosphates increase the pH slightly and enhance the salt solubilization of myofibrillar protein. Phosphates reduce the viscosity of the paste allowing a better machinability of the paste.

When salt added surimi paste is maintained at room temperature (32-43^{0}C) the viscous paste loses its viscousness and turns to an elastic gel. This phenomenon is called 'suwari' (setting) (Kammuri and Fujita,1985). A controlled suwari is popular among Japanese Kamaboko processors. Seki *et al.*, (1990) proposed that the cross linking of myosin is promoted by transglutaminase contained in fish muscle.

2.4.2. Drying of Surimi Sol

2.4.2.1. Spray Dried Surimi

Niki *et al.*, (1992)have elaborated the processing conditions where by surimi may be spray dried into a functional (good gel forming ability) powder. Sugars are added as anti denaturants along with coldwater to surimi and the slurry is passed through a colloid mill. The dispersion is filtered to remove bits of skin, scales or bone and spray dried at 150-180^{0}C inlet air temperature and 50- 80^{0}C outlet air temperature.

The gel forming ability of spray dried surimi is improved as the myofibrillar protein concentration is increased. The protective effects of sucrose, sorbitol and glucose during spray drying was studied by Oizumi *et al.*, (1981) and found that sucrose was the most effective. Grantham (1981) reported that polysaccharides have a greater protective effect than monosaccharides. And sugars have a greater effect than polyols. Okada (1992) reported the importance of sugar compounds in reducing the denaturation of muscle protein during dehydration. The dehydrated surimi thus obtained had better binding capabilities than traditional binders of meat and fish fillets such as alginates and vegetable proteins.

Niki and Igarashi (1982) studied the relationship between the pH of the milled muscle dispersion and the Ca^{2+}ATPase activity (an index of gel forming property) of the rehydrated product and found that greatest stability of protein was obtained at near neutral pH. They also recommended that the ionic strength of the dispersion be low, to maximize the stability of the proteins

A lower temperature of drying results in a more functional product. However lowering the inlet air temperature decreases the efficiency of spray drying. Lowering the outlet air temperature increases the moisture content of the product thereby decreasing the recovery rates (Niki and Igarashi,1982).

Ease of storage and transport are two of the main advantages of dried surimi as compared with the frozen product. Niki *et al.*,(1983) studied the effects of storage at several temperatures (5,18 and 30^0C) and the stability was very good at 5^0C. However removal of oxygen from the product was effective in improving the stability at higher temperatures. Lower moisture content imparted greater stability during storage.

One of the most troublesome problems in spray drying of surimi is the high viscosity of the milled muscle dispersion, which requires special high pressure pump to feed the spray drier. High viscosity is found to relate to the high water holding capacity (WHC) of the proteins at near zero ionic strength. Niki *et al.*, (1984) found that addition of small amounts of salt or Ca^{2+} as calcium chloride or water soluble proteins had some negative effects on protein stability. Lowering the pH to near isoelectric point lowers the WHC but at the expense of gel forming ability. But the use of carbonic acid as the acidulent avoided this problem,as it is decomposed to CO_2 and water during drying, thus restoring the pH of dried powder to neutrality.

In the case of gelation dependent thermo stable water dispersions, the solubility and stability of proteins in the dispersions at high temperatures could be taken advantage of, for the development of functionally active protein powders by spray drying. Since there was no need for any additive such as

carbohydrate the powder prepared had more than 90% protein (Venugopal *et al.*,1994).

Niki *et al.*, (1982) reported the development of a spray dried powder from Alaska pollock surimi through milling in presence of sorbitol and carbonic acid.

Venugopal *et al.*, (1996) reported that the colourless and odourless spray dried thread fin bream powder had 2-3 times more oil emulsification capacity and water solubility as compared to the FPC prepared from the same fish by conventional method.

Venugopal *et al.*, (1997) described the preparation of a shark protein powder by using acetic acid to lower the pH to 4.0. The non hygroscopic powder could be stored at 30^0C for 2 months or up to one year under refrigeration. It was soluble in water containing 0.2% STPP.

Grantham (1981) suggested drum drying to impart a flake structure to the product and thus increase its acceptability.

2.4.2.2. Freeze - Dried Surimi

As with spray drying, the addition of cryoprotective sugars or sugar alcohols is necessary to stabilize the proteins during drying and storage. Freeze dried surimi is presently commercially produced in Japan for use as a binder in preparing fabricated herring roe and commercial fillet blocks (Niki *et al.*,1992).

During freezing and drying, the water environment surrounding the protein molecules, which stabilizes the hydrogen and hydrophobic bonds, holding the molecule in its native structure is removed. This can lead to interaction of neighboring molecules (aggregation). In order to prevent this denaturation, the water movement must be controlled. Sucrose added with polyphosphates protect the protein from denaturation during drying (Matsuda,

1979a). Among the sugars that have been tested, sucrose was the most effective during storage and did not induce browning due to Maillard reactions as reducing sugars did (Matsuda, 1979b). Some sugars that are effective in stabilizing proteins during frozen storage, such as arabinose and sorbitol are less effective in stabilizing fish muscle during storage of freeze dried product (Matsuda,1981). The difference in effectiveness is likely related to the phenomenon of *collapse*, that is encountered in freeze drying. Collapse can occur in the freeze drying of an amorphous matrix of liquid material along the freeze drying front, resulting in the softening of the matrix or drying by direct evaporation. It has been reported that a solution of eutectic forming solutes collapse at their eutectic temperatures during sublimation (MaeKenzie, 1975).

Freeze dried materials have 80-160 times more surface area than air dried materials. Accordingly they are much more susceptible to the influences of the storage environment. Low temperature storage is important for retaining functionality in freeze dried surimi (Matsuda,1983).

In a study conducted by Reynolds *et al.*, (2002) freeze dried surimi showed the highest salt extractable proteins and the lowest DMA values after 9 months of storage. Leyva-Mayorga *et al.*, (2002) conducted a study to determine the feasibility of employing freeze dried surimi as a functional additive in the formulation of low fat meat emulsions. Lowering the fat content of the meat emulsion decreased the hardness, chewiness and WHC. Employment of freeze dried surimi increased the values of mechanical properties lost by the decrease in the fat content.

Huda *et al.*, (2000) reported that freeze dried surimi powder prepared from thread fin bream , had superior nutritional properties compared to fish flesh and oven dried surimi powder.

Suzuki *et al.*, (1978) described the production of dry textured surimi (Marinbeef®) by ethanol extraction of washed mince followed by extrusion and its incorporation into different meat products.

2.5. MICROBIOLOGICAL CONSIDERATIONS

Aerobic plate counts of surimi reported by Elliot (1987) ranged from 1.6×10^3 to 8.3×10^6 per gram. In general factory ships produced surimi has lower microbial counts compared to shore produced surimi. This could be due to the microbial growth that occurred in the fish prior to processing on shore (Lee, 1992). Hobbs (1983) reported the microbial counts of the fish skin from 10^3 to 10^5 per cm^2; the gills 10^3 to 10^4 per g and the intestine 10^2 to 10^9 per g.

Lee (1992) discussed the application of HACCP concept (the Hazard Analysis Critical Control Points) to surimi manufacturing process. The critical control points identified were the unloading of catch, fillet washing step, screw press dehydrator, addition of cryoprotectants, packaging and freezing.

Grantham (1981)described the possibility of food poisoning through poor handling practices. Niki *et al.,* (1992) observed that there was a reduction in the bacterial counts as the spray drying process progressed. However the manufacturing process did not include a pasteurization step, as this would lead to denaturation of proteins. Therefore good sanitation is essential in conducting the process.

Matches *et al.,* (1987) reported that most of the bacteria in the frozen surimi were inactivated during product manufacture that involved heating or pasteurization.

2.6. WATER ACTIVITY AND EFFECTS OF DRYING

Moisture content and water activity (a_w) affect the progress of chemical and microbial spoilage reactions in food. Dried or freeze dried foods having water content in the range of 5 -15% have great storage stability. Reduction of a_w can be obtained by drying or by adding water soluble substances such as sugar and salt (Grantham,1981; deMan, 1990).

Most enzymes are inactive when a_w falls below 0.85 (eg. amylase, phenol oxidase and peroxidase). However lipases may remain active at values as low as 0.3 or even 0.1. The substrate (oil) has to be in the liquid form (deMan,1990).

Non-enzymic browning are strongly dependant on a_w and reach a maximum rate at a_w of 0.6-0.7 i.e., intermediate moisture foods. (In the intermediate range the reactants are all dissolved and that further increase in moisture content leads to dilution of the reactants). Even at low a_w sucrose may be hydrolysed to form reducing sugars which may take part in browning reactions (deMan, 1990).

In general, food materials at higher temperatures show an increase in a_w at constant moisture content. As the temperature is increased, the hydrogen bond between water molecule and the OH groups of food solid, breaks and the water becomes free. This increases the water activity. However in foods containing sugars an opposite trend is observed. At higher temperatures dissolution of sugars take place in the newly formed free water. This results in the decrease in a_w, with the increase in temperature. Stickiness is a property of sugar containing hygroscopic amorphous powders, occurring at a combination of temperature and moisture content (Jaya *et al.,* 2002).

Oxidation of lipid is maximized at very low a_w, probably because of the concentration of metal catalysts. The reaction of the oxidized lipids with amino acids and proteins lead to damage to the four most limiting amino acids - cystine, methionine, tryptophan and lysine (Olley *et al.*,1988). Carbonyl compounds from lipid oxidation, in presence of mild heat can react with the epsilon-amino group of lysine in a manner, similar to that of sugars to complete the Maillard reaction (Bligh *et al.*,1988).

