DEVELOPMENT OF CONVENIENCE PRODUCTS FROM MINCED MEAT OF BIG EYE (*Priacanthus hamrur*) FISH

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CERTIFICATE

This is to certify that the thesis entitled "**Development of convenience products from minced meat of big eye (*Priacanthus hamrur*) fish**" submitted by **Mr. Chandan.C.** for the degree of **Master of Fisheries Science** in **Industrial Fishery Technology** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of research work done by him during the period of his study in the University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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Introduction

I. INTRODUCTION

Fish in general, is considered as important food from the nutritional point of view (James, 1984; Kent, 1987; Sikorski *et al.*, 1990). It provides about 14% of the worlds needs for animal proteins and 4-5% of the total protein requirements (Whittle, 1984). Fish supplies a good balance of proteins, vitamins and minerals and relatively low calories (Sikorski *et al* 1990). In addition, certain species of seafood are excellent sources of n-3 polyunsaturated fatty acids, which have therapeutic value (Kinsella, 1987).

Although fish production from tropical countries has shown an increasing trend, In terms of consumer preference, a significant portion of total available fish constitutes commercially unimportant and hence underutilized species. This has resulted in an industry, processing only a few selected items leaving several fish species not utilized to full extent. Considering the present global scenario, where commercially important fish stocks are fast depleting, throughout the world, there is an urgent need for better utilization of local fish catch.

Several problems exist in full utilization of underutilized fish resources as source of human food such as 1. Seasonal nature or lack of steady supply of individual items, 2. Smaller size and compositional nature, 3. Extreme heterogenecity of composition, bony structure, dark flesh, small size, unattractive appearance and texture, strong flavour and possible presence of toxic species (Connel and Hardy, 1981). Due to these reasons, it has become a common practice that such fishes are discarded at sea in preference to high value species (Bell, 1978). Since fish in general is nutritionally rich and there is a desperate need for better utilization in the present global scenario, there is a need for devising suitable processing methods to utilize these kinds of species and present to the customer in a proper way.

The processing of fish involves firstly, the application of preservation techniques, in order to retain quality and increase shelf life and secondly it may also involve value adding to produce a wide variety of products. The processing of fish into a wide variety of value added products is now common

with the increase in demand for food products that are ready to eat or require little preparation before serving.

Fishes with low economic value and underutilized fishes can be industrially processed into a wide array of products to increase their economic value and allow the fishing industry and exporting countries to reap full benefits of their aquatic resources. In addition, value addition processes generate employment and hard currency earnings. This is more important nowadays because of societical changes that have led to the development of out door catering, convenience products and food services requiring ready to eat fish products or little preparation before serving.

Enormous data are available on the fishery wealth in deep waters off the West coast (Bande *et al.*, 1989). Some of the notable species which are available on a fairly abundant scale are *Priacanthus hamrur*, *Chloropthalamus agassizi* and *Centrolophus niger* (Dhananjaya*, et al.,* 1984). Among these species, 40% of *priacanthus* sp., popularly known as Disco fish forms one of the major deep sea varieties.. It is comparatively a new addition to our fishery resources having fairly thick skin and large eyes with high flesh content. Therefore in the present investigation efforts have been made to utilise this species by suitable processing for human consumption. Minced meat preparation offers an effective way for utilising the most species of fish and hence can be incorporated as a base into a wide variety of sea food products like sausages, fish fingers, fish cutlets, fish steaks, fish cakes, Kamaboko etc.

The objectives of present investigation are

1. To prepare two mince based fish products.
2. To standarise the levels of ingredients and method of preparation
3. To evaluate quality of prepared products.

Landings of Big eye (*Priacanthus hamrur*) off Mangalore coast

Month / Year	2000	2001	2002	2003	2004	2005
January	61897	375021	487841	216584	9910	115887
February	189693	214183	637609	232114	59658	155255
March	133878	185849	446799	69156	79431	169427
April	129761	177360	99083	126210	117647	39535
May	159053	175780	267920	155645	81131	118749
June	NA	58643	73605	32713	7707	98770
July	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
August	**NA**	3559	9045	**NA**	16173	60445
September	218016	239528	99146	270825	366500	84407
October	107346	239231	86220	112329	72459	66122
November	502193	580939	58695	8175	56175	23052
December	154613	426982	47434	22865	124595	29730

All values are in Kgs

Source :CMFRI Annual Report 2006

NA : Not Available

Review of Literature

II REVIEW OF LITERATURE

2.1 Quality changes during frozen storage of fish

Fish is a highly perishable commodity which demands suitable preservation techniques in order to be consumed later. Of many conventional and unconventional techniques adopted for preserving the fish, freezing of fish results in an end product which is very close to fresh fish in term's of quality. Preserving of fish by freezing is not a new idea. Man learnt that fishes could be preserved by freezing, very long back before artificial refrigeration techniques were found out. But it was only possible in countries with cold climates through natural freezing in the open air.

Several scientists have worked on frozen storage studies of different fish species, at different levels of temperature. Aubourg and Medina (1999) have studied the influence of temperature and time on deterioration of lipid in cod and haddock during frozen storage. Marshal (1989) has studied different processing and freezing methods influencing the consistency and quality of fresh and peeled craw fish meat. Schubring (2002) has studied the influence of freezing of cod fillets on sensory, physical and chemical attributes. Benjakul *et al.* (2005) have reported on the effect of frozen storage on chemical and gel forming properties of thread fin bream in Thailand. Benjakul *et al.* (2003) have carried out a comparative study on physiochemical changes of muscle proteins from some tropical fish during frozen storage. Badii and Howell (2001) have carried out a study on biochemical changes occurring in cod and haddock during frozen storage. Jiang *et al.* (1985) studied the effect of temperature fluctuation on the meat quality of frozen Mackerel (*Scomber tapeinocephalus*). Similarly frozen storage of chub mackerel (*Rastrelliger kanagurta*), Nile perch (*Lates niolicus*), White Sardine (*Esuloya thoracata*), anchovies, Antarctic Krill (*Euphausia superba*), Skipjack tuna (*Katsuwonous pelamis*) were studied by Abdullah and Yu (1985), Namulema *et al.* (1999), Park and Lee (1989), Badonia and Devdasan. (1980) and Jaya Singe *et al.*(1997) respectively.

Nilsson (1997) observed the effects of freezing and thawing on the muscle structure of formed rainbow trout (O*ncorhynchus mykiss*) and reported

that rapid thawing was of greater importance to both sensory and biochemical quality in frozen fish and also reported that rapid thawing resulted in less membrane disintegration irrespective of length of frozen storage. Similarly during freezing and frozen storage of fish, a number of chemical and physiochemical changes take place. These changes bring about an alteration in the sensory quality of frozen fish. The various changes during frozen storage of fish and their impact on quality of fish are reviewed in the following sections.

2.2 Prepared fish products / processed fish products

There is an account of historical background and development of processed fish products industry over year's, which has been given by Sammartian and Tressler in their book "The freezing preservation of foods" (Tressler *et al.*, 1968). The idea of fish sticks was first conceived by owner of Redifoods, New York in 1949. Teeny and Niyauchi (1972) have reported on the preparation of fish sticks from the modified minced blocks of black rockfish and on their storage characteristics. Baker and Darfler (1979) prepared battered and breaded smelt and investigated their storage stability.

Bremmer and Snell (1978) studied the suitability of minced flesh of six tropical fish species, using Australian taste panel. They concluded that the minced meat of pink perch (*Nemipterus japonicus*) was the most acceptable among the six species tested. Rao (1984) described the preservation of fish cake at low temperature. He concluded that the product when stored at 2°C was acceptable for a period of 8 days and at -20°C for 60 days. Joseph and Perigreen (1983) studied on the preparation and storage of fish cutlet from low priced fish. They concluded that flash fried products had a shelf life of 22 weeks at - 22°C.

In terms of consumer preference, a significant portion of total available fish constitutes commercially unimportant and hence underutilized species most of which are landed as by catch of fishing operations are aimed at targeted species. This has resulted in an industry, processing only a few selected items leaving several species not utilized to full extent. In the pretext

of present global scenario of gradual depletion of commercially important fish species, a need has arisen to process these under utilized species, through value addition into diversified fish products.

Utilization of these underutilized fish species, which almost have same nutritive value in comparison to commercially important species can be, encouraged through development of ready-to-cook convenience pre-processed products. One of the major factors that stimulated frozen fish trade was introduction of fish sticks. This product alone created a whole industry which has now been further expanded by the addition of battered and breaded portions. Development of fish mince technology actually accelerated the practice of utilization of underutilized products. De-boned meat from underutilized fish species can be used as a raw material for production of variety of texturised and paste products which have a wider acceptance all over the world.

In our country a lot of work has been done in the utilization of fish species for human consumption. Varieties of fish products have been developed in the laboratories of Central Institute of Fisheries Technology, Cochin, Central Food Technological Research Institute, Mysore, College of Fisheries, Mangalore, Central Institute of Fisheries Education, Mumbai and other research institutes.

Value added products from fish mince such as chip-like cod based crackers have been prepared by Omayama (1993). He has studied its acceptability and chemical composition during frozen storage.

Sehgal and Sehgal (2001) have prepared some battered and breaded products such as fish sticks, fish fingers. They have studied their acceptance and biochemical characteristics during the period of frozen storage. Tokur *et al.* (2005) have reported on preparation, chemical and sensory quality changes of fish fingers made from mirror carp during frozen storage (-18°C). Cakli *et al.* (2004) have reported on production and quality of fish fingers produced from different fish species. They have found through the results of microbiological and chemical analysis that fish fingers were found to be within "acceptable limits" during frozen storage of 8 months. Standardization and

frozen storage of fish cutlet from bleached and unbleached Mackerel mince meat has been reported by Kamat *et al.* (2003). They have reported that fish cutlets prepared from bleached mince meat were acceptable for two months and those from unbleached mince meat were acceptable up to one month, from the point of view of organoleptic and biochemical qualities.

The utilization of oil sardine has been a major object of research and analysis for scientists in India. Muraleedharan (1980) developed ready-to-serve products from oil sardine to suit Indian consumers. The need for diversification in Indian fishery products industry is discussed by Girija and Ravinath (1986). Various processing methods and diversified products with respect to Indian market are analyzed by them. Marketing challenges to increase seafood sales with respect to quality, preparation, promotion of fresh and frozen foods are examined by Olafsson (1988).

2.3 Minced meat

Fish mince forms an important base material for the development of number of value added products. Minced fish represents a significant advance in an effort aimed at better utilization of underutilized species. Minced fish is the flesh separated in comminuted form from the skin, bones, scales and fin's of source material. A wide range of machines is available for the purpose of deboning. The color, flavor and functional properties of the mince depend upon the initial nature and quality of fish apart from conditions of deboning. Prolonged ice storage of fish, frozen storage of mince prior to processing affects protein solubility, emulsification capacity, water binding capacity, cooking loss, drip loss and texture (Reddy and Srikar, 1991; Reddy *et al* .,1992).

The yield of mince obtained ranges from 50% to 75% and having a content of few mg % of bone. Low bone content is desirable for better functional properties. The minced meat obtained is less stable than the intact muscle. The disruption of tissue membrane and exposure of the meat to air accelerates oxidative process during storage. Refrigerated storage may affect the lipid stability of mince from fatty fish species. The frozen mince has an

average storage life of about 6 months depending upon the fish. Longer storage may affect the protein quality, with significant loss in functionality.

The technology of fish mince production has been discussed by several authors (Babbit, 1986; FAO 1977; Grantham, 1981; Rengenstein and Rengenstein,1991; Mackie 1985 *et al*; Suzuki 1981; Venugopal and Shahidi, 1995). Several products can be manufactured using the whole fish mince. One popular item is the composite fillet (Connell and Hardy, 1981). Traditional products such as sausages, fish fingers, fish cakes, pastes, patties, balls wafers, burgers can be prepared (Grantham, 1981).

Crawford (1972) has studied the yield and acceptability of separated flesh from orange rock fish, yellow tail rock fish, Dink cod and True cod. Joseph and Perigreen (1983) have prepared minced meat from lizard fish (*Saurida tumbil*), Pink perch (*Nemipterus japonicus*), Jew fish (*Johinus sp)* and other miscellaneous fishes Muraleedharan *et al.,* (1994), used *Trichiurus lepturus*, *Epinephelus diacoanthus* and *Priacanthus hamrur* caught on board FORV Sagara Sampada in preparation of minced meat.

Shih-Bin-lin *et al.* (2005) have reported on change of thermal gelation properties of horse mackerel mince led by protein denaturation occurring in frozen storage and consequential air flotation wash. Leelapongwattana *et al* (2004) have studied the physical and biochemical changes of minced flesh of lizard fish (*Saurida micropectoralis*) kept in air and vacuum during frozen storage at -20°C for 24 weeks. Verma *et al.* (1995) have investigated the effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine mince.

Benjakul *et al.* (2003) have studied the effect of frozen storage on chemical and gel forming properties of fish commonly used for surimi production in Thailand.

Tokur *et al.* (2005) have prepared fish fingers from minced meat of *Cyprinus carpio* and have recorded chemical and sensory quality changes of fish fingers during frozen storage. Kose *et al.* (2004) have carried out studies on storage properties of refrigerated whiting mince after mincing by three different methods. Cakli *et al.*(2004) have carried out studies on production

and quality of fish fingers from minces of different fish species. Suvanich *et al* .(2000) have carried out studies on changes in selected chemical quality characteristics of channel cat fish frame mince during chill and frozen storage.

Minces prepared under the most controlled conditions are extremely susceptible to post mincing contamination (Liston, 1980). The minces of high levels of initial contamination have extreme risk of spoilage on handling, storage and further processing. Rao (1984) has emphasized the microbiological aspects with reference to diversified products.

2.4 Subsidiary ingredients

Ingredients such as salt, starch, batter mixture, spices etc. are added for the preparation of fish cakes, to improve taste, quality as well as shelf life of the product.

2.4.1 Common salt (sodium chloride)

The amount of salt added in the preparation of these products, to obtain proper taste is around 2.3%. When salt at 2-3% level is added to the fish mince and grounded, the minced meat will turn into a viscous paste, wherein the sodium chloride increases ionic strength of meat to solubilise the actomyocin in the muscle into "sol" form (Amano, 1962; Suzuki, 1981). Addition of salt to fish mince and grinding will rupture the intermolecular salt linkage among myofibrillar proteins (Niwa *et al.*, 1986). Further, higher concentration of salt will affect the solubility of the myofibrillar protein and destabilizes their molecular structure to thermal denaturation as noticed by differential scanning calorimetry (DSC) studies (Wu *et al.*, 1985). Effect of different levels of salt in the preparation of paste products is studied by Joshi and Shetty (1994).

2.4.2 Starch

Starch is used as an ingredient in prepared fishery products mainly to influence the texture and bulkiness of the product. The starch added serves the purpose of improving elasticity and bulkiness of the product. Usually starch is added at the rate of 10% of bulk weight either in wet condition or powder form. Various kinds of starch powders such as rice flour, corn flour, wheat flour are added. Potato starch in wet condition has shown better effect than wheat starch because of its greater hydration capacity.

According to Tanikawa (1971), added starch strengthens the meat gel structure after the ground meat is heated to 80-85°C. But according to Okada (1959), when starch is mixed with ground meat, starch molecules will swell due to heating, thereby strengthening the meat gel. The starch added to the fish product results in reduced production cost and help in binding together the various proteins in fish. Nishino *et al.* (1990) have studied the effect of different kinds of starches on adhesion of fish meat products.

2.4.3 Spices

The spice added to the fish product imparts taste and flavour and enhances the products overall quality. Spice oil has preservative effect on fresh water fish fillets at both room temperatures, as well as under refrigerated conditions (Vishweswaraiah and Lahiry, 1985). Cinnamon oil is effective in extending shelf life of foreign prawn (Yermal *et al.,* 1972). It is well known that spices and spice mixtures carry heavy loads of bacteria, especially spore formers (Krishnaswamy *et al.* 1979).

In fish fingers prepared from Mackerel, application of batter imparted some protective effect. According to Hoiston (1955), the hue of finished fish sticks is primarily determined by the type of breading material. As the ratio of bread crumbs to wheat cereal base mix is increased the shade of color obtained at a given temperature and cooking time is deepened. Fish sticks prepared from croaker meat do not deepen in shade noticeably even during extended storage.

2.5 Proximate composition

Knowledge on the nutritive value of any food product is important. The proximate composition of fish gets affected by mechanical deboning. During the process of deboning, considerable quantities of lipid and heme components get released from bone marrow and get accumulated in mechanically separated products. The fraction thus released during this process dilutes the protein fractions and increase the lipid fraction in the mechanically deboned fish. Crawford (1972) has reported compositional data on several species of mechanically deboned fish. Fat levels varied from 1.9 to 8.2%, difference in moisture content was proportional to the variance in fat content. Webb *et al.* (1976) have compared mechanically separated fish muscle with hand separated muscle from Atlantic croaker. The shearing action of mechanical deboner was believed to be removing more fatty tissue from the skin than was obtained by hand skinning. Bone marrow apparently has contributed to the increase in fat content of the mechanically separated fish tissue. Ultimately, mechanically deboned fish have protein quality comparable to that of hand deboned sources. Thus, mechanically deboned source of fish can be used in products without sacrificing protein quality. Protein content varying from 15% to 22% has been reported in the literature. The differences in protein content have been attributed to variation in species, season, age, sex and food availability (Love, 1992).

Tokur *et al.* (2005) have carried out studies on proximate composition of fish fingers prepared from mirror carp (*Cyprinus carpio L, 1958*). The moisture, crude protein, lipid and crude ash contents of fish fingers from unwashed fish mince was found to be 68.50%, 15.5%, 6.00% and 2.20% respectively. Sayar (2001) has reported similar results for fish fingers from hake fillet (*Merlangus merlangus*). In fish fingers produced from washed mince of mirror carp, the moisture, crude protein, lipid and crude ash contents were found to be 70.23%, 10.8%, 2.14% and 1.80% respectively, where lipid content has decreased significantly as a result of washing treatment. Similarly, a decreasing effect of washing treatment on proximate analysis parameters was established by Biscatchin-gryschek *et al.* (2001), Park and Lee (1996) and Adu *et al* (1983).

The fat content in fish fluctuates over a wide range depending upon the season, a definitive quantification is not cited anywhere, but fish are often classified according to their fat content. Lean fish have less than 0.5% fat, semi-fatty fish contains 0.5 – 2% fat and fatty fish have more than 2% fat (Clucas, 1981). Among the many different species of fish, normally lean fish is used for preparation of minced meat.

2.5.1 Protein changes

Protein deterioration is a major problem during freezing and frozen storage of fishes and shell fishes and has therefore been a subject of much research and numerous review articles (Connell, 1964; Sikorski *et al.,* 1976; Matsumoto, 1979; Shenouda, 1980). Sikorski *et al.*(1976) have defined protein denaturation as a "complex phenomenon involving alteration of the secondary and tertiary structures of protein due to the breakage of the bond, that contribute to the stability of the native protein conformation without rupture of the covalent linkage between carbon atoms in the polypeptide chain".

The changes occurring during frozen storage has been studied by Arai (1975). The effect of freezing on protein changes leads to the loss of the ability of fish to regain tissue fluids resulting in drip loss (Aitken *et al.*, 1980; Rengenstein and Rengenstein, 1991). This effect is primarily due to common phenomenon of protein denaturation. There are various factors which influence the protein denaturation, such as effect of ice crystals (Fellows, 1990), binding of fatty acid and lipid oxidation products to proteins, oxidation and interaction of third group and chemical reactions of amino acid residue in the protein with formaldehyde (Love, 1966; Sikorski *et al.*, 1976; Matusmoto, 1979; Shenouda, 1980 and Haard, 1992). The denaturation of protein during frozen storage is due to the aggregation of myofibrillar proteins in which hydrogen bonds, ionic bonds, hydrophobic bonds and disulphide bonds are formed. Protein changes in the frozen and stored croaker with respect to muscle protein were reported by Srikar (1979). As the water is slowly frozen, dehydration occurs resulting in conformational changes and simultaneously

the products of lipid oxidation will react with protein giving more cross linking (Jiang, 1985; Sikorski *et al.,* 1976 and Shenouda, 1980).

Benjakul *et al.* (2003) have carried out studies on physicochemical changes of muscle proteins from some tropical fish during frozen storage. They have concluded that, extended frozen storage caused the denaturation of protein as well as the cell disruption in all species, but the degree of changes was dependent upon species.

Bajakul *et al.* (2000) have carried out studies on physiochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. Leelapongwattana *et al.* (2005), have carried out studies on physiochemical and biochemical changes during frozen storage of minced flesh of lizard flesh. They have concluded that, greater changes in muscle proteins were observed in the samples kept under vacuum than those in air. They have found that, packaging of mixed flesh of lizard flesh, under conditions lacking oxygen, might induce a quality loss during frozen storage. Tokur *et al.* (2004) have carried out studies on quality changes of tilapia burger during frozen storage.

2.5.2 Salt soluble protein

Fukuda *et al.* (1981) reported the denaturation of myofibrillar protein in deep sea fish muscle by freezing and frozen storage Borderais *et al.* (1982) studied the changes in trout (*S. iredeus*) muscle during frozen storage in different styles viz, whole, fillets and minced meat. They also observed that salt soluble proteins obtained after 225 days of storage were 45% in mince and 60% in fillets and whole fish. Radhakrishnan *et al.* (1982) observed a decrease in myofibrillar protein fraction with increase in storage period in oil sardine and mackerel. Joseph and Perigreen (1983) observed a considerable decrease in solubility during the first 14 weeks of storage followed by a relatively constant decrease during further storage in the threadfin bream. Oshima *et al.* (1984) reported a definite negative interrelation between the FFA content and the salt soluble protein extractability. Doong (1988) found a

clear correlation between myofibrillar protein solubility and the change in ice crystal size.

During frozen storage, the increase in PV, FFA and TBA correlated significantly with the decrease in SSP (Reddy ad Srikar, 1996). According to Sarma *et al.* (1998), lipid oxidized and hydrolyzed products had an equally adverse impact on protein solubility in case of sardine.. Similar conclusions were drawn regarding the decrease in the salt soluble protein fractions during the period of frozen storage by several scientists in different fish species (Namulemo *et al.*, 1999; Suvanich *et al.*, 2000; Verma and Srikar 1994; Ahmed *et al.*, 1989; Agarwal *et al.*, 1986; Wang *et al.*, 1994 and Sarma *et al.*, 1994).

Lizard fish (*Saurida spp.*) have been considered as a potential raw material for high grade paste products due to their high gel forming ability. However Benjakul *et al.* (2005) have reported that gel forming ability of the fish decreased rapidly during post-harvest handling. This was associated with proteolysis as well as formation of FFA.