Drying in the sun causes only slight lowering of digestibility due to protein damage and to the formation of enzyme-indigestible substrates, compared with hot air-drying. Extraction of lipids as in the case of leached

mince, reduces the formation of enzyme-indigestible material (Olley *et al.*,1988). They also suggested that whenever there is a choice between processing temperature and processing time, reduction in temperature (below 70^0C) should have priority over a reduction in processing time. This recommendation does not include dryers such as spray-dryers in which drying is completed within a fraction of a minute.

2.7. INGREDIENTS

White sugar extracted from sugar cane is very nearly pure sucrose, with traces of mineral matter and water. The mineral compounds entrapped in the crystals produce a buffering effect in solution and prevent the rapid inversion of sucrose to invert sugar in the presence of any acidic ingredient (Stansel, 1988). Sucrose has a higher 'glass transition temperature '(Tg), (62^0C) than fructose (5^0C). Tg is the temperature at which an adhesive substance loses its flexibility and becomes hard, inflexible and 'glass like'. The low Tg value of fructose increases the stickiness in foods containing more fructose than sucrose (Jaya *et al.*, 2002).

Cocoa is the powdered product from roasted and ground cocoa beans from the cocoa tree. The roasted cocoa bean has 19.8% protein and a high content of natural fat (24.5%) known as 'cocoa butter', which determines the characteristics of the chocolates. The stable crystal from cocoa butter melts at 37^0C. After extraction of cocoa butter from the refined beans, the residual cocoa press cake is processed to produce cocoa powder. It still contains about 8-10% cocoa butter (Stansel, 1988; Catsberg and Dommelen, 1990).

The anti-oxidative substances in cocoa, separated by high performance liquid chromatography (HPLC) and identified by mass spectra, were epicatechin and catechin. The chocolate is stable against oxidative deterioration on account of these poly phenolic compounds (Osakabe *et al.*, 1998).

2.8. PACKAGING AND STORAGE

A powdery material is usually stored in flexible packages. Sealability, low moisture permeability, opaqueness and printability are the qualities needed for the packaging materials. Since a single material cannot meet the above qualities, several materials arranged in layers (lamination) are often used but cannot be made 100% moisture proof (Wu,1992; Jaya *et al.*,2002). Therefore the moisture content of the powder stored, increases due to migration of moisture from atmosphere. As stickiness is a function of moisture content and temperature, the 'sticky point moisture content' at a particular storage temperature can be termed as 'critical moisture content' of the powder (Jaya *et al.*,2002). They also described a method to determine the time in days required for the moisture content of the powder to increase from an initial value to its critical value.

Wu (1992) and Yokoyama (1992) described in detail the characteristics of different packaging materials used for surimi and surimi based products. According to Gopal *et al.*, (1998) commonly used packaging materials for dried (fish) products are low density polyethylene (LDPE) or poly propylene (PP). These materials are cheap, readily available and have good tearing and bursting strength. Disadvantages are high water vapour and gas transmission rate and proneness to puncture or damage. Shelf life is also limited. A recent development is the use of polyester-polythene laminated pouches for consumer packaging.

Materials and methods

3. MATERIALS AND METHODS

3.1. RAW MATERIAL

In this study big eye snapper (*Priacanthus hamrur*, Forskal, 1775) was used. The fish was procured from the fisheries harbour, Cochin, iced and brought to the laboratory without any delay. The fish was washed thoroughly to remove slime and sand, and re-iced. The size ranged from 100-300 g and length from 18-31 cm. The average weight was 238g per fish.

3.2. DEBONING MACHINE

A belt-drum type, deboning machine was used. The fishes were fed in between the rubber belt and the outside of a revolving perforated drum. The flesh was forced through the perforations in the drum and was collected as a coarse mince. In order to increase the leaching efficiency, a mincing machine further reduced the particle size of the mince.

3.3. COMMINUTION EQUIPMENTS

In this study a silent cutter was used for kneading, which consists of a revolving bowl, which holds the surimi and other ingredients and rotating knife blades, which cut the moving surimi mass.

3.4. DRIER

Here in this study a tray type electric drier was used for drying the surimi paste. The paste was spread on trays and loaded on to the racks. A blower circulates hot air from the heater, the temperature of which is controlled by a thermostat. A portion of the humid air is let out through a vent at the top and corresponding fresh air is sucked in through the bottom hole.

3.5. PREPARATION OF SURIMI

Surimi is the basic raw material for the sweetened fish powder. When properly prepared and frozen-stored, surimi can be kept in good condition upto six months or more. So when the suitable fish is available in plenty and cheap,

it can be converted to surimi and frozen-stored, which can be used as and when required, for the preparation of the product.

3.5.1. Filleting and Mincing

Throughout the production process, the temperature of fish was kept below 10^0C. Since the average size of the fish was higher, filleting was resorted to, instead of beheading and gutting. By this, improved quality mince was obtained, at the expense of yield. Flesh was removed from either side of the fish, and fillets were washed thoroughly to remove blood and visceral parts. The fillets were fed to the mincing machine, and the mince was collected.

3.5.2. Leaching and Dewatering

The undesirable compounds were removed from the fish mince by water washing. Potable water was used for washing. The temperature of the water was brought below 10^0C by using ice. The fish mince was weighed to determine the quantity of water to be mixed with.

A two washing cycle was applied here, with 5min agitation time. In the first washing cycle, 1:3 and in the second 1:2, meat : water (w/v) proportions were used. After stirring, the upper portion of the water with lipid content was decanted. The remaining material was squeezed in a double layered 'cora' cloth for dewatering. In the final washing cycle, 0.2% salt was added to the water, to prevent hydration of the meat particles and thereby to effect easy removal of water. The final dewatering was carried out by a screw press, and thereby the moisture content of the meat was brought down to around 80%.

3.5.3. Mixing of Cryoprotectants and Frozen Storage

Generally 4.5% sucrose and 4.5% sorbitol are used together, along with 0.2-0.3% polyphosphates, as cryoprotective additives. 10% sucrose was used along with 0.15% STPP and 0.15% sodium pyrophosphate in the present study. The additives were uniformly dispersed and dissolved before the meat was frozen, by using a silent cutter. The temperature of the equipment was brought

down initially by using ice, to avoid a rise in temperature during blending and thus protecting the myofibrillar protein from heat-denaturation. After thorough blending, the surimi was weighed into portions, packed in high molecular weight low density polyethylene bags, sealed and frozen. The frozen packets were given an additional layer of packaging, and stored at -20^0C in a cabinet freezer.

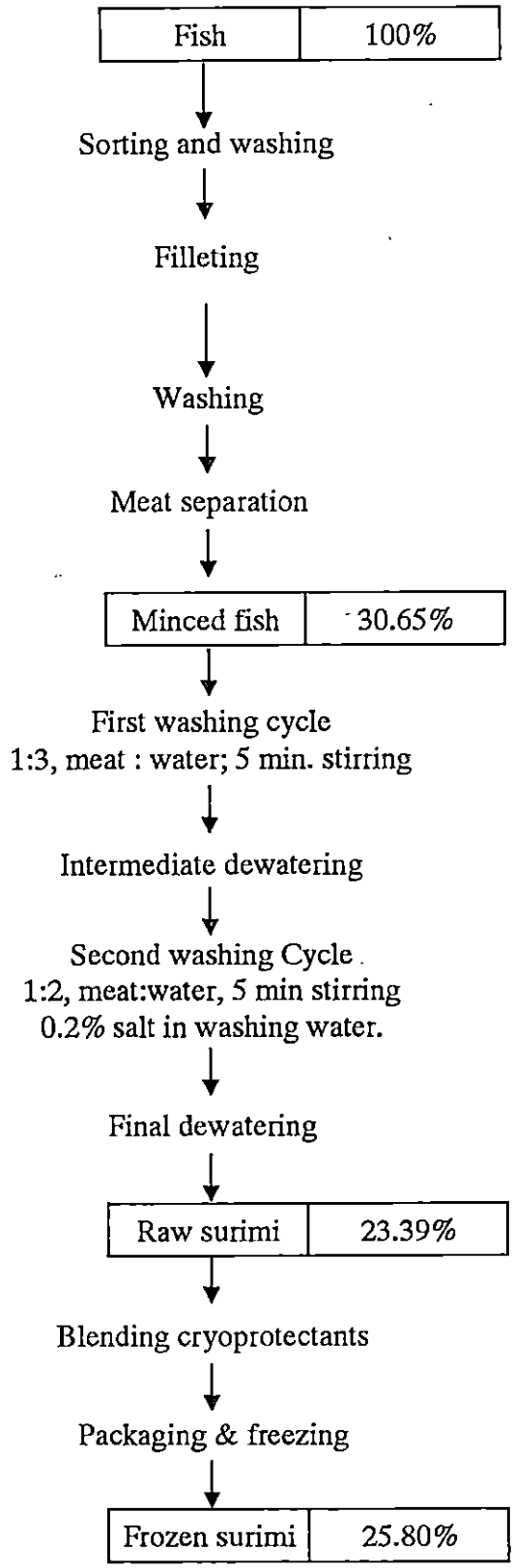

Fig. 1: Flow chart for the preparation of surimi

Plate 1: Raw material: Big eye snapper

Plate 2: Frozen surimi : prepared from big eye snapper

3.6. STANDARDIZATION OF THE METHOD

3.6.1. Tempering and Solubilization of Surimi

Tempering of the surimi was carried out by keeping the frozen surmi-blocks at refrigerated temperatures, so that the surimi-temperature was brought to around -4^{0}C.