2.5.3 Non-protein nitrogen

Simudu (1962) has discussed the importance of non-protein nitrogenous compounds in fish, crustaceans and molluscs. The contribution of non-protein nitrogenous matter to taste, variations between different groups of fishes and animals, seasonal changes etc, also have been dealt with though it is present in small quantities which play a key role in metabolic process of aquatic animals and their spoilage, besides contributing to flavor (Zaitsev *et al.*, 1969).

Webb *et al.*(1976) have shown that, during mechanical separation of croaker muscle tissue, there is an increase in NPN values compared to hand separated meat and the explanation given for this is due to incorporation of more free amino acids associated with skin and possibly bone marrow by mechanical separator. On the contrary a decrease in NPN content was observed in minced meat from ribbon fish by Badonia and Devdasan (1980), in clam meat by Mishra and Srikar (1989) and in pink perch meat by Reddy

(1988) during frozen storage. Sarma (1992) have reported a decline in NPN content in pink perch during frozen storage. Tokur *et al.* (2005) have observed an appreciable increase in the NPN values in the early stages of frozen storage followed by a gradual decrease as the period of storage increases.

2.6 Lipid quality

Fish fats are characterized by their high levels of long chain polyunsaturated fatty acids (Ackmen *et al.*, 1976) which are highly susceptible to enzyme hydrolysis and non-enzyme oxidation. Dawson and Prince (1979) and Ghadgi (1977) described that mincing process accelerated these reactions through physical surface effects and through dispersion of catalytic contaminants. Wu (1976) has reported the causes and preventive measures of rancidity of frozen fish during storage in a review.

During cold storage of fish products, mono and polyunsaturated fatty acids are autooxidised in the presence of oxygen to hydro peroxides through radical chain mechanism. Hydro peroxides in turn decompose to volatile aldehydes, ketones and acids which are responsible for rancid odors (Watts, 1961; Banks and Hardy, 1965). Kurode and Baranowske (1987) predicted the shelf life of frozen minced flesh in terms of oxidative rancidity using TBA number. In general, measurements of both peroxide value and malanoldehyde content can be of help in objectively assessing the progress of rancidity of lipid in frozen fish muscle.

Effect of freezing and thawing on the auto oxidation of blue fin tuna myoglobin was studied by Chow *et al.* (1985). Effect of maillard reaction products on the stability of minced herring in frozen storage was studied by Beckel *et al.*(1985). Results showed that Maillard reaction products did not significantly inhibit oxidation at any time during entire period of storage. Hsih and Kingsella (1986) studied the lipoxygenase catalyzed oxidation of N-6 and N-3 polyunsaturated fatty acids. Influence of atmospheric oxygen and freshness on the oxidation of fish lipids during their extraction was studied by Watanabe *et al.* (1986).

Acceleration of lipid oxidation in the skin of fish by the disintegration of connective tissue was reported by Yamaguchi *et al.*(1984). Effect of freezing and thawing on the auto oxidation of blue fin tuna myoglobin was studied by Chow et *al.* (1985). There is an influence of storage time and temperature on lipid deterioration in case of haddock and cod (Aubourg and Medina, 1999). Undeland and Lingert (1999) noticed the influence of pre-freezing on the lipid oxidation in fillets of herring during frozen storage. Richards *et al.* (2002) have reported about studies on contributions of blood and blood components to lipid oxidation in fish muscle.

Lipid peroxidation and chemical changes in cat fish muscle was studied by Eun-Jong-Bang *et al.* (1994). Miki *et al.* (1994) studied the relation between protein denaturation and lipid oxidation in the fish muscle during frozen storage and reported that both phenomena appeared to occur independently. Fernandez *et al.* (1995) have studied the change in lipids of whole and minced ray fish muscle during frozen storage and reported that mincing hastened the hydrolytic and oxidative process which was slowed down at the lower storage temperature. Reddy and Srikar (1996) have reported that lipid parameters and SSP are useful for determining the stability of mince during frozen storage. Benjakul *et al.* (2005) have carried out studies on lipid oxidation and influence of oxidized products in whole lizard fish during frozen storage.

2.6.1 Free Fatty Acids (FFA)

Hydrolysis of lipids is a common post mortem feature in fish and shell fish products. In recent years attention has been focused mainly on the interactions of the fatty acids formed as a result of lipolysis with protein thereby affecting the textural qualities (Sikorski *et al.,* 1976). Hydrolysis of both triglycerides and phospholipids proceeds with the liberation of FFA.

FFA and melanoldehyde formed during storage are known to increase protein cross linking reactions leading to loss of water holding capacity and textural changes (Sikorski *et al.*, 1976). Effect of accumulated free fatty acid on reduction of salt soluble protein of cod flesh during frozen storage was

described by Oshima *et al.* (1984). Joseph and Perigreen (1983) reported that the rate of change of PV and FFA were low in the early stages of storage, followed by an increased rate of a maximum value and then a decrease. Reddy and Srikar (1996) observed a significant correlation between increase in PV, FFA, and TBA with the decrease in SSP during frozen storage.

Tokur *et al.* (2005) have carried out studies on the effect of lipid oxidation products during frozen storage of fishery products. The TBA value has been used by them to indicate the degree of lipid oxidation. In their study, they found the TBA values increased significantly during frozen storage. The increasing of TBA value during frozen storage has been demonstrated by Chuapoehuk and Raksa-Kulthai (1982) for fish fingers (made from lizard fish, thread fin bream and barracuda), by Cakli *et al.*(2004) for fish fingers made from (*T. kinca*) by Yanar and Fenercioglu (1998) for fish balls made from carp. Tokur *et al.* (2004) have also reported an increase in TBA value for fish burgers made from tilapia during frozen storage. Undeland *et al.* (2003) have found that the development of lipid oxidation products was faster in washed cool muscle than in unwashed cool mince within 4 days.

Several attempts have been made by many workers on the frozen storage life of different fish species with regard to free fatty acid content (Balasundari *et al.*, 1997; Reddy and Srikar, 1996; Xue-Changhu *et al.*, 1995; Suvanich *et al.,* 2000; Paavar *et al.*, 2002; Tokur *et al.*, 2004 and Undeland *et al.*, 2003).

2.6.2 Peroxide value

Peroxide value is a measure of extent of oxidation of lipids and has been used as criteria for measuring the freshness of fish and shell fishes. Oxidative rancidity is the major limiting factor for the storage life of fish and shell fish products and is a major cause for the off-flavor and odor deterioration in fish. Greig (1968) observed high positive correlation between rancidity scores of last panels and peroxides and also TBA values of frozen fish. Even though PV increases during storage of common murrel, rancidity flavor and odor could not be detected by the panel members during storage

(Perigreen *et al.*, 1988). Undeland and Lingert (1999) reported that ice storage has a greater effect than frozen storage on changes in PV. A significant difference between PV, FFA and TBA was observed by Simeonidou *et al.* (1997). Verma and Srikar (1994) have noticed an inverse correlation between SSP and PV. Informed opinion considers rancidity a major cause of flavor deterioration in frozen stored fish (Bilinski *et al.*, 1979). A good correlation between peroxide value and organoleptic quality is often observed which greatly depends on the fish species and storage condition.

Similar observations where an increase in peroxide value during frozen storage were obtained from the study of different scientists in different fish species (Lee., 1989; Wang *et al.,* Ahmed *et al.,* 1989 Joseph *et al.*, 1992; and Reddy *et al.,* 1992 Miki *et al.,* 1994; Park and 1994; Sarma *et al.,* 1994; Sharma *et al.*, 1994 Reddy and Srikar, 1996.,; Balasundari *et al.*, 1997; Aubourg and Medina, 1999;).

2.6.3 Nitrogenous constituents (TMA-N and TVB-N)

TMA-O present in fish gets readily degraded during postmortem to TMA and FA by microorganisms or endogenous systems. A measure of ammonia, TMA and DMA has been used to determine early stages of spoilage (Whittofgel, 1958) whereas formal titration, bacterial count and pH were unsatisfactory.

Jendrush (1967) suggested TVB-N content in combination with sensory qualities can be used for assessing quality of frozen fish. Reddy *et al.* (1992) found that protein solubility (PS) and TVB-N content in frozen stored meat were inversely related as TVB-N increased with storage period. Jebsen and Riaz have reported the prefreezing period as well as storage of fillets with skin enhanced the formation of DMA and FA from TMA-O. An inverse correlation has been observed between SSP and VBN and TMA by Verma and Srikar (1994). Several research workers have reported an increase in TVB-N and TMA-N in different fish species during frozen storage (Suvanich *et al.*, 2000; Sarma *et al.*, 1998; Simeonidou *et al.,* 1997; Balasundari *et al.*, 1997; Orlick *et al.,* 1991; Wang-shu-Jen *et al.*, 1994 and Ciarlo *et al.,* 1985). Tokur *et al.*

(2005) during their studies conducted on biochemical analysis of fishery products have reported that protein solubility in mince did significantly decrease from 60.7% to 27.9% throughout frozen storage. Similarly numerous reporters have reported an increase in TVB-N during frozen storage of fish mince (Benjakul *et al.*, 2005).

The speed of TMA-0 degradation depends upon many factors, such as storage temperature, species, muscle integrity and reducing conditions (Parkin and Hulkin, 1982). Kidney of lizard fish contain high amount of TMAOase, which can contaminate fish muscle during processing (Benjakul *et al.,* 2004). The absence of oxygen has also been shown to accelerate the rate of DMA and FA production. FA accelerates the formation of high molecular weight polymers from isolated myosin and actomyosin during freezing and frozen storage.

2.6.4 Change in pH

During frozen storage, both an increase as well as decrease in pH has been recorded. According to Poulter (1977) there is an increase in pH of cod, whiting sole and skate muscle during frozen storage. Curran *et al.* (1986) made a similar observation in Bolivian fish. In addition to these, several scientists (Simeonidou *et al.*, 1997; Park and Lee, 1989 and Ciarlo *et al.,* 1985) have also found an increase in pH of different fish species stored in frozen storage.

Suvanich *et al.* (2000) observed no change in pH of channel cat fish mince. Tokur *et al.* (2005) have recorded pH values during frozen storage of fishery products. They have found that, initially during the storage period the pH values have not increased. But a sharp increase has been reported at the beginning of 4th month onwards. They have noted that when the pH increased, the TBA-values decreased during the 4th month and that the TBA values increased while pH decreased in the fifth month. It has been observed that hemoglobin (Hb) can show strong pro-oxidant activity for some species between pH 6 and pH 7 and it can retard oxidation at pH values above 7 (Richard and Hultin, 2002; Tokur *et al.,* 2004).

2.7 Microbiological quality.

During freezing and frozen storage some bacteria get destroyed, but the speed with which the bacteria get destroyed varies (Green, 1949). Wesier and Ostercud (1945) have found that there is an immediate death caused by freezing and a storage death is a direct function of time and temperature of storage. Freezing causes destruction of 60-90% bacterial population of pure culture (Kiser and Beckwith, 1942; Pivinick, 1949). During storage a further fall in bacterial number occurs, exponentially for the first period, then a more gradual decline.

Gunderson (1956) and Humber *et al.* (1958) have reported that a total plate count standard of less than 10,000 bacteria per gram should be easily met by any producer. These authors stressed the importance of sanitary provision in the quality assurance programmed and the importance of starting with good quality raw materials to ensure a finished product with a low bacterial count.

Vandenbruck *et al.* (1984) studied on the microbiological quality of retail fish fillets. Verma *et al.* (1986) have studied the bacteriological quality of frozen seafoods for export with special reference to salmonella.

Balasundari *et al.* (1997) have studied the bacterial quality of farm grown oyster at harvest and after processing by various methods such as depuration, shucking, antioxidant treatments, freezing and frozen storage at -18°C. Verma *et al.* (1986) have given the incubation temperature for total bacterial count of frozen sea foods. They concluded that incubation at ambient temperature (37°C) gave better results than at 5-10°C. Wang-Shu-Jen *et al.* (1994), Rebach *et al* .(1990) and Eyo (1989) recorded a decrease in bacterial count during frozen storage in different fishes. Tokur *et al.* (2005) have carried out studies on microbiological quality of frozen battered and breaded products. Sehgal and Sehgal (2001) also have reported on microbiological quality of some value added products prepared from fishes. Cakli *et al.* (2004) have carried out studies on the microbiological quality of fish fingers from different fish species.

2.8 Fry bath practices

Hoiston (1955) determined the effect of cooking time and oil temperature on the shade of color. According to him, recommended temperatures for the fry bath vary from 350°c to 390°c. A fry bath temperature of 375 (190°C) is common (Robertson, 1953).

Quammen (1955) lists the six common "trouble factors" encountered in frying food as following:

a. Excessive heat
b. Metallic contamination
c. Soap and caustic residues left after cleaning equipment.
d. Burnt food particles
e. Inadequate filtering and
f. Insufficient turnover of frying fat.

The author also indicated that higher temperatures speed up colour and free fatty acid development. Prevention of development of FFA formation is important, if a long storage life of the product is required.

2.9 Organoleptic evaluation

Organoleptic evaluation is a very important method in determining the acceptability of all food products. Govindan (1972) and Iyer (1972) documented that it is also a most reliable method for evaluation of the freshness of raw and processed fishery products which exhibit their own characteristic flavor and aroma which are mostly complex in nature. These characteristics cannot be analyzed totally by any of the objective method developed so far. Farber (1965) developed numerical scoring system for the sensory assessment of freshness.

Sensory evaluation panel can be a precise tool of assessing the quality of fish if tests are designed properly and trained personnel are selected with a meaningful statistical analysis (Kramer, 1952).

The relation between sensory and chemical tests has been discussed by Jenson (1956). Several investigators have reported a favorable correlation

between sensory judgement and amino nitrogen as a measure of early fish spoilage (Farber, 1965). A good correlation was observed between chemical and microbiological parameters and organoleptic evaluation in determining the refrigerated shelf life of croaker, white trout, Spanish mackerel and King mackerel. Farber (1965) strongly feels that the line dividing fish that are still fresh from those with some early signs of spoilage is not very well defined and is most often subject to difference in personal opinion.

Some feel that objective tests should be used only to confirm taste panel findings and should not themselves be used to determine acceptability (Antoacapolous, 1969). Taste panel methods do not always differentiate clearly between the changes occurring in iced fish and those occurring in frozen fish (Baines *et al.*, 1989). There are drawbacks, however, because experienced people are required and this experience takes a long time to acquire, though the period can be shortened by formal training. Reddy and Srikar (1996) have reported that lipid parameter and SSP influenced the quality of thread-fin bream mince with respect to flavour scoring.

Materials and Methods

III MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

Minced meat of Big eye (*Priacanthus hamrur*) locally known as 'disco fish' was used in the preparation of plain as well as battered and breaded fish cakes. The fishes were collected from fish landing center in Mangalore and brought in iced condition to the laboratory. The fishes were then washed in clean water, dressed and washed again in chilled water and stored at 5°C temperatures till next use. .

3.1.2 Subsidiary materials

The subsidiary materials used in preparation of fish cakes were corn flour, chilli powder, pepper powder, coriander powder, garam masala, salt, garlic paste, ginger paste, onion paste, bread crumbs, maida flour, oil and water. All these materials were obtained from local market.

3.1.3 Equipments

Fish meat picking machine, model: SG, Type: I supplied by Tokyo Seikan Kasha limited, Japan, was used for separation of meat from bone and skin. Meat mincer, chopper, Model M- 3, Type: 42 supplied by the same company was used to reduce the size of the picked meat of fish, with disc having perforations of 1/8" diameter.

3.1.4 Packaging material

Polyethylene bags (200 gauge) were used for packing the products.

3.1.5 Chemicals

The chemicals used in the analysis were either analytical reagent or laboratory reagent grade of BDH make. Hi- media plate count agar was used for estimating total plate count of bacteria.

3.2 Methods

3.2.1 Preparation of minced fish meat.

Big eye (*Priacanthus hamrur*) brought in fresh and iced condition from landing center was washed thoroughly in fresh water in the laboratory. Then the fishes were dressed and washed hygienically. After dressing and washing, the meat was separated from bone and skin by using meat picking machine. Then the picked meat was minced using meat mincer.

3.2.2 Preparation of plain fish cakes

The minced meat obtained from fish was thoroughly mixed with a standardized proportion of corn flour. Then calculated quantities of subsidiary ingredients such as salt, chilli powder, pepper powder, garam masala, water, ginger paste, garlic paste, onion paste was added to the minced meat and mixed thoroughly. The prepared paste was moulded into a shape of fish cake having thickness of nearly 1cm and dimension of 5cm X 4cm.These fish cakes were frozen using air blast freezer at - 40 ° C. Frozen fish cakes were packed in Polyethylene bags and kept in cold storage at- 20 ° C(Fig. 1).

3.2.3 Preparation of battered and breaded fish cakes

The minced meat obtained was thoroughly mixed with subsidiary ingredients such as corn flour, chilli powder, pepper powder, garam masala, onion paste, ginger paste, garlic paste, salt and water, to obtain a paste of desired consistency. Then prepared paste was moulded into the shape of fish cakes having 1 cm thickness and dimension of 5 cm X 4 cm (LXB) using suitable moulds. These fish cakes were dipped in batter solution of maida flour and rolled on bread crumbs. Then the fish cakes were frozen in air blast freezer at -40 ° C. After freezing, the frozen cakes were packed in polythene bags and stored in cold storage at - 20 ° C (Fig .2).

3.3 Standardization of recipe

By adopting a standard batter mix formulate and a basic formulae of fish fingers and fish sticks (Reddy,1989) the starting recipe was formulated for plain as well as battered and breaded fish cakes,(Table.3 and Table 4) . In the present study suitable recipe was found out by using varying levels of different ingredients such as corn flour, rice flour, gram flour, mixed meat, salt, garam masala, pepper powder and bread crumbs (Table 7and Table 8). The effect of these variations on organoleptic attributes like colour, texture, flavour, appearance, taste, odour and overall acceptability were determined using trained panelists. The prepared products were presented to taste panelists after frying in refined groundnut oil at 140 – 150 °C for 4 minutes till a golden brown colour had developed. A sample score sheet provided to the panelists showing the sensory scores for various attributes is given in Appendix – I.

3.4 Storage Studies

The plain fish cakes and battered and breaded fish cakes were examined for quality changes during frozen storage by means of chemical, microbiological and sensory evaluation methods at a regular interval of 1 month.

FIG 2. Preparation of battered and breaded fish cakes

Fresh fish minced meat

↓

Mixing with corn starch (flour), salt, chilli powder, pepper powder, garam masala, garlic, onion and ginger paste and water.

↓

Shaping into rectangular shape (using moulds)

↓

Dipping in batter solution of maida flour

↓

Breading

↓

Freezing at - 40° C

↓

Packing in polyethylene bags.

↓

Storage at - 20° C.

FIG 1. Preparation of Plain Cakes

Fresh fish minced meat

↓

Mixing with corn starch (flour), salt, pepper powder, garam masala, chilly powder, garlic, onion, ginger paste and water

↓

Shaping into rectangular shape (using moulds)

↓

Freezing at - 40° C

↓

Packing in Polyethylene bags.

↓

Storage at - 20° C

3.5 Analytical methods

3.5.1. Chemical parameters

3.5.1.1 Moisture

Moisture was determined following the standard hot air oven method of AOAC (1995). About 5.0 grams of fish meat taken in a moisture bottle was dried in a hot air oven maintained at 100 ± 2°C for 16-18 hrs and desiccated to a constant weight. The weight lost during the drying process is expressed as moisture per 100gram of sample.

$$\% \text{ Solid} = \frac{\textit{Wt. of solids (after drying)}}{\textit{Wt. of sample (before drying)}}$$

% Moisture = 100 - % solid

3.5.1.2 Crude protein:

The total nitrogen was estimated by Kjeldahl method as per AOAC (1995). About one gram of meat sample was transferred to 250ml of digestion flask and 10 to 12 ml of sulfuric acid was added with 0.2gram of digestion mixture and digested on a digestion chamber till a clear digest was obtained. After cooling the volume was made up to 100ml with distilled water. 5ml of solution was taken for distillation in Kjeldahl distillation unit. The liberated ammonia was absorbed in 2% boric acid solution containing mixed indicator (2% methyl red and 2% methyline blue in 1:1 ratio dissolved in ethyl alcohol), the pink colour of boric acid solution turns to green. Then the boric acid was titrated against N / 140 standard hydrochloric acid until a pink colour end point was obtained.

$$\textbf{Total nitrogen \% =} \frac{\textit{14/1000 Normality of 1/140 N HCl x (Volume run down of 1/140 N HCl – Blank) x 100 x100}}{\textit{5X wt. of the sample}}$$

Crude protein = 6.25 x *total nitrogen*

3.5.1.3 Total Lipid

Lipid extraction

The total lipid from the fish meat sample was extracted according to the procedure of Bligh and Dyer (1959). 50gm of minced fish meat sample was taken and 80ml of chloroform, 160ml of methanol were added, homogenized for 2 minutes and filtered. To the residue further 80ml of chloroform was added and homogenized for 30 seconds and filtered. The lipid extract was separated by using Buchner Funnel filtration with suction, and the lipid extract was separated using separating flask. Total amount of lipid extract was measured using stoppered flask measuring cylinder. Aliquots (25ml) of lipid extract were evaporated to dryness under vacuum and the weight of the lipid determined. Evaporation was facilitated by drying under vacuum in a water bath maintained at 40-50°c. The total lipid of fish meat sample was calculated as

$$\textbf{Total lipid (\%)} = \frac{\textit{Wt of lipid in aliquot x total volume of } CHCl_3 \textit{ extract x100}}{\textit{Volume of aliquot x Wt. of meat used for extraction}}$$

3.5.1.4 Ash

The total ash content of the product was determined by heating an incinerated sample in a muffle furnace at 550 to 600°c for about 5 to 6 hours as per AOAC (1995), and expressed as percentage of the sample.

3.5.1.5 pH

pH of the sample was measured by the method described by Suzuki (1981). About 5gm of sample was blended with 45ml of distilled water and pH of the homogenate was measured by pH meter.

3.6 Determination of lipid quality

3.6.1 Free Fatty Acid (FFA):

The free fatty acid (FFA) content in the lipid extract was determined by the improved titrimetric method of Ke *et al.*(1976) as described by Takagi *et al* .(1984). To 8ml chloroform extract, 4ml of methanol and 8ml of isopropanal (Chloroform; Methanol; Isopropanol = 2:1:2) were added and mixed in a 100ml conical flask. 3 drops of 0.5% metacresol purple was added as a indicator. The FFA was titrated to purple end point with 0.05N aqueous sodium hydroxide. The percentage of free fatty acid was calculated (as oleic acid percentage) as follows.

$$\textbf{FFA (\%)} = \frac{(\textit{Titre value of sample} - \textit{Titre value of blank}) \times \textit{N of NaOH} \times 28.2}{\textit{Wt. of sample}}$$

$$\textbf{Percentage of FFA} = \frac{\textit{Acid value}}{1.99\ (\textit{Oleic acid})}$$

3.6.2 Peroxide value (PV):

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

$$\textbf{PV} = \frac{1000 \times (\textit{Titte value of sample} - \textit{T.V of blank}) \times \textit{Normalityof } Na_2S_2O_3}{\textit{Wt. of lipid}}$$

3.7 Determination of nitrogenous constituents

Preparation of Trichloro acetic acid (TCA) extract.