Surimi was first chopped into very small particles with out the addition of any ingredients for a few minutes in a comminution equipment like silent cutter. Salt (2%) was then added to the surimi and again chopped for a few minutes. At this stage, because of the solubilization of myofibrillar proteins, a very viscous paste was formed. The product temperature was maintained below 10^{0}C.

3.6.2. Determination of the preferred level of sugar.

The optimum level of sucrose that gave the maximum acceptance for the product, was determined by sensory evaluation. The solubilized surimi paste was apportioned to three and their sugar levels were adjusted to 10%, 15% and 20% respectively. Each portion was then spread as a thin layer of 2-3 mm thickness on aluminium trays. The trays were loaded to the electric tray drier and dried at 55 to 60^{0}C. The dried paste was recovered from the trays as a thin film, broken to pieces and pulverized. The fine powder thus obtained was mildly warmed to further reduce its moisture content, at a very low flame for a few minutes. To each ground and dried portion, an arbitrary level of cocoa powder (5% by the weight of surimi) was added and mixed. The products were then presented to a taste panel for sensory evaluation to determine the most preferred level of sugar in the product. The sensory evaluation-sheet format is given in Appendix I.

3.6.3. Determination of the preferred level of cocoa-powder

Dehydrated surimi powder with the most preferred level of sugar, was prepared as above and then divided into three lots and mixed with three levels

of cocoa-powder : 5%, 10% and 15% (of the weight of surimi). The three lots were presented for sensory evaluation to determine the preferred level of cocoa-powder in the product.

3.6.4. Preparation of Standard Product

The product was prepared for storage as per the recipe given below.

Ingredients	Percentage
Surimi	73
Sugar	15
Cocoa powder	10
Salt	2

3.6.5. Packaging and Storage

The final product was prepared according to the standardized procedure. Since the expected moisture content of the product was below 10% and also because of its sugar and salt contents; the product would be highly hygroscopic. Moreover the aroma of the cocoa powder had to be retained on storage. So a packaging material with high moisture and gas barrier properties was selected. A 50 gauge plain polyester, laminated with 300 gauge low density polythene film was used in this study. The product was packed in the bags and heat - sealed. A storage study was also conducted to get an idea about the shelf life of the product and also to understand the various chemical, microbiological and organoleptic changes that might be taking place. Samples were drawn on the 0th day, 30th day, 60th day and 90th day for analysis.

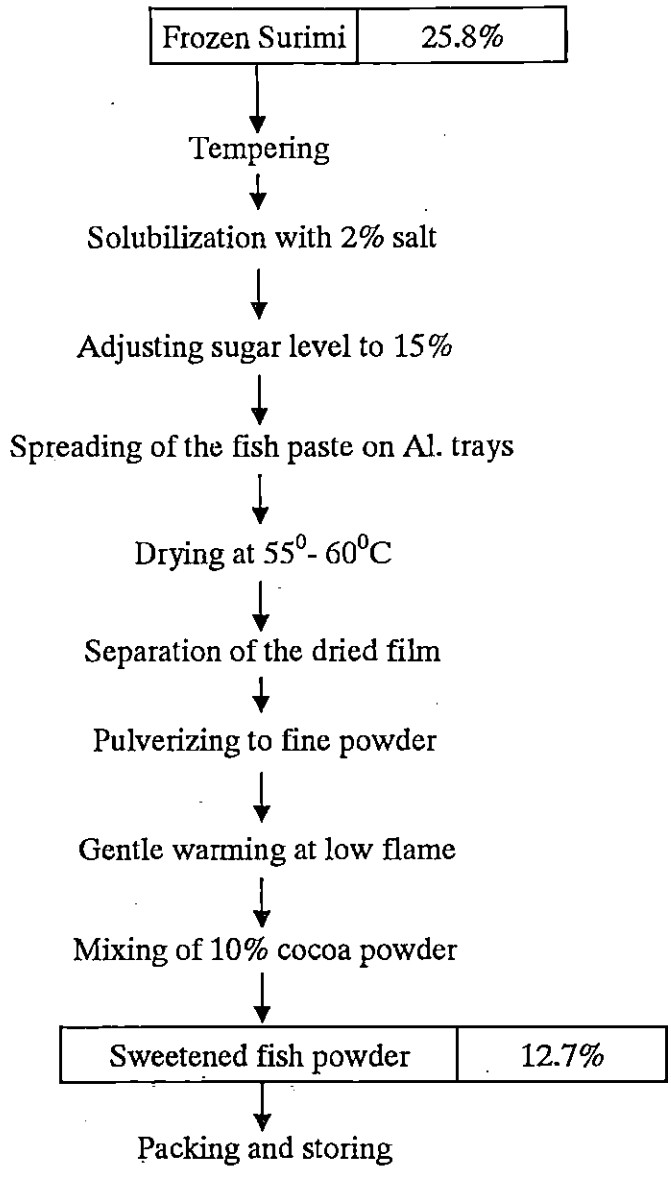

Fig. 2: Flow chart of the standardized procedure for the preparation of sweetened fish powder

* Frozen surimi forms 25.8% of the basic raw material (100%).

Plate 3: Drying surimi sol: in an electric tray drier

Plate 4: Product : Sweetened fish powder

3.7. BIOCHEMICAL TESTS

3.7.1. Moisture Content

The moisture content of the product was determined by the AOAC (1984) oven drying method, utilizing an overnight (18hrs) drying period at 100^0C. About 5g of the sample was accurately weighed in an analytical balance at room temperature and was dried to a constant weight. The dried sample was cooled in a desiccator before taking the weight . The moisture content was calculated as the percentage loss of weight upon drying.

3.7.2. Protein Content

Protein content of the product was estimated by the Microkjeldahl's method (AOAC,1984). The principle of the method is as follows: The sample is digested with concentrated sulphuric acid in presence of a suitable catalyst, so that the protein nitrogen is converted to ammonium sulphate. The ammonium sulphate formed is, distilled with alkali and ammonia evolved is absorbed in boric acid containing Tachirhos' indicator. The ammonia absorbed is then titrated against N/70 H_2SO_4. From the titre value, the percentage of the nitrogen in the sample is calculated. Since proteins on an average contain 16% nitrogen, the crude protein content of the sample is obtained by multiplying the percentage of total nitrogen by 6.25.

0.5 g sample was accurately weighed and digested in a Kjeldahl's flask with 10ml concentrated sulphuric acid and a pinch of the digestion mixture ($CuSO_4$: K_2SO_4 ; 1 : 8). About 25 ml of distilled water was carefully poured into the flask along the sides. The flask was swirled to dissipate off the heat evolved. When the solution attained room temperature, it was quantitatively transferred to a 50ml standard flask, with distilled water washings. The solution was then made up to 50ml using distilled water and mixed thoroughly. Five ml of the made up solution was used for distillation in Kjel Plus (Distil-M) equipment, with 10ml NaOH solution. The ammonia fixed by boric acid as

ammonium borate was titrated against *N*/ 70 H_2SO_4 back to the original pink colour.

3.7.3. Lipid Content

The Soxhlet method of fat estimation was followed (AOAC,1990). Five gram sample was weighed, made moisture free and transferred to an extraction thimble. The electrical heating unit was adjusted so that the solvent, petroleum ether (60-80^{0}C) siphons over 5 to 6 times per hr. The extraction was carried out for about 16 hrs. The solvent was then transferred to a pre-weighed beaker and evaporated off on a boiling water bath, then cooled to room temperature in a desiccator and weighed. The difference in weight was expressed as a percentage of the sample weight.

3.7.4. Ash Content

Ash content of the product was determined by the method of AOAC (1984). About 0.5 g of the sample was weighed accurately in a pre-weighed silica crucible. It was then ignited in a muffle furnace at a temperature of 550^{0}C until the sample was free of carbon. It was then cooled in a desiccator and weighed. The difference in weight was expressed as a percentage of the sample-weight to denote the total ash.

3.7.5. Carbohydrate Content

Carbohydrate content was indirectly calculated by using the following formula:

% carbohydrate = 100 - (%moisture + %protein + % fat + % ash.)

3.7.6. Determination of Total Volatile Base Nitrogen (TVBN)

TVBN was determined by Conway's microdiffusion method (Conway, 1962), as a test for spoilage. The tissue is extracted in the trichloro acetic acid (TCA) solution to obtain the non protein nitrogen (NPN) compounds. The extract is treated with saturated solution of sodium carbonate, to liberate the

volatile bases in the outer chamber of the Conway's microdiffusion apparatus. The liberated volatile bases are absorbed in H_2SO_4 present in the inner chamber. From the amount of acid consumed by the liberated bases, TVBN content is calculated.

A four gram sample was homogenized with 10% TCA, filtered and made upto 50ml. One ml of the TCA extract was taken in the outer chamber of the Conway's unit. One ml of the saturated sodium carbonate solution was also added to the outer chamber. One ml of N/50 H_2SO_4 was taken in the inner chamber. The unit was closed air tight immediately. The solution in the outer chamber was mixed by slow rotation of the apparatus. It was then incubated at room temperature overnight. The amount of unreacted acid in the inner chamber was determined by titration against N/50 (0.02*N*) NaOH, using Tachirho's indicator. A blank was also run using one ml of 10% TCA in the outer chamber.