About 10gm of fish meat was homogenized with 10ml of 20% TCA for 3 minutes in a tissue homogenizer. The homogenate was filtered through Whatman No.4 filter paper and made up to 100ml. The TCA extract was used for the estimation of TVB – N and TMA – N.

3.7.1 TVB – N and TMA – N:

1ml of the above TCA extract was used for the estimation of TVB – N and TMA- N by the procedure of Beatty and Gibbons (1937) using Conway's micro diffusion technique. 2ml of boric acid containing mixed indicator into the inner chamber and 1ml of sample into the outer chamber of Conway's unit were added. The unit was covered with greased cover glass of the unit leaving a little gap for adding K_2CO_3. Immediately, 1ml of K_2CO_3 was added to outer chamber as quickly as possible and the lid was closed and kept for incubation at 37°C for 90 minutes after mixing the contents gently. After absorption, cover was removed and titrated the contents inside the chamber against 0.02N H_2SO_4 kept in micro burette until a pale pink colour appeared as the end point. For TMA – N estimation 1ml of formaldehyde was used to trap the ammonia, before the addition of K_2CO_3. The results were expressed as mg/100gm of sample.

$$\textbf{TMA – N/TVB-N (mg \%)} = \frac{14 \times N \text{ of standard acid} \times (\text{Titre value of sample} - \text{TV of blank}) \times 100 \times 100}{\text{Wt. of sample} \times 1}$$

3.7.2 Alpha Amino Nitrogen

AAN in the sample was estimated following the copper method of Pope and Stevens (1939) using TCA extract. The amino acids react with excess copper present in the form of copper phosphate from soluble copper compounds and the amount of copper taken in to the solution by amino acids

is determined iodometrically. The AAN content is expressed as mg/100 gm of sample.

3.7.3 Non protein nitrogen (NPN)

About 10gm of the sample was homogenized with 50ml of 10% TCA for 3 minutes in a tissue homogenizer. The homogenate was filtered through Whatman No. 1 filter paper and made up to 100ml with distilled water. 5ml of TCA extract was digested by Micro – Kjeldahl method. The digested sample was used for estimation of NPN by Kjeldahl distillation procedure.

$$\textbf{SSP or NPN (mg \%)} = \frac{N x V x 100 x\ 100 x\ 1000}{W x 5 x 5 x 1000}$$

3.8 Determination of protein quality

Quality of proteins was determined by estimating the salt soluble protein and non protein nitrogen using Kjeldahl method (AOAC,1995).

3.8.1 Salt soluble protein (SSP)

Salt soluble protein was estimated by the method of Dyer *et al* (1950). 5gm of minced fish meat was homogenized at 0°C to 5°C in a tissue homogenizer for 3 minutes using chilled 5% sodium chloride solution, buffered with 0.02M by using 0.1N HCl. The total volume of the homogenate was adjusted to 100ml taking into account the moisture content of the sample. Salt soluble protein was determined by Kjeldahl AOAC (1995).

3.9 Microbiological analysis

All the products were tested for total plate count. Appropriate dilutions of homogenate were made in physiological saline (0.85%) and plated in duplicates on to plate count agar by pour plate method. Incubation was done plate count agar by pour plate method. Incubation was done at room temperature for 24 – 48 hours.(A.P.H.A).

3.9.1 Sensory Evaluation

Prepared fish products were fried at 140- 150 °C for 4 minutes and assessed by trained panelists based on 9 point hedonic scale for appearance colour, texture, flavour, taste, odour and overall acceptability (Appendix-1).

3.9.2 Statistical Analysis

The data was analyzed statistically using analysis of variance and correlation of coefficient techniques wherever necessary (Mahony, 1986).

Experimental Results

IV EXPERIMETNAL RESULTS

The fish used in the present study was big eye (*Priacanthus hamrur*), which is primarily a demersal fish. The dressed fish was analyzed for proximate composition, various biochemical, microbiological and sensory characteristics. The picked and minced meat of this fish was used for the preparation of plain cakes and battered and breaded fish cakes. The quality characteristics of the products were analyzed by physical tests and sensory evaluation. The results obtained are presented in the following sections

4.1 Raw material Characteristics of fresh fish

4.1.1 Physical characteristics

The fish used in the present study had an average total length of 22.5 ± 2.36 cm with an average standard length of 17.67 ± 1.87 cm and an average total weight of 156.78 ± 3.68 gms (Table 1).

4.1.2 Yield at different stages of processing

The yield percentage of fish at various stages of processing is presented in Table.1. The percentage yield of picked meat meat was 39 %and minced meat was 29.52 %based on dressed fish weight (Table1).

4.1.3 Proximate composition of fish meat

The fish is a lean variety with low fat content (total lipid 1.28 %) and high protein content of 19.31 ± 0.63%. Moisture and ash contents of the fresh fish were 77.52 ± 0.4 % and 1.43 ± 0.21 % respectively (Table.2).

4.1.4 Biochemical quality of fresh fish

The chemical characteristics of the raw material are given in Table 2. The Total volatile base nitrogen (TVB – N) and Trimethyl Amine (TMA – N) of fresh *Priacanthus hamrurur* were found to be 6.23 mg% and 2.87 mg % respectively. The AAN content of the fresh fish is found to be 13.42 mg %. The pH of the fish meat was 6.23. The salt soluble protein (SSP) and non protein nitrogen (NPN) of the fish were 13.13 gm / 100gm of meat and 321.23 mg/100 gm of meat of meat respectively on wet weight basis. Peroxide value (PV) and Free fatty acid (FFA) contents were 15.34 milliequivalents of O_2/ kg of fat and 3.36 % of oleic acid respectively.

4.2 Microbiological characteristics

Total plate count (TPC) in the fresh fish is shown in Table 2 and was found to be 6.43 x 10^4 cfu / gm of meat.

4.3 Standardization of recipe

In the present experiments suitable variations in the type of composition and quantity of ingredients to be added were worked out, to develop a basic formula for plain fish cakes and battered and breaded fish cakes (Table.3& 4). Along with trying out different recipe, studies on effect of using different kinds of starches (Table. 5 & 6) on overall acceptability of the product were also carried out. Based on the highest average score of texture, flavour, taste and overall acceptability obtained for different starches, the product where corn flour was used as starch was selected for further experiments. After selecting the kind of starch to be used in the product, variations in the levels or quantities of ingredients to be added and their influence on overall acceptability of the product was worked out.

Table 7 and 8 gives the standardization of different recipe for plain fish cake and battered and breaded fish cakes respectively. The sensory scores

for plain fish cakes of three different recipes (I to III) are shown in Table 9 and the average overall acceptability score obtained was 7, 8.8 and 7.6.

Similarly sensory scores for battered and breaded fish cakes of three different recipes (I to III) are shown in Table 10 and the average overall acceptability scores obtained was 8, 8.5 and 7.5 respectively. Based on the highest average score for texture, flavour , taste and overall acceptability, IInd (Table.7) recipe for plain fish cakes and IInd recipe of (Table.8) for battered and breaded fish cakes were selected and the modified formula were further used in the preparation of plain fish cakes and battered and breaded fish cakes . The standardization of battering mixture for drip treatment of battered and breaded fish cakes was carried out using gram flour, Maida flour and rice flour (Table.11) out of which maida flour was selected for further processing based on organoleptic scores.

4.4 Changes during frozen storage.

4.4.1 Proximate Composition

Changes in proximate composition such as moisture, protein fat and ash content of the prepared products like plain fish cakes and battered and breaded fish cakes, before and at the end of cold storage is given in Table - 12.

4.4.2 Peroxide Value

The peroxide value for plain fish cakes and battered and breaded fish cakes are presented in Table 13 and Fig 4a.

The initial peroxide value of plain fish cakes and battered and breaded fish cakes were 13.20 and 12.80 milliequivalents of oxygen per kg of fat respectively. At the end of 5th month of storage period, plain fish cakes and battered and breaded fish cakes showed a maximum value of 39.48 and 33.43 milliequivalents of oxygen per kg of fat.

Fig 4a. shows the variation in peroxide value of plain fish cakes and battered and breaded fish cakes, stored at – 20 ° C for 5 months

4.4.3 FFA

The FFA content of plain fish cakes and battered and breaded fish cakes stored at 20 ° C is presented in Table 14 and Fig 4b. The initial FFA value of plain fish cakes and battered and breaded fish cakes was 3.06 and 3.43 as percent of oleic acid respectively. The FFA value moderately increased during the storage, in both the samples.

FFA values at the end of cold storage period of plain fish cakes and battered and breaded fish cakes were 9.43 and 7.43 as percent of oleic acid.

Fig 4b. indicates that FFA values were slightly low for battered and breaded fish cakes sample throughout the period of study. Gradual increasing trend of FFA value can be seen in both the samples.

4.5 Changes in nitrogenous constituents during frozen storage of the prepared product.)

4.5.1 TVB-N

The variation in TVB-N in prepared products during storage is presented in Table 16 and Figure 6a.At the beginning of storage, the TVB-N content for plain fish cakes and battered and breaded fish cakes were 8.32mg % and 8.43 mg %. At the end of the 5 months of storage period, the value of TVB-N for plain fish cakes and battered and breaded fish cakes had increased to 41.68 mg % and 39.78 mg%.

4.5.2 AAN

Alpha amino nitrogen is the protein quality parameter studied during frozen storage (Table 15 & Fig 6 b) .The AAN content initially before the commencement of frozen storage for plain fish cakes and battered and breaded fish cakes were 15.34 mg % and 19.16 % respectively. This increased to 31.23 mg % and 36.34 mg % respectively at the end of 5th month of storage.

4.6 Changes in protein quality parameters of prepared products during frozen storage.

4.6.1 SSP

The SSN content of prepared products before cold storage was 12.26 gm/100 gm of meat (plain fish cakes) and 13.73 gm/100 gm of meat (battered and breaded fish cakes) .After the end of cold storage period of 5 months the SSN values had decreased to 8.78 gm /100 gm of meat (plain fish cakes) and 7.85 gm /100gm of meat (battered and breaded fish cakes) respectively (Table 18.and 7 a).

4.6.2 NPN

Non protein nitrogen is the protein quality parameter studied during frozen storage (Table-17 & Fig 7 b) The NPN content, initially before the commencement of frozen storage for plain fish cakes and battered and breaded fish cakes were 1.56 gm/100gm of meat and 1.34 gm/100 gm of meat respectively. This decreased to 0.73 gm /100 gm of meat and 0.76 gm /100 gm of meat respectively at the end of 5th month of storage

4.7 Changes in Total plate count (TPC) of prepared products during frozen storage.

Initially TPC of the bacteria for plain fish cakes and battered and breaded fish cakes was 5.43×10^4 cfu/gm of meat and 6.23×10^4 cfu/gm respectively and finally at the end of storage period it was 2.23×10^4 cfu / gm of meat and 2.12×10^4 cfu/ gm of meat respectively (Table 19 and Fig 5 b).

4.7.1 pH

There was not much change in the pH of the prepared products. pH at the commencement of storage for plain fish cakes as well as battered and breaded products was 6.21 and 6.28 and at the end of the 5th month of storage the pH values of plain fish cakes and battered and breaded fish cakes increased to 6.47 and 6.51 respectively (Table 20 and Fig 5a).

4.8 Sensory Evaluation

Tables 21 and Table 22 shows the organoleptic qualities of two prepared products during cold storage at - 20 ° C for a period of 5 months. For all the prepared products gradual reduction in scores is observed with increase in storage period time.

4.8.1 Statistical results

Statistical interpretation of biochemical and sensory quality of prepared products from big eye fish (*Priacanthus hamrur*) is shown in Table-24. .The results of analysis of variance technique on the mean over all acceptability scores of two products are given in Table- 25. .

There was a significant difference between both the products and a negative co- relation between sensory, protein quality parameters and different biochemical tests. (Table 23 and Table 24).

Correlation between overall acceptability scores and freshness parameters are shown in Table-22. In that sensory scores are decreasing during storage in both the products. From the table it is clear that overall acceptability scores are significantly different due to period and due to different products, tried in the experiment.

Discussion

V. DISCUSSION

5.1 Quality of Raw material

5.1.1 Physical and Sensory quality

The fishes used in present study were of medium size with mean total length of 22.5cms, standard length of 17.67cm and average weight of 156.78gms. The fishes used in the present study have lengths slightly higher, when compared to the study by Dhananjaya *et al.* (1984) and Murthy (1999) who have used fishes having mean length 18.2 cms and 22.02cms and weight of 76.90 and 132.60gms respectively. Whereas the fishes used for studies by Bhatta (2001), had lengths and weights similar to lengths and weights of fishes of present study.

The percentage of debonded meat from dressed fish was about 31%. The picked meat yield of dressed fish was 39% for the same species. Bhatta (2001) obtained 67% dressed yield, 41% picked meat yield and around 32% minced yield. The value obtained for physical characteristics in this study are some what slightly lower when compared to values reported by Bhatta (2001) as the fishes used in the present study were slightly smaller in size.

5.1.2 Chemical parameters

The assessment of freshness of fish can be carried out effectively by determining TVB – N content in the fish. The determination of TVB-N for assessment of freshness of fish is widely used because of its good correlation with sensory changes during storage (Nair *et al.*, 1971). In the present study, the TVB-N content of fresh fish was 6.23mg%. The fishes used by Murthy (1999) and Bhatta (2001) for the preparation of value added products from *Pricanthus hamrur* were also of similar quality with a TVB – N content of 7.4 mg % /100 gm of meat and 5.88 mg % of meat respectively. The marine fishes contain substantial amount of TMAO. So bacterial spoilage of fish can be determined by determining TMA content in the fish, which is the most specific index of bacterial spoilage. The TMA content of the fish used was

2.87mg%, comparable to that of 3.37mg% reported by Murthy (1999) and 2.12mg%, Bhatta (2001). Connell (1975) has stated that a limit of 30-40 mg % of TVB – N and 10-15 mg % of TMA – N can be considered border line for acceptance. The value obtained above indicates that the fishes used in the present study were of good quality.

The changes occurring in lipid, during storage is conventionally used as an index of quality in fish and fishery products (Connel and Hardy, 1981). The PV as an index of oxidative rancidity (Labuja, 1971), was found to be 15.34 milliequivalents of O_2/kgfat. The free fatty acid content as % of oleic acid as an index of hydrolytic rancidity was 3.36%. The PV and FFA content of fresh fish used by Bhatta (2001) were 15.21 milliequivalents of O_2/kgfat and 3.4% of oleic acid which is very close to the values found in the present study. pH of fish was found to be 6.23 showing that fish was in rigor stage. The protein quality parameters i.e. SSP and NPN content of fresh fish were 13.3 mg % and 321.12 mg/100 gm of meat respectively (Table – 2).

5.1.3 Microbiological characteristics

The prevalence of microorganism in freshly caught fish is natural. The fish may contain micro-organisms in areas such as gills, guts and skin. These micro organisms may enter into the product during post harvest handling and processing. Since, microbes being the most important agents of food spoilage, keeping the initial microbial load low are important. Total plate count (No. of cfu/gm meat) of 6.43×10^4 (Table 2B) was observed in fresh fish. Bhatta (2001) has reported initial count of 5.65×10^4 cfu /gm meat.

5.1.4 Proximate composition

Proximate composition of fishes depends on several factors like diet, size, sex, physiological state of fish and ecological conditions (Connell, 1975). The proximate composition of fresh fish (Table 1c) indicates that bull's eye

fish is a lean variety fish having proteins 19.31% and lipid of 1.28 % content. The proximate composition of bull's eye given by Murthy (1999) and Bhatta (2001) were protein content of 18.6% and 19.31%, fat content of 1.38% and 1.28% and ash content of 1.2% and 1.51 % respectively. The results of present investigation on proximate composition agree closely with the above mentioned values given by research workers, considerably.

5.2 Standardisation of recipe

In the development of any convenience products from fish, standardization of process as well as product quality is very much essential. Preliminary experiments were carried out on the desired level of minced meat to be added to the products, on levels of important subsidiary ingredients like starch, water, salt and spice mixture to be added to get a desired taste in the products recipe, suitable to the taste and traditions of our people.

The ingredients used in the present studies were, minced meat, corn starch, water, salt, maida flour (batter material), chilli powder, pepper powder, coriander powder, ginger paste, garlic paste, onion paste and bread crumbs. The effect and characteristics of these ingredients are studied at different levels.

Based on the average taste panel scores and comments (Table 9 & table 10) salt 2%, minced meat 75%, corn starch 10%, water 10%. coriander powder 0.5%, chilly powder 1.5%, pepper powder 0.25%, garam masala 0.25% ginger paste 0.16%, onion paste 0.16% and garlic paste 0.18% were used for preparation of plain fish cakes and battered and breaded fish cakes.

5.3 Storage studies of prepared products from big eye (*Priacanthus hamrur*) fish

Two products namely plain fish cakes and battered and breaded fish cakes were evaluated for quality changes during cold storage up to 5 months.

Both the products were stored at -18°C. The changes in the products quality as indicated by biochemical, sensory and microbiological changes are discussed under six following headings.

I. Changes in proximate composition

II. Changes in nitrogenous constituents

III. Changes in protein quality characteristics

IV. Changes in lipid characteristics

V. Changes in sensory characteristics

VI. Changes in microbiological characteristics

VII. Statistical analysis

5.3.1 Changes in proximate composition during storage

The proximate composition (Table 12) indicates a slight decrease in moisture content in frozen samples with corresponding increase in protein content. Moisture content decreases in all the samples at the end of storage period in this study. These findings are in accordance with the reported findings of Sehgal and. Sehgal (2001), Tokur *et al.* (2004), Cakli *et al.* (2004) during the frozen storage studies of fish fingers and fish burger. The results obtained in the present study agree with the above reports within reasonable limits. After the addition of ingredients to minced fish meat the moisture contents of plain fish cakes and battered and breaded fish cakes were found to be 70.82% and 67.86% respectively. The moisture content of these products at the end of the storage period was 66.43 % for plain fish cakes and 66.98 % for battered and breaded fish cakes.

In the present study the protein content in plain fish cakes and battered and breaded fish cakes before cold storage was 15.73 % and 15.08 % respectively. A small decrease in protein content was observed for all the

samples. At the end of cold storage period of 5 months the protein content of plain fish cakes was 14.87 and that of battered and breaded fish cakes was 14.52. A similar decrease in protein has been observed by Sehgal and Sehgal (2001), Tokur *et al.* (2005) Cakli *et al.* (2004), Maruthi (1989) during frozen storage of battered and breaded products of fish.

Ash content showed a slight increasing trend during the frozen storage of prepared fish products. This may be due to the loss of water soluble inorganic constituents in the products during frozen storage (Kamat *et al.*, 2003).Battered and breaded fish cakes have shown a slight increase in total content than plain fish cakes due to the presence of batter covering and presence of bread crumbs over it.

The total lipid content in both plain fish cakes and battered and breaded fish cakes has increased slightly from initial content of 0.60% (plain fish cakes) and 0.83% (battered and breaded fish cakes) to 1.43 % and 1.54 % respectively.

The remaining percentages of the total proximate analysis are thought to be due to carbohydrate. In general, fishes are known to have low amounts of carbohydrate in their muscle. However, the higher amounts of carbohydrate in both the products may be derived from corn flour (starch) added in both plain fish cakes and battered and breaded fish cakes. In case of latter one it also includes coating materials which may contain carbohydrate rich ingredients such as maida flour (batter) and bread crumbs. This has been confirmed by Sayar (2001) who has found 15.2% carbohydrate levels in fish fingers.

5.3.2 Changes in nitrogenous constituents during storage

5.3.2.1 TVB-N

The changes in nitrogenous constituents such as TVB-N and AAN are shown in Table (16 and 17) and Fig (6a and 6b). The volatile base of nitrogen

indicates the production of ammonia, mono, di and trim ethylamine nitrogen and are found in the common pattern of spoilage. It is generally recommended for assessing the quality of frozen fish. The TVB-N content has shown an increase in both the frozen prepared products. In case of plain fish cakes initially it was 8.32mg% and at the end of 5th month it had increased to 41.68mg% indicating the formation of volatile bases. In case of battered and breaded products the level of T-VBN initially was 8.43 and increased the 39.78 during 5 months of storage indicating the formation of volatile bases. The increase in volatile bases in both the products may be due to enzymatic activity. Mincing has possibly contributed to the high TVB-N values (Connell, 1990). Several workers have observed similar increase in TVB-N of prepared products during storage, (Reddy *et al.*, 1992, Verma and Srikar 1994 and Kamat *et al.*, 2003). Verma and Srikar (1994) have co-related TVB-N content with organoleptic scores and a negative correlation was observed between SSP and TVB-N in pink perch mince.

5.3.2.2 Alpha Amino Nitrogen

The Alpha – amino nitrogen is an index of free amino acids present in the meat and is also a measure of proteolysis. The increase in AAN content can be attributed to break down of protein, during postmortem changes. The increase in AAN may also be due to the bacterial and autolysis enzyme activity leading to proteolysis, before the inhibition of their activity during freezing.

In the present study AAN content showed a steady increase in all the samples of the both the prepared products. Similar increase was found by Reddy *et al.* (1992), Cakli *et al.* (2004), during frozen storage studies of fish fingers from different fish species. This may be due to above said reasons.

5.3.3 Changes in protein quality characteristics

5.3.3.1 SSP &NPN

During freezing and subsequent storage, a number of microstructural and chemical alterations occur, resulting in drip from thawed muscle.such as decrease in NPN, SSP, loss of succulence and development of flavours (Sikorski *et al.*, 1976, Morrison 1993 and Matsumoto 1979). A decrease in protein solubility with freezing of muscle is due to specific denaturation or loss of natural properties of actomyocin fractions. Extractability of muscle proteins using salt solutions have been attempted to study the alterations in proteins during frozen storage of fish meat by many workers (Poulter and Disney, 1978; Sikorski 1976 and Srikar 1979). It has been pointed out by Dyer and Fraser (1959) that lean fish are more prone to denaturation than fatty fish when held under similar conditions of storage. Since, *Pricanthus hamrur* may be grouped under lean variety due to its low lipid content, the above reason may hold good for this species during frozen storage.