3.7.7. Determination of Thio Barbituric Acid Reactive Substances (TBARS)

Oxidative rancidity of lipids is often measured by TBARS test. Malon dialdehyde (MDA) is formed from polyunsaturated fatty acids It serves as a convenient index for determining the extent of peroxidation reaction. Malon dialdehyde reacts with thio barbituric acid to give a red colour absorbing at 535 nm. The method adopted here, is that of Beuge and Aust (1978).

0.2g of the product was weighed accurately into a homogeniser, 3 ml of distilled water was added and homogenized. It was then transferred to a centrifuge tube, added 0.15 ml 0.01 % butylated hydroxy anisole (BHA) and 6 ml of TCA-TBA-HCl solution. (During heating, the haem proteins and metals enhance the colour formation by promoting the breakdown of hydroxy peroxides. The BHA added abolishes the metal- catalyzed auto-oxidation of lipids during heating). The mixture was then vortexed and incubated in water-bath at or below 80^0C for 15 min to develop the colour. It was then cooled in water for 10 min and centrifuged at 1000 g for 10 min. The absorbance of the

sample was measured at 535 nm against a blank that contained all the reagents except sample. The concentration of malon dialdehyde in the sample was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. A sample blank was used without adding TBA, to nullify the effect of the natural colour of the product, due to the presence of cocoa-powder.

3.7.8. Determination of pH

There is a possibility of lowering the pH in products containing sugar or carbohydrate, on storage. NFI (1991) method was adopted for measuring the pH. 45 ml of distilled water was added to 5g sample and blended. The pH was measured with a pH meter. Attention was given to the proper calibration of the meter and cleanliness of the electrode.

3.8. MICROBIOLOGICAL ANALYSIS

3.8.1. Total plate count (TPC)

All media and diluents were sterilized by autoclaving at a temperature of 121°C for 15 minutes and all glasswares at 160°C in hot air oven for two hours.

Total plate count of the sample was determined according to the method of Maturin and Peelei (1995). A sample of 1g was aseptically transferred to a sterile blender and homogenized with 9ml phosphate buffer (pH-7.2) as a diluent. Appropriate serial decimal dilutions of 1ml of the homogenate were made using phosphate buffer. Appropriate dilutions were plated, in duplicate, by pour plate technique using Plate count agar medium. Plates were incubated at a temperature of 37^0C for 48hours. Plate showing 30 to 300 colonies were counted. Counts were expressed as colony forming units (c.f.u) per gram sample.

$$\text{TPC (cfu/ g of sample)} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Weight of the sample}}$$

3.8.2. Total Yeast and Mould Count (TYM)

Fungal count was determined according to the method of Detroit (1971). The homogenate dilutions prepared for TPC determination were used for determining the fungal count also. Appropriate dilutions were plated, in duplicate, by pour plate technique using potato dextrose agar medium containing 10% tartaric acid and incubated at a temperature 20–25°C for 5 days. Plates having colonies ranging from 30 to 300 were selected for determining fungal count.

$$\text{TYM (cfu/g of sample)} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Weight of sample}}$$

3.9. SENSORY EVALUATION

The organoleptic quality parameters like odour, taste, texture and the over all acceptability of the product on storage, were evaluated periodically by a taste panel. A five -point hedonic scale was used for this purpose. The sensory evaluation sheet format is given in Appendix II.

3.10. STATISTICAL ANALYSIS

The results of the above evaluations, conducted periodically, were analyzed statistically using Student's t-test, to determine whether the variations were significantly different (Rangaswamy, 1995). The data of individual evaluation were also plotted on graphs, to understand the trends.

Results

4. RESULTS

4.1. STANDARDISATION OF THE METHOD

The sweetened fish powder was developed from surimi, which in turn from minced fish meat. The fish was first filleted and then minced. The yield of skinless fillets from the whole fish was 30.65%. The yield of leached fish mince was 23.39%, and after the addition of cryoprotectants (surimi), the yield was 25.80%.

When samples prepared with different levels of sugar, were subjected to sensory evaluation, 15% sugar level was preferred by the majority of the panelists. Later majority of the panelists preferred 10% cocoa level in the product when samples with 5,10 and 15% cocoa levels were evaluated. The results are shown in Table 4. The final product was thus prepared with these levels of ingredients and the yield obtained was 12.70% from the whole fish and 49.40% from the frozen surimi.

Table 4a: Different levels of sugar in samples with an arbitrary level of 5% cocoa powder subjected for sensory evaluation for determining the most preferred level of sugar.

Sugar (%)	% panelists preferring the different sugar levels.
10	18.75
15	**56.25**
20	25.00

Table 4b: Different levels of cocoa powder in samples with the most preferred level of sugar (15%) subjected for sensory evaluation for determining the most preferred level of cocoa powder.

Cocoa powder (%)	% panelists preferring the diferent cocoa powder level
5	27.80
10	**44.40**
15	27.80

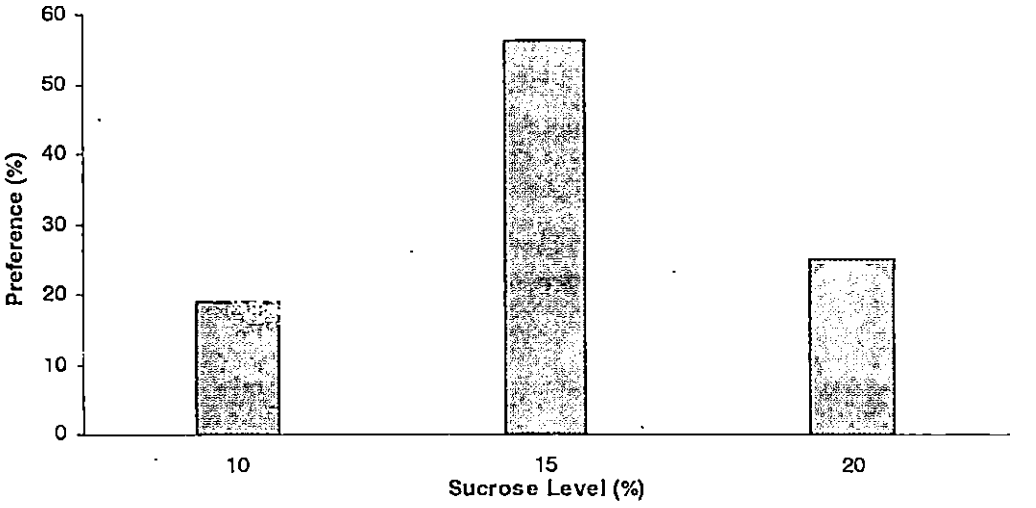

Fig. 3: Preference to different sugar levels

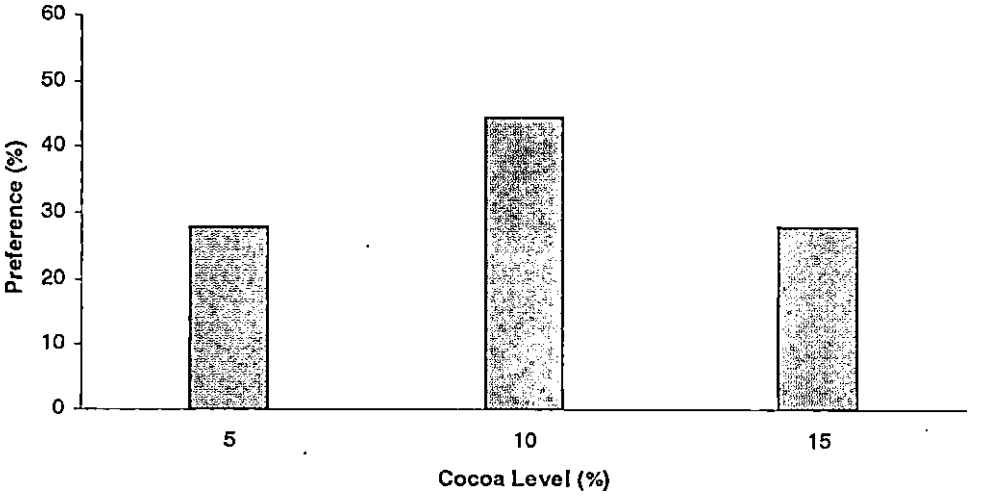

Fig.4: Preference to different cocoa levels

4.2. STORAGE STUDIES

4.2.1. Proximate Composition

The variations in the moisture content and protein, fat, ash and carbohydrate contents (on dry weight basis) of the sweetened fish powder during the storage period are given in Table 5.

During the storage period, the moisture content increased from an initial value of 2.33% to 4.58% at the 90th day. Significant difference was noted between the initial and final moisture levels.

The protein content showed a variation from 29.18% to 29.08% in 90 days, which is not statistically significant at 5% level.

There was a slight variation in the fat content from 1.13% to 1.15% which was also not statistically significant.

The ash content varied from 5.27% to 4.94% but was not statistically significant.

In the case of carbohydrate content, there was only a slight variation from 64.42% to 64.83% during the storage period.

Table 5: Proximate composition of sweetened fish powder during the storage period (%) [Values are mean ± SD of six determinations].

Storage period (days)	0	30	60	90
Moisture	2.33±0.26	3.50±0.06	4.58±0.08	4.58±0.10
Protein (dry weight basis)	29.18±3.10	29.27±2.90	29.60±2.70	29.08±3.30
Lipid (dry weight basis)	1.13±0.10	1.14±0.12	1.26±0.13	1.15±0.12
Ash (dry weight basis)	5.27±0.48	5.32±0.50	5.28±0.51	4.94±0.47
Carbohydrate (dry weight basis)	64.42	64.27	63.86	64.83

Statistical Analysis

1. Moisture content

The computed value of **t** is18.00, which falls within the critical region at 5% level of significance at degrees of freedom 10 i.e., $P \leq 0.05$. So the difference in the moisture content from the initial value to the final value is statistically **significant.**

2. Protein content

The computed value of **t** is 0.049, i.e., $P>0.05$. Hence the difference in protein content from the initial to the final value is **not significant.**

3. Lipid content

The calculated value of **t** is 0.29, i.e., $P>0.05$. So the difference is **not significant.**

4. Ash content

The calculated value of **t** is 1.098, i.e., $P>0.05$. So the variation is **not significant.**

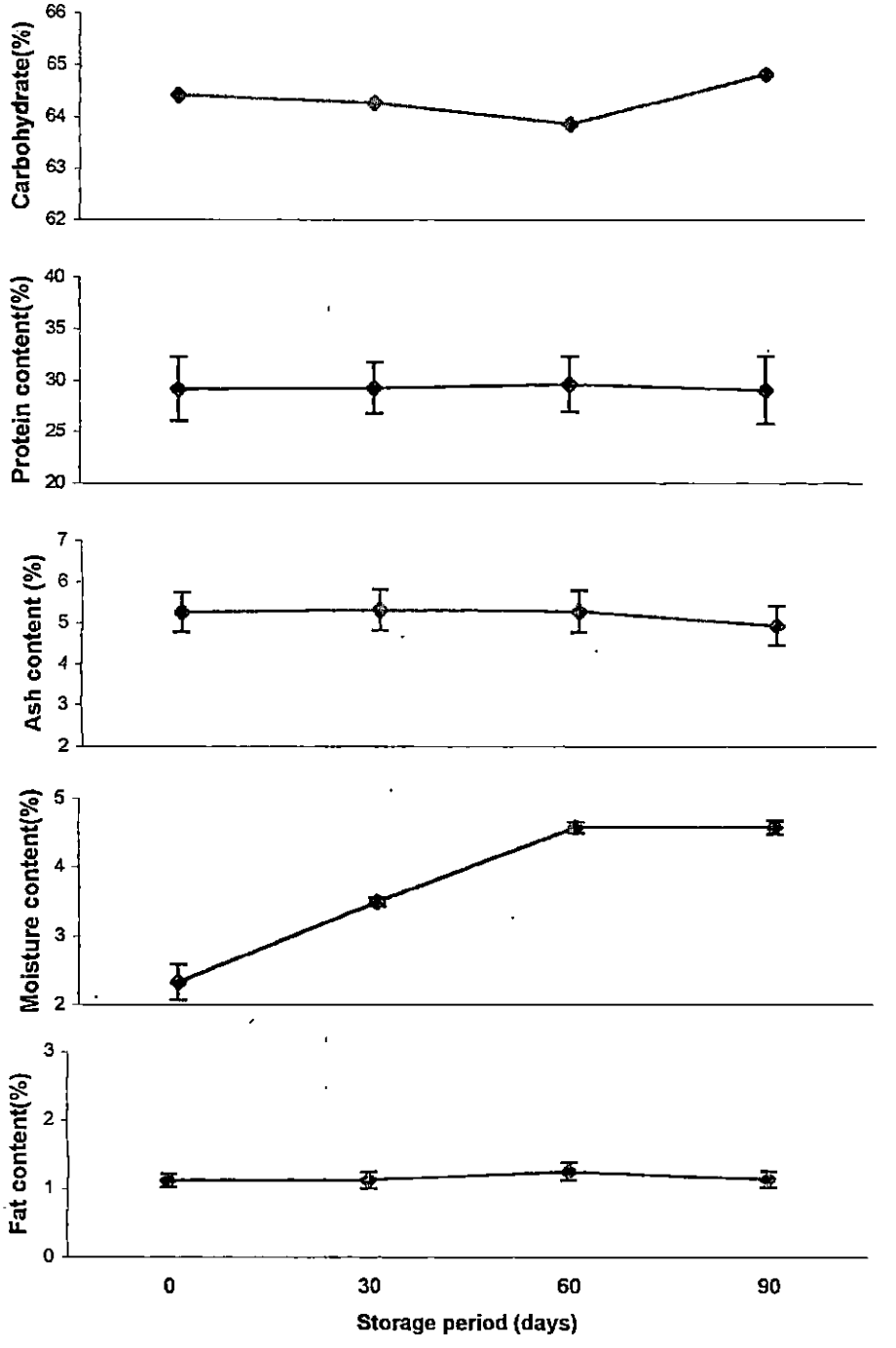

Fig. 5: Variations in the proximate composition of the product during storage at room temperature.

4.2.2. Total Volatile Base Nitrogen

The TVBN values for the storage period are shown in table 6. There was no difference in the TVBN content in the first three observations (28.00 mg%) but slightly increased to 29.75 mg% at the end of the storage period. But statistically this variation is found to be not significant.

4.2.3. Thio Barbituric Acid Reactive Substances

During the storage period the TBARS increased from 14.31 mg malon dialdehyde/ kg to 16.27 mg/kg. This increase is found to be statistically significant.

4.2.4. pH

There was no change in pH (6.40) upto the second month. During the third month, though it was slightly reduced to 6.38, the same was not found to be significant.

Table 6: TVBN, TBARS and pH values of the sweetened fish powder during the storage period [Values are mean ± SD of six determinations].

Storage period (days)	0	30	60	90
Total volatile base nitrogen (mg%)	28.00±2.02	28.00±2.86	28.00±2.02	29.75±1.75
Thiobarbituric acid reactive substances (mg MDA/ kg)	14.31±0.47	14.52±0.36	15.12±0.55	16.27±0.32
$\overline{pH}$	6.40±0.03	6.40±0.02	6.43±0.64	6.38±0.03

Statistical analysis

1. TVBN

The computed value of t is 1.46, i.e., P>0.05. So there is **no significant** variation from the initial to the final values.

2. TBARS

The calculated value of t is 7.71, i.e., P≤0.05. So the increase in the TBARS content from the initial value to that, at the end of the storage period is statistically **significant.**

3. $\overline{pH}$

The computed value of t is 1.05 ie P> 0.05. So the variation in the pH values from the initial to the final is **not significant.**