The non nitrogenous extractive components include the organic acids, sugars and inorganic salts. Relatively few studies have been made of their distribution in fish and shell fish when compared with those of nitrogenous compounds. The NPN values obtained in the present study appear to be high. Shoji Konosu and Yamaguchi (1982) have given a detailed account on the NPN content of various sea foods and have reported that its content is highly variable depending on growth, season, salinity, culture or wild fish, part of body and freshness. NPN The denaturation of protein leads to loss of drip and there by loosing water soluble odour giving components which are grouped under extractives. The decrease in NPN content was observed in minced meat of ribbon fish (Badonia and Devadasan, 1980) in clam meat (Mishra and Srikar, 1989) and pink perch meat by Reddy (1988) and Sarma *et al.* (1998). In the present study, in case of both the prepared fish product that were frozen stored NPN content decreased gradually. In case of plain fish cakes, initially the NPN content was 1.56 and at the end of 5 months of storage time it was 0.73gm/100gm of meat.

In the case of battered and breaded fish cakes initially the NPN content was 1.34 and at the end of 5 months of storage period at -18°c, it had decreased to 0.76gm/ 100 gm of meat. These values recorded during frozen storage studies, comply with the values reported by Raju (1994), Murthy (1999), Tokur *et al.* (2005), Sehgal and Sehgal (2002), Sarma *et al.* (1998) and Benjakul *et al.* (2004) considerably.

5.3.4 Changes in lipid quality parameters during storage

5.3.4.1 Lipid hydrolysis (FFA)

Usually the fish lipids which contain a high proportion of unsaturated fatty acids are liable to breakdown or undergo further changes which affect the odour, flavour, colour and taste of the fish due to hydrolytic rancidity. These changed are detected by estimating the FFA content in the prepared fish products. As the fish becomes stable and spoiled, the FFA content increase gradually and therefore has been used as a quality index for different fishes.

In the present study free fatty acids increased in both the prepared products during cold storage at -20°C (Table 14). In case of plain fish cakes, free fatty acid content was found to increase from 3.06% to 9.43% as % of oleic acid (Table 14 and Fig 4b). In case of battered and breaded products it increased from 3.43% to 7.34% of oleic acid (Table 14 and Fig 4b). Increase in FFA during frozen storage of prepared fish products has been observed by Maruthi (1989) and Raju (1994). Several workers have reported a similar increase in FFA content during cold storage of prepared products (Tokur *et al.,* 2005: Sehgal and Sehgal 2002).

5.3.4.2 Lipid Oxidation (PV)

Lipid oxidation is common phenomenon during frozen storage. Oxidation of fats results in the formation of peroxide and aldehydes the

estimation of which serves as a spoilage index. In addition to the problems of rancidity, oxidized lipids interact with proteins causing undesirable changes in the nutritional and functional properties of proteins. The extent of oxidation varies from species to species. In fatty fishes lipid oxidation becomes the dominant spoilage mechanism during cold storage where as in lean fish, lipids are oxidized slowly contributing to the spoilage during frozen storage. The maximum permissible limit for PV is between 13-30 milliequivalents of oxygen per kg of fat (Lajalina, *et al.,* 1985).

In the present study, the prepared fish products have shown susceptibility of lipids for oxidative rancidity during storage. Fish lipids are of great importance due to their tendency to develop off flavour during storage which is caused by atmospheric oxidation. Love (1992), found that oxidisability of fish lipids co-related with the contents of polyunsaturates. Here in case of plain fish cakes, PV has shown an increasing trend from 13.20 to 39.48 milliequivalents of 0_2/kg of fat (Table 13). Similarly is battered and breaded fish cakes, the PV has shown a increasing trend from 12.80 to 33.43 milliequivalents of 02/kg of fat (Table 9). It is known that the metals such as iron catalyse lipid oxidation and involvement of enzymes in lipid oxidation also cannot be ruled out. The decrease in sensory values may be attributed to increase in PV and FFA content. The susceptibility of fish lipids to auto oxidation even at reduced temperature is attributed to larger degree of unsaturation. An increasing trend of PV has been observed even by Joseph *et al.* (1992), Sarma .*et al.*(1998); Balasundari *et al.* (1997), Kamat *et al.*(2003) and Benjakul *et al.*(2002) in several products.

5.3.5 Changes in Microbiological characteristics

Spoilages in fish and fishery products is brought about chiefly by autolysis and bacterial decomposition.

The Total plate count of both the prepared fish products during frozen storage is shown in Table 18 and Fig5b. It can be seen in table that the number of colony forming unit have decreased during initial period of storage

and increase slightly in the later period of storage. In frozen samples temperature might be a factor for inhibiting proliferation. Some bacteria are destroyed by freezing and frozen storage, but the speed with which bacteria are destroyed varies.

In the present study there was not much change in TPC. In case of plain fish cakes, TPC decreased from 5.43×10^{4} to 2.23×10^{4} cfu /gm meat at the end of storage period. In case of battered and breaded fish cakes. The TPC similarly decreased from 6.23×10^{4} to 2.12×10^{4} cfu /gm meat. The extent of initial decrease and constant phase depends on temperature (Steurart, 1943). Kamat *et al.* (2003), Tokur *et al.* (2004), Rebach *et al.* (1990) observed a decrease in the bacterial load in frozen stored fish products.

5.4 Changes in sensory characteristics

Sensory attributes such as appearance, colour, texture, and flavour have shown decreasing trend in both products. The OAS has decreased from 9 to 7.25 in battered and breaded products and in plain fish cakes from 8.25 to 6.80 which lie in acceptable region in 9 point hedonic scale.

In both the products there was negative co-relation between OAS and freshness parameters.

5.5 Statistical results

From the results of analysis of variance techniques it can be seen that over all acceptability score are significantly different due to storage period and also due to different products tried. There existed a negative correlation between sensory scores, protein quality parameters and different biochemical tests. Co-relation between overall acceptability scores and freshness parameters also shower a negative co-relation, it can be seen that sensory scores are decreasing during storage period and fresh ness parameters are increasing in both the products.

From this study it can be concluded that storage at -20ºC has proven to be an effective method to maintain the quality of frozen plain fish

cakes and battered and breaded fish cakes for at least 4 months of storage. Battered and Breaded fish cakes have slightly longer shelf life in comparison to plain fish cakes during frozen storage as they have a coating over it and they were acceptable even after 4 months of storage. As the demand for ready to eat foods is increasing these products may satisfy the demands in the coming time.

Summary

VI Summary

The present study deals with the preparation of value added products such as "plain fish cakes and battered and breaded fish cakes" from minced meat of Big eye fish.. During the storage the physical chemical and microbiological properties of the raw materials and final products were studied. The moisture, protein fat and ash content of the prepared products were analyzed. The other parameters studied during cold storage at -20°C were proximate composition, FFA, PV, NPN, SSN, AAN TVB-N, TPC and organoleptic quality of the product. The average weight of the plain cakes and battered and breaded fish cakes were in the range of 18 to 22 gm and 26 to 32 gm respectively.

- The storage period has little influence on the proximate composition of the products.
- The change in the PV and FFA indicate that hydrolysis and oxidation of lipids has occurred during frozen storage.
- The values of TVB-N and AAN showed a steady increase in all the samples.
- There was decrease in NPN values during frozen storage.
- There was a gradual reduction in the bacterial load of prepared products during frozen storage.
- Organoleptic scores indicate that both the products were within acceptable limits till the end of 4 months of storage time. After 4 months parameters such as TVB-N, FFA, PV, had crossed their maximum permissible limits.
- The statistical analysis of the results of over all acceptability scores indicate there is a significant difference between the different products .Sensory and protein quality characteristics with biochemical tests have shown a negative correlation.

From this study it can be concluded that storage at -20°C has proven to be an effective method to maintain the quality of frozen plain fish cakes and battered and

breaded fish cakes for at least 4 months of storage. Battered and Breaded fish cakes have slightly longer shelf life in comparison to plain fish cakes during frozen storage as they have a coating over it and they were acceptable even after 4 months of storage. As the demand for ready to eat foods is increasing these products may satisfy the demands in the coming time.

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Appendix

APPENDIX-I

KARNATAKA VETERNARY, ANIMAL HUSBANDARY AND FISHERY SCIENCES, BIDAR.COLLEGE OF FISHERIES MANGALORE.

DEPARTMENT OF FISH PROCESSING TECHNOLOGY

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

Characteristics	**Plain fish cakes**	**Battered and breaded fish cakes**
Appearance		
Colour		
Texture		
Taste		
Flavour		
Odour		
Over all acceptability		

Hedonic scale

Like extremely : 9

Like very much : 8

Like moderately : 7

Like slightly : 6

Neither nor dislike : 5

Dislike slightly : 4

Dislike moderately : 3

Dislike very much : 2

Dislike extremely : 1

Name :

Date :

Signature :

Comments:

Table 1. Raw materials characteristics of fresh fish

A. Physical Characteristics			
Average total length (cm)		22.5 (2.36)	
Average standard length (cm)		17.67 (1.87)	
Average total weight (gm)		156.78 (33.68)	
B. Yield at different stages			
Total weight of the fish (kg)		40	
Dressed yield (%)		64	
Picked meat yield (%)		39	
Minced meat yield (%)		29.52	
C. Proximate composition			
Moisture (%)	Protein (%)	Total lipid (%)	Ash (%)
77.52 (0.40)	19.31 (0.63)	1.28 (0.01)	1.43 (0.21)

All value expressed in wet wt basis

Table 2. Biochemical and microbiological characteristics of fresh fish

A. Chemical characteristics	
Total volatile base nitrogen (mg %)	6.23 (0.01)
Trimethyl amine	2.87 (0.27)
Alpha amino nitrogen (mg %)	13.42
Salt soluble proteins (gm / 100gm of meat)	13.13 (3.12)
Non protein nitrogen (mg/100gm of meat)	321.23
Ph	6.23
Peroxide value (milliequivalents of O_2 /kg of fat)	15.34 (3.08)
Free fatty acid (% of oleic acid)	3.36 (0.30)
B. Microbiological characteristics	
Total plate count (cfu/ gm of meat)	6.43×10^4

All values are expressed on wet weight basis

* Standard deviations are shown in parenthesis

Table 3. Basic recipe formula for plain fish cakes

Ingredients	Percentage
Fish mince	75
Corn flour (starch)	10
Water	10
Salt	2
Chilli powder	1.5
Garam masala	0.25
Pepper powder	0.25
Coriander powder	0.5
Ginger paste	0.16
Garlic paste	0.18
Onion paste	0.16

Table 4. Basic recipe formula for battered and breaded fish cakes

Ingredients	Percentage
Fish mince	75
Corn flour (starch)	10
Water	10
Salt	2
Chilli powder	1.5
Garam masala	0.25
Pepper powder	0.25
Coriander powder	0.5
Ginger paste	0.16
Garlic paste	0.18
Onion paste	0.16

Table 7. Standardisation of fish mince, starch and water in the preparation of plain fish cake

Recipe ingredients %	I	II	III
Fish mince	70	75	80
Starch	13	10	8
Water	12	10	7
Spice mixture			
Chilli powder	1.5	1.5	1.5
Garam Masala	0.25	0.25	0.25
Coriander powder	0.50	0.50	0.50
Pepper Powder	0.25	0.25	0.25
Ginger paste	0.16	0.16	0.16
Onion paste	0.16	0.16	0.16
Garlic paste	0.18	0.18	0.18
Salt	2.0	2.0	2.0

Table 8. Standardisation of fish mince, starch and water in preparation of battered and breaded fish cakes

Recipe ingredients %	I	II	III
Fish mince	70	75	80
Starch	13	10	8
Water	12	10	7
Spice mixture			
Chili powder	1.5	1.5	1.5
Garam Masala	0.25	0.25	0.25
Coriander powder	0.50	0.50	0.50
Pepper Powder	0.25	0.25	0.25
Ginger paste	0.16	0.16	0.16
Onion paste	0.16	0.16	0.16
Garlic paste	0.18	0.18	0.18
Salt	2.0	2.0	2.0

Table 9. Average panel scores of plain fish cake recipe with different % of fish mince, starch & water

Attributes	Recipe		
	I	II	III
Appearance	9	9	8
Colour	8	9	8
Odour	8.5	9	8
Texture	7	8.5	7
Taste	6	8	7
Flavour	8	9	7
Overall acceptability	7	8.8	7.6

Table 10. Average panel scores of battered and breaded fish cakes prepared with different % of fish mince, starch & water

Attributes	Recipe		
	I	II	III
Appearance	8	9	7.5
Colour	7	9	8
Odour	8	7	8
Texture	8	8.5	7
Taste	8	9	6
Flavour	7	8	8
Overall acceptablity	8	8.5	7.5

Table 5. Average panel scores of plain fish cake for different kinds of starches used.