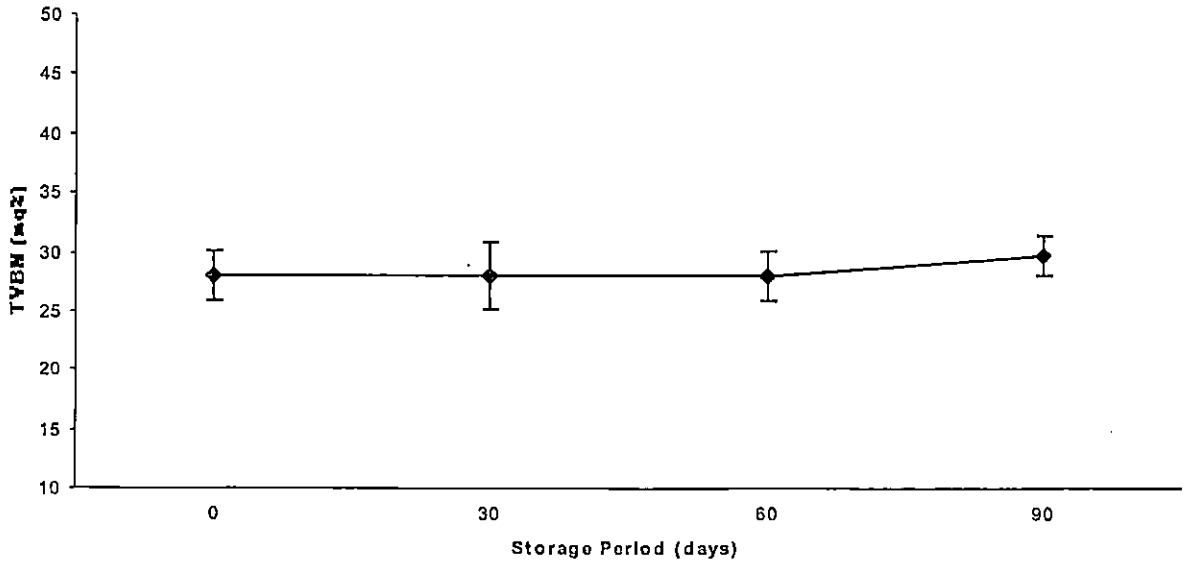

Fig. 6: Variations in the TVBN content during storage at room temperature

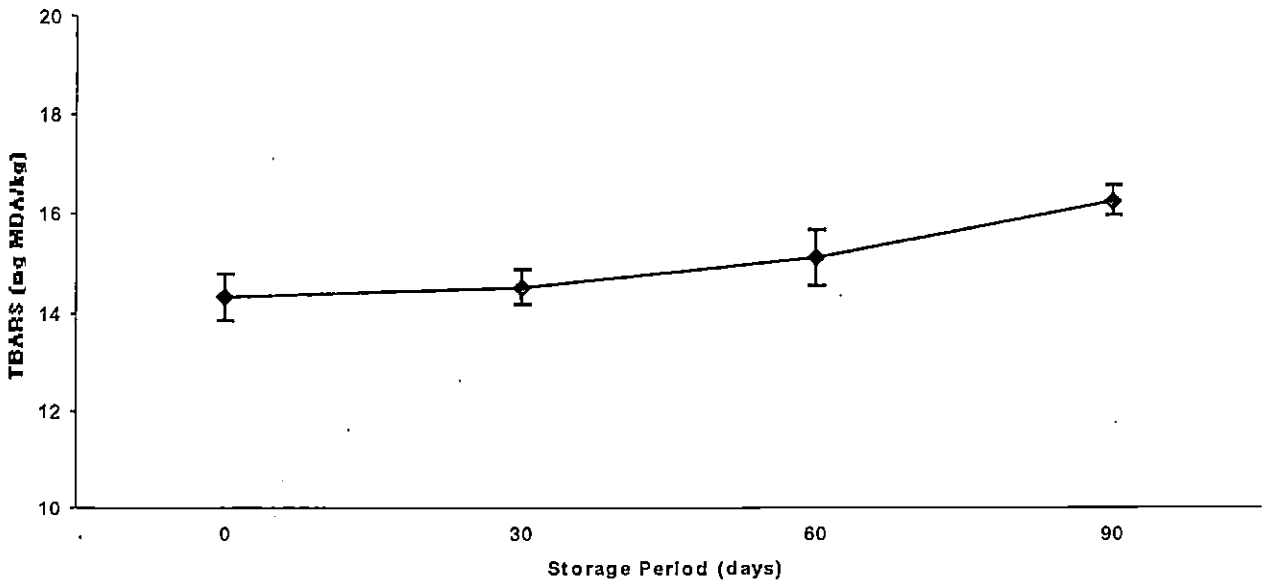

Fig. 7: Variations in TBARS values during storage at room temperature

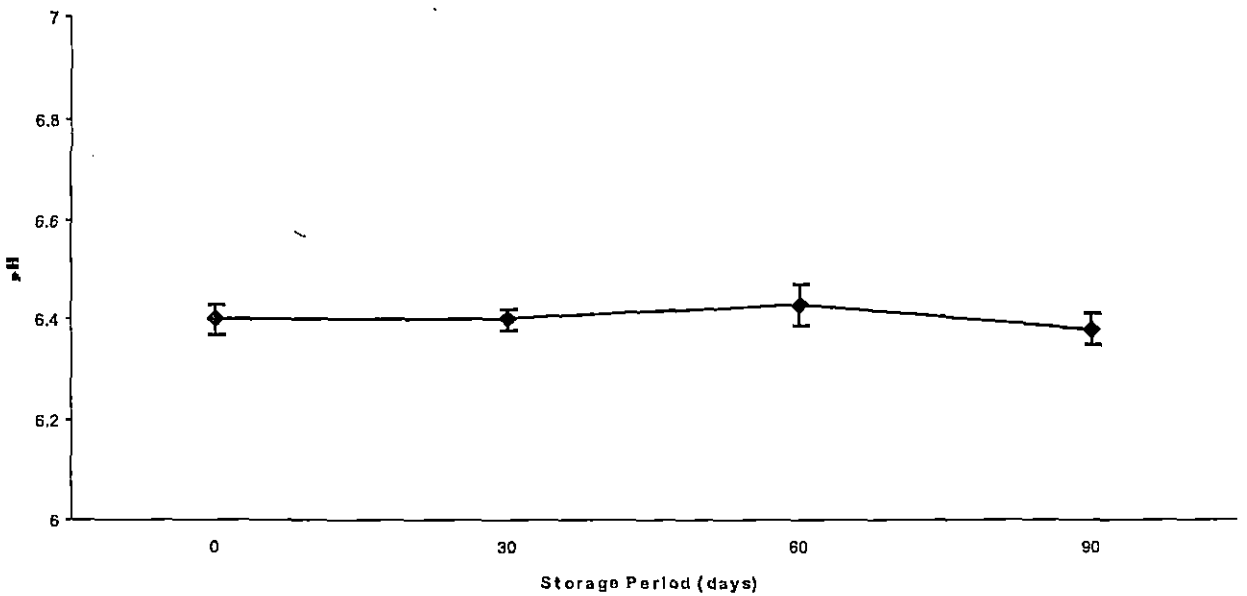

Fig. 8: Variations in the pH values during storage at room temperature

4.2.5. Microbiological Analysis

The total plate count (TPC) and the total yeast and mould count (TYM), during the storage period are given in Table 7. The TPC decreased from the initial value of 2.36×10^5 to 5.3×10^4 on the 90^{th} day of storage. In the case of TYM no observation was there on the 0^{th} day, less than 30 on the 30^{th} day and then slowly increased to 3.4×10^2 on the 90^{th} day of storage.

Table 7: Total plate count and Total yeast and mould count during the storage period, cfu/g (colony forming units/g).

Storage period (days)	0	30	60	90
Total plate count	2.36×10^5	2.74×10^5	1.94×10^5	5.30×10^4
Total yeast and mould count	ND	< 30	3.20×10^2	3.40×10^2

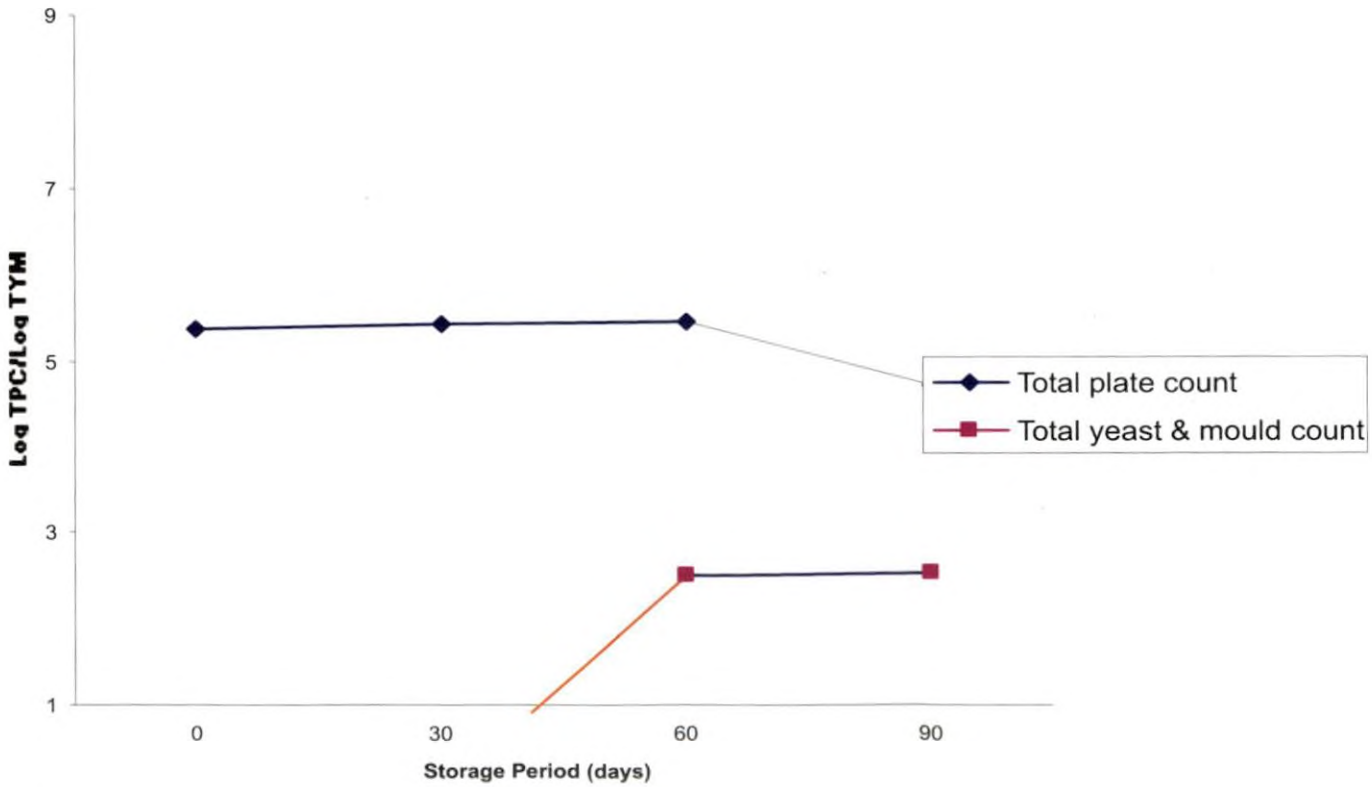

Fig. 9: Variations in the TPC and TYM during storage at room temperature

4.2.6. Sensory Evaluation

Odour, taste and texture as well as the overall acceptability of the product was evaluated organoleptically. The means of the scores are given in Table 8. There were no significant changes in the scores of any of the sensory parameters. The overall acceptability of the product remained in 'very good' condition (score >4.0) throughout the storage period. All the values were slightly decreased from the initial level (odour 4.50 to 3.81; texture 3.93 to 3.44 and overall acceptability from 4.14 to 4.06), except taste in which case, the means score was slightly increased from 4.00 to 4.06.

Table 8: Sensory evaluation values of the sweetened fish powder during the storage period ['mean score' ± SD of minimum 14 values]

Storage period (days)	0	30	60	90
Odour	4.50±0.63	4.00±0.73	3.93±0.70	3.81±0.81
Taste	4.00±0.65	3.87±0.72	4.29±0.45	4.06±0.66
Texture	3.93±0.59	3.67±0.47	3.50±0.63	3.44±0.61
Overall	4.14±0.52	3.80±0.65	4.14±0.52	4.06±0.66

Statistical analysis

The computed values of 't' for odour, taste, texture and overall acceptability are 1.500, 0.145, 1.290 and 0.213 respectively, i.e., in all the cases $P>0.05$. So the variations in all the sensory parameters from the initial to the final values are **not significant**

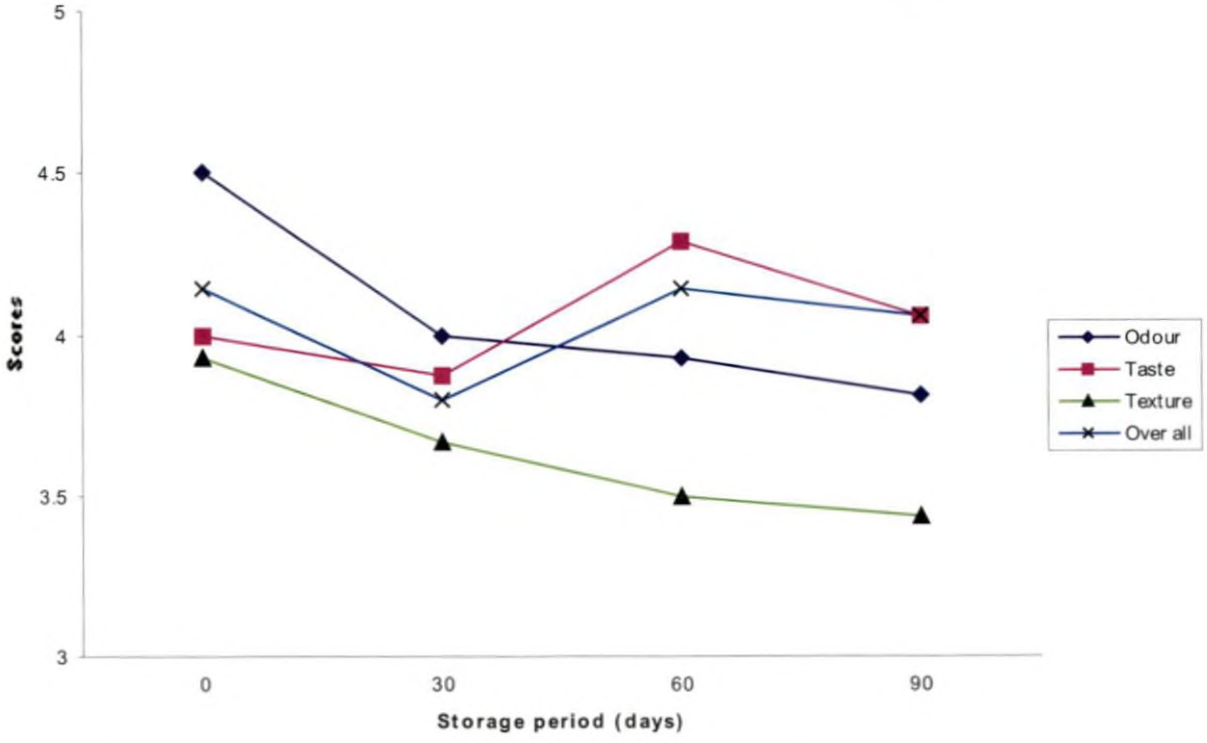

Fig. 10: Variations in the sensory quality parameters of the product during storage at room temperature.

Discussion

5. DISCUSSION

5.1. FORMULATION OF PRODUCT

The importance of protein in the diet, especially at the growing stage has been well accepted. The superiority of seafood protein is also well known. But, most of the children are reluctant to eat fish, because of the fear for the presence of bones and such other reasons. Hence a study was planned to develop a product from fish, devoid of its undesirable properties and at the same time, retaining its advantages. The leached fish mince/surimi, has the desirable properties and so, was selected as the raw material for the product.

Most of the children prefer food items, based on taste, flavour and appearance. Children generally like sweet foods. So it was decided to sweeten the surimi with sugar. The sugar also has an added advantage of protecting the protein from heat-denaturation. Several natural and artificial food grade flavours are available, like vanilla, chocolate, orange, etc. Here in this study, it was decided to exploit the weakness of children towards chocolate flavour. So cocoa powder was used as the flavouring agent. The resultant product was planned as a dried one, because of convenience of storage, transportation and utility.

In the production of all surimi-based products, surimi is first solubilized into an actomyosin sol, by kneading with salt. This makes it convenient and easy for the mixing of ingredients with surimi, and also for moulding to shapes in the case of imitation products. Here also, sugar was mixed with the surimi sol, made into thin film and dried in an electric tray drier. The cocoa powder was not blended along with sugar, because of the possible loss of flavour and chance of melting, during drying process. Hence it was added at the final stage, after drying and pulverizing. A more attractive, 'flaky' nature of the product could have been possible with drum-drying. But here, a powdered product was prepared because only tray drier was available

Any product developed must have consumer acceptance. The consumer appeal can be reflected in sensory analysis (Joseph and Iyer, 2002). So the standardization of the levels of ingredients, was done by conducting sensory evaluation. At first, the sugar level was determined, by the sensory evaluation of samples with different sugar levels, and an arbitrary level of cocoa powder. The cocoa level was then determined by the sensory evaluation of samples with different proportions of cocoa powder and with the most preferred level of sugar. The most preferred levels thus determined were, 15% sugar and 10% cocoa powder (of the surimi weight). Most of the panelists have expressed that, a '10% sugar and 5% cocoa' combination could not effectively mask the fish- flavour. At the same time, according to some of them, with higher levels of sugar (20%) and cocoa (15%), the product was too sweet. Moreover, this higher level of ingredients, has an indirect negative effect on the final protein content of the product.

In addition, many of them remarked that the particle size of the product should have been smaller. Reducing the particle size at the mince level itself could solve this problem. This would effect, a more efficient leaching as well as would provide a finely powdered product. The flavour of the product could be modified by adding spices as well, with an added advantage of its anti-oxidative property.

Children's preference also has been ascertained. It was given to children of the age between two and ten. The product was seemed to be liked by them, from their attitudes and responses. Interestingly none of them could identify the original material behind it.

Instead of using it directly as a powder, it could be incorporated into other snack foods like biscuits, kulfis, etc. and taken. It was used, mixing with hot milk also, without further flavouring/ sweetening. The only problem noted

was the settling of the particles, if kept for some time. To keep the particulate matter in suspension, stabilizers such as lecithin, alginates, etc. can be used. A similar beverage was developed by CIFT from FPH powder. To counter the bitterness of peptides, 20% sugar, 5% cocoa powder, 10% milk powder and 20% malt were used (Gopakumar, 2002). The FPH powder has the advantage of digestibility, but the production process is not simple but involve the use of enzymes, its incubation at specified temperature for specified time, and finally its inactivation.

The sweetened fish powder could also be developed from the minced fish directly, thereby simplifying the process and increasing the yield. Even though the big eye snapper, the fish used in this study is not a fatty fish, the sensory qualities of the product, if prepared directly from its mince, may not be as acceptable as the one prepared from its surimi.

All the negative aspects of leaching are applicable here also, which include the loss of fat, vitamins, minerals, sarcoplasmic protein and also a reduction in yield. The product can be made as a wholesome food, by fortifying it with vitamins, minerals etc. without changing its basic sensory qualities.

This product had a protein content of 29.18%(on dry weight basis) and carbohydrate content of 64.42%. In the normal spray-dried and freeze-dried surimi, the carbohydrate content was 24% and 20% respectively (Niki *et al.*, 1992). This carbohydrate consisted almost entirely of the sugars added as anti-denaturants prior to drying, which corresponded to about 5% sucrose level in the fully rehydrated product. But here in this product, the carbohydrate content was higher because sucrose was added not only as an anti-denaturant but mainly as a sweetening agent. The energy requirements for the increased physical activities at childhood could be met from this carbohydrate. But excess energy intake from any source will cause obesity, which in turn may increase the risk of heart

disease. However a high fat intake (9 Kcal energy per gram fat) is more likely to promote obesity than a high sugar intake (4 Kcal energy per gram) (Paul *et al.*, 2002).

There is a correlation between the feasibility of a manufacturing process and cost of production. The product yield has a direct bearing on the cost of production. The yield of this product was influenced by the surimi manufacturing and drying processes. To achieve maximum yield of surimi, a careful control of the filleting and meat separation, and preventing the loss of myofibrillar protein as fine meat particles through wash-water, are required. Yield may vary with species, size, season and the type of processing machinery used. The yield of fillets obtained in this study was 30.65% from big eye snapper. Venu (2002) had obtained an yield of 25.12% for fillets from big eye snapper of the size range 200-300 g /fish. The size of the fish used in this study, weighed nearly 300g. This might be the reason for the higher yield obtained. The yield of leached mince obtained in this study was 23.39%, whereas Venu (2002) obtained a higher yield of 28.67%. The increased number of washing cycles, higher meat: water proportion and longer agitation time, as employed by him, might have increased the water-retentivity of the meat particles. This could be the reason for the higher yield. The yield of surmi obtained in this study was 25.80%, which is comparable with the findings of other works, for examples, 23.80% by Lee (1986b); 26.00% by MacDonald and Lanier (1988) and 22.00% by Gwinn (1992). The yield of the dried product obtained was 12.70% from the whole fish.

5.2. STORAGE STUDIES

The product was packed and sealed in pouches of polyester (50 gauge) laminated with low density polyethylene (LDPE 300 gauge) film. Lamination of two or more films improves the appearance, barrier properties and mechanical strength of the package (Gopal *et al.*, 1998). Changes during storage, were studied based on various tests. In addition to the biochemical

and microbiological tests, the consumer acceptability of the product was determined by sensory evaluation.

The moisture content of the product was 2.33%. During the storage period, the moisture content was slightly increased by 2.25%. But these variations were confined to below 10% moisture level, under which the stability of dried products would not be much affected. The variation was limited, possibly because of the high water vapour barrier property of the laminate used. There were no significant variations in the protein, lipid, ash and carbohydrate contents during the storage period.

The total plate count of fungi (TFC) of smoked fishes as reported by Lilabati and Vishwanath (2000), was from 10^2 to 10^4 cfu per gram and by Sindhu (2004) was 3.2×10^1 to 6.8×10^2 cfu/g. The TYM here, though not detected initially, rose to 3.4×10^2 cfu/g at the 90^{th} day of storage. Some yeast and mould can grow even at a_w of 0.65 and in a temperature range of 10 to 45^0C. But here, considering the low moisture content of the product and the presence of salt and sugar in it, its a_w is expected to be below the level required for mould growth. But the experiment was conducted during the monsoon season and at least the surface layer of the powder might have absorbed just sufficient moisture to allow mould growth there. The source of contamination could be the cocoa powder (which did not receive any heat treatment) and the packaging material.

In a normal food product, the presence of sucrose may support microbial growth (Ingram and Potter, 1987). Lee (1992) had suggested the possibility of a protective effect of cryoprotectants on microorganisms. But in this study there was a reduction in the microbial load, from 2.36×10^5 to 5.30×10^4 cfu/g. The sources of microorganisms in raw surimi could be raw fish, human handling and unclean equipments. Niki *et al.*, (1992) have reported a reduction in the bacterial count from 1.10×10^5 to 1.0×10^4 cfu/g, when raw surimi was spray-dried. The source of comparatively higher initial microbial load of the product under study might be sucrose and cocoa powder, especially the latter, which was added after the drying process. Matches *et al.*, (1987) have reported that heat-resistant

bacteria in surimi products could have been introduced by ingredients such as starch, sucrose, sorbitol and flavouring agents. Yokoyama (1992) had reported the packaging materials and containers as a possible source of contamination. The bacteria thus introduced by ingredients and packaging materials, and also those survived from the raw surimi, might have remained dormant in the product because of the very low moisture content and then gradually got reduced in number because of phenomenon like auto-sterilization.

If the bacteria in the product had grown, it would have converted the sucrose to lactic acid and thus reduced the pH level. But the pH remained almost the same during the storage period. This correlates with the TPC of the product and can be inferred that there was no bacterial growth. In addition, the acidic pH of the product and lower moisture content have a protective effect on lysine, from malondialdehyde (MDA) reacting with it, similar to reducing sugars (deMan, 1990).

Total volatile base nitrogen (TVBN) is a measure of ammonia, trimethyl amine, dimethyl amine etc., produced mostly by bacterial action and therefore used as an indicator of spoilage of fish and fish products. Increase in the TVBN content with fish spoilage has been reported in many studies (Chakrabarti, 1998; Joseph, 2001). The TVBN content of this product was 28.00 mg% with a slight ($p>0.05$) increase of 1.75 mg% on storage. The almost stable TVBN values also correlate to the TPC values of this product, confirming that there was no growth of bacteria during storage. The TVBN contents of smoked fish products have been reported in many studies: 26.2 to 66.4 mg% (Fukuhara and Kuroda, 1950), 40-80 mg% (Lilabati and Vishwanath, 2000) and 18.29 mg% (Kyriazi – Papadopoulou *et al.*, 2003). The TVBN content of edible fish powder was increased from 19.13 to 32.25 mg% in five months (Chattopadhyay *et al.*, 2004). The acceptable limit of TVBN for salted and dried fish as recommended by Connell (1980) is 100 to 200 mg per 100g.

The TBARS is a measure of fat rancidity and has high application in storage studies. The MDA, a fat oxidation end product has a direct bearing on the odour and flavour characteristics of the product and also has an indirect effect on texture, by denaturing actomyosin (Buttkus,1967). None of the panelists in this study had reported rancid flavour or taste during the sensory analysis. Moreover there was no significant reduction in the sensory quality of odour. There could be several reasons for this:

° The low gas transmission barrier property of the laminated pouch might have minimized the entry of oxygen to the package.

° The lower lipid content of the product.

° The antioxidative effect of polyphosphates in surimi (Weilmeier, 1999).

° The antioxidative property of the polyphenolic compounds in cocoa-powder (Osakabe *et al.*, 2000; Wang *et al.*, 2000).

Lower levels of TBARS were obtained in frozen fish, eg. 0.906 to 3.296 mg MDA/ kg (Joseph, 2001). The TBA values of dehydrated rohu steaks (15.10% moisture content) during storage were in the range of 13.0 mg MDA/ kg to 18.0 mg MDA/ kg (Smruti *et al.*, 2003). Compared to frozen fish, lipid oxidation is maximized at very low a_w in dried products, probably because of the concentration of metal catalysts (Olley *et al.*, 1988). The influence of sucrose on the susceptibility of lipids to peroxidation and thus affecting the TBARS estimation, has been studied by many workers (Shlafer and Shepard, 1984; Knight *et al.*, 1988). The higher values of TBARS in this study may be attributable to the presence of sucrose and to the effect of lower moisture content.

From the results of various tests conducted, it could be seen that, most of the parameters were interrelated. During the storage study, even though there were slight deterioration in the sensory parameters, the variations were not statistically significant. This was supported by the results of chemical and microbiological tests conducted. The product remained acceptable for the period of storage study.

Summary

6. SUMMARY

1. The objective of the study was to develop a sweetened snack food for children, from a lean and cheap variety of fish.

2. For developing the product, emphasis was given to the following considerations: a food that could be prepared by a simple method, retaining the nutritional quality of fish; that could be stored at room temperature; which would be attractive and palatable to children; that could be eaten directly or incorporated into other snack foods; and could meet the increased nutritional and energy requirements at childhood.

3. Surimi, prepared from minced fish meat has high quality protein and at the same time, is devoid of any fishy odour and taste. Because of these desirable qualities, the product was developed from surimi.

4. Surimi was prepared from big eye snapper (*Priacanthus hamrur* Forskal, 1775), which was meat-picked, the picked mince was water-washed, dewatered, cryostabilized and frozen stored, for further use.

5. Salt was used, for solubilizing the surimi into an actomyosin sol and also for enhancing the taste. Sucrose was used to prevent heat-denaturation of protein and also to sweeten the product. Cocoa–powder was used as the flavouring agent. The most preferred levels of sucrose and cocoa powder as determined by sensory evaluation were 15% and 10% (based on surimi weight) respectively.

6. The final product was prepared with the standardized ingredient levels, dried, ground, packed in polyester-LDPE laminated pouches, sealed and stored at room temperature. The product was presented to children to understand their attitude. Their responses could not be quantified but were really encouraging. Moreover they were unable to identify the basic raw material of the product.

7. A storage study was carried out on the keeping quality of the product, for a period of 90 days. The quality changes were monitored based on the following tests conducted periodically: proximate composition, thiobarbituric acid reactive-substances, total volatile base nitrogen, total plate count, total yeast and mould count, pH and sensory quality parameters, *viz.* odour, taste, texture and overall acceptability.

8. There were no significant variations ($P>0.05$) in the TVBN and pH values during storage. The TBARS and moisture content were slightly increased. The TPC showed a declining trend, might be because of the low a_w level in the product.

9. Though there was an insignificant decrease in the sensory quality parameters, the product was in acceptable condition throughout the storage period.

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DEVELOPMENT OF A READY TO CONSUME SWEETENED FISH POWDER FOR CHILDREN

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ABSTRACT OF THESIS

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ABSTRACT

A sweetened snack food was developed from a lean and cheap fish (*Priacanthus hamrur* , Forskal, 1775) for children, to meet their increased requirements for protein and energy, corresponding to their higher growth rate and physical activities. The food was planned to be attractive & palatable to children; without any fish flavour and bones; and that could be stored at room temperature. The product was developed from surimi, since the latter was devoid of all the undesirable characteristics of fish flesh but retained its nutritive qualities. Surimi was solubilized with salt, into a protein-sol and sweetened it with sucrose, which also had a protective effect on protein from heat-denaturation. The sol was dried, ground and flavoured with cocoa–powder. The most preferred levels of sucrose and cocoa-powder were standardized by sensory evaluation of samples prepared with different levels of sucrose and cocoa powder.

The final product was prepared by the standardized procedure, packed in polyester-LDPE laminated pouches and stored. Children's response to the product was also tested. A 90day's storage study was conducted and the quality changes during storage were monitored periodically. There were no significant variations in the pH and TVBN values ($P>0.05$). The moisture and TBARS values showed a statistically significant increase. A decreasing trend was observed in the aerobic plate counts. The sensory quality parameters like odour, texture, taste and overall acceptability, did not decrease significantly. The product was acceptable throughout the storage period.

APPENDIX – I

SCORE SHEET FOR SENSORY EVALUATION

Please evaluate the given samples, according to your preference. Taste each sample, and record your judgment by putting a '✓' mark against the appropriate score.

	SCORE									
	10 Excellent Taste	9	8	7	6	5	4	3	2	1 Very poor Taste
SAMPLE I										
SAMPLE II										
SAMPLE III										

Date :

Name & Signature

Remarks if any :

SCORE SHEET FOR SENSORY EVALUATION

Please taste the given sample for each of the following sensory characteristics and record your judgment by giving appropriate score, based on the scale given below, against each characteristic.

Sensory Characteristic	Score
Odour	
Taste	
Texture (Crispness)	
Overall acceptability	

SCALE:

Excellent	-	5
Very good	-	4
Good	-	3
Fair	-	2
Poor	-	1

Remarks if any:

Date :

Name & Signature