Quality/ ingredients	Corn flour	Rice flour	Wheat flour
Appearance	8	7	8
Colour	8	8	7
Odour	9	8	8
Texture	9	8	8
Taste	9	8	9
Flavour	9	9	9
Overall acceptability	9	8	8

Table 6. Average pannel scores of battered and breaded fish cakes prepared with using different kinds of starches

Attributes /Ingredients	Corn flour	Rice flour	Wheat flour
Appearance	9	8.0	8.5
Colour	9	8.0	7.5
Odour	8.5	8	8
Texture	9	7.5	8.5
Taste	9	8	9
Flavour	9	8	8
Overall acceptability	9	8	8

Table 11. Average panel scores of battered and breaded fish cake prepared with different types of battering mixtures

Attributes/ ingredients	Gram flour	Maida flour	Rice flour
Appearance	8	9	9
Colour	8	9	8.5
Odour	8.5	8.5	9
Texture	8	8.5	8
Taste	8.5	8.5	9
Flavour	8	9	8.5
Overall acceptability	8	9	8.5

Table 12. Proximate composition of fish products

Samples	Moisture %	Protein %	Fat %	Ash
1. Plain fish cakes (before cold storage	70.8280	15.73	0.6082	3.6356
2. Plain fish cakes (at the end of cold storage period	66.4348	14.87	1.4346	4.5689
3. Battered and breaded fish cakes (before cold storage)	68.56	15.08	0.83	3.9869
4. Battered and breaded fish cakes (at the end of cold storage)	66.98	14.52	1.5483	4.9324

Table 13. Changes in peroxide value (Milliequivalents of O_2 per kg of fish cakes) during storage at - 20°C

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	13.20	12.80
1	14.45	14.56
2	19.36	18.93
3	25.44	26.38
4	34.22	30.32
5	39.48	33.43

Table 14. Changes in Free fatty acid (as percent of oleic acid of fish cakes) during frozen storage at -20 °C

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	3.06	3.43
1	4.56	3.83
2	5.18	4.03
3	6.48	4.87
4	7.94	6.73
5	9.43	7.34

Table 15. Changes in AAN values of fish cakes during frozen storage at -20°C (gm/100gm of meat).

Storage period in months	Plain fish cakes	Battered and breaded fish cakes.
0	15.34	19.16
1	21.72	23.9
2	24.84	27
3	26.92	32.62
4	28.47	34.87
5	31.23	36.34

Table 16. Changes in TVB – N values of fish cakes during frozen storage at -20°C (gm/100 gm of meat).

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	8.32	8.43
1	10.03	10.27
2	14.36	14.73
3	21.23	20.87
4	33.43	32.83
5	41.68	39.78

Table 17. Changes in NPN values of Fish cakes during frozen storage at -20°C (gm/100gm of meat).

Storage period in months	Main fish cakes	Battered and breaded fish cakes
0	1.56	1.34
1	1.43	1.23
2	1.02	0.93
3	0.97	0.87
4	0.89	0.83
5	0.73	0.76

Table 18. Changes in SSP values of fish cakes during storage at -20°C

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	12.26	13.73
1	11.85	12.52
2	10.87	10.93
3	10.63	1.02
4	9.71	8.57
5	8.78	7.85

Table 19. Changes in Total plate count of fish cakes during storage at -20°C)cfu/ gm of meat.

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	$5.43x10^4$	$6.23x10^4$
1	$3.87x10^3$	$4.16x10^3$
2	$1.28x10^3$	$1.48x10^3$
3	$7.80x10^3$	$8.68x10^3$
4	$1.16x10^4$	$1.34x10^4$
5	$2.23X10^4$	$2.12x10^4$

Table 20. Changes in the values of pH of fish cakes during frozen storage at -20°C

Storage period in months	Plain fish cakes	Battered and breaded fish cakes
0	6.21	6.28
1	6.23	6.34
2	6.30	6.38
3	6.36	6.41
4	6.39	6.44
5	6.47	6.51

Table 21. Changes in sensory scores of plain fish cakes prepared from big eye fish (*priacanthus hamrur*) during frozen storage at -20°C

Storage in months	Appearance	Colour	Texture	Taste	Flavour	Odour	Over al acceptability
0	8.55	8.75	8.75	8.34	8.25	8.34	8.25
1	8.72	8.25	8.25	8.40	8.34	8.37	8.05
2	8.18	8.34	7.65	7.43	8.45	8.23	7.80
3	8.05	8.12	7.30	7.33	7.75	7.75	7.45
4	7.57	7.70	6.75	7.12	6.84	7.12	6.80
5	6.75	7.12	6.40	6.70	6.53	6.73	6.25

Table 22. Changes in sensory scores of batterd and breaded fish cakes prepared from big eye fish (*priacanthus hamrur*) during frozen storage at - 20°C

Storage in months	Appearance	Colour	Texture	Taste	Flavour	Odour	Over al acceptability
0	8.8	8.73	9	8.82	8.56	8.5	8.73
1	8.7	8.60	8.5	8.45	8.45	8.40	8..60
2	8.45	8.45	8.35	7.50	8.25	8.25	8.45
3	7.75	7.40	7.8	7.8	7.36	7.70	7.75
4	7.20	7.25	7.48	7.32	7.21	7.12	7.42
5	7.02	7.60	7.13	7.O8	7.12	6.87	7.25

Table 23. Correlation coefficients between sensory scores of fish cakesand freshness parametersof fish cakes.

Parameter	Battered and breaded fish cake	Plain fish cake
OAS vs. PV	- 0.9977	- 0.9758
OAS vs. FFA	- 0.9484	- 0.9872
OAS vs. TVB-N	- 0.9601	- 0.9698
OAS vs. AAN	- 0.9884	- 0.9396
Odour vs. PV	- 0.9840	- 0.9898
Flavour vs. PV	-0.8733	- - 0.8604
Odour vs. FFA	- 0.9914	- - 0.9608
Texture vs. SSN	- 0.9833	- - 0.9643

Table 24. Correlation coefficients between protein quality parameters and freshness parameters of fish cakes

Parameter	Battered and breaded fish cake	Plain fish cake
SSP vs. PV	- 0.9800	- 0.9825
SSP vs. FFA	- 0.9419	- 0.9853
SSP vs. TVB-N	- 0.9583	- 0.9776
SSP vs. AAN	- 0.9871	- 0.9399
NPN vs. PV	- 0.9275	- 0.9099
NPN vs. FFA	- 0.8213	- 0.9286
NPN vs. TVB-N	- 0.8577	- 0.9651
NPN vs. AAN	- 0.9577	- 0.9651

Table 25. Statistical interpretation of biochemical and sensory quality of fish cakes prepared from big eye ,(*priacanthus hamrur)* during storage.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio	P value	Critical value
Due to storage period	5	5.826167	1.16523	39.8597	0.000496	5.050339
Due to product	1	1.484033	1.4840	50.7651	0.000845	6.607872
Error	5	0.14616	.02923			
Total	11	7.4536				

APPENDIX-I

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DEPARTMENT OF FISH PROCESSING TECHNOLOGY

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

Characteristics	**Plain fish cakes**	**Battered and breaded fish cakes**
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Flavour		
Odour		
Over all acceptability		

Hedonic scale

Like extremely : 9

Like very much : 8

Like moderately : 7

Like slightly : 6

Neither nor dislike : 5

Dislike slightly : 4

Dislike moderately : 3

Dislike very much : 2

Dislike extremely : 1

Name :

Date :

Signature :

Comments:

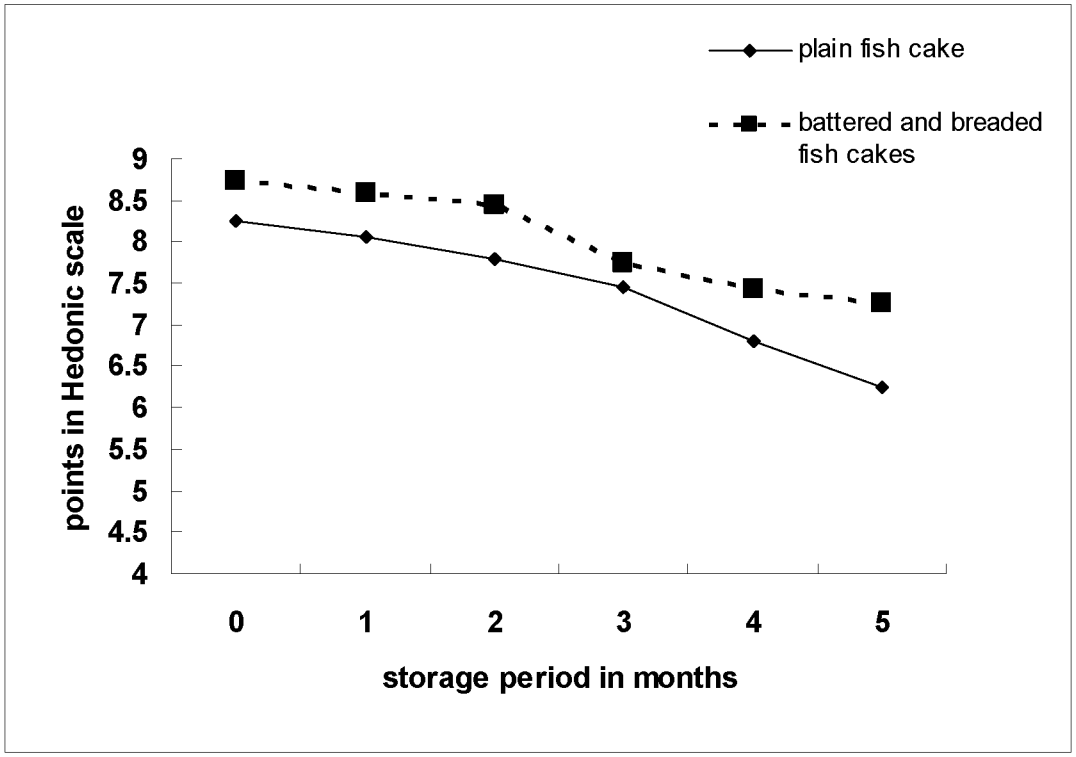


FIG. 8 Changes in OAS of fish cakes during frozen storage period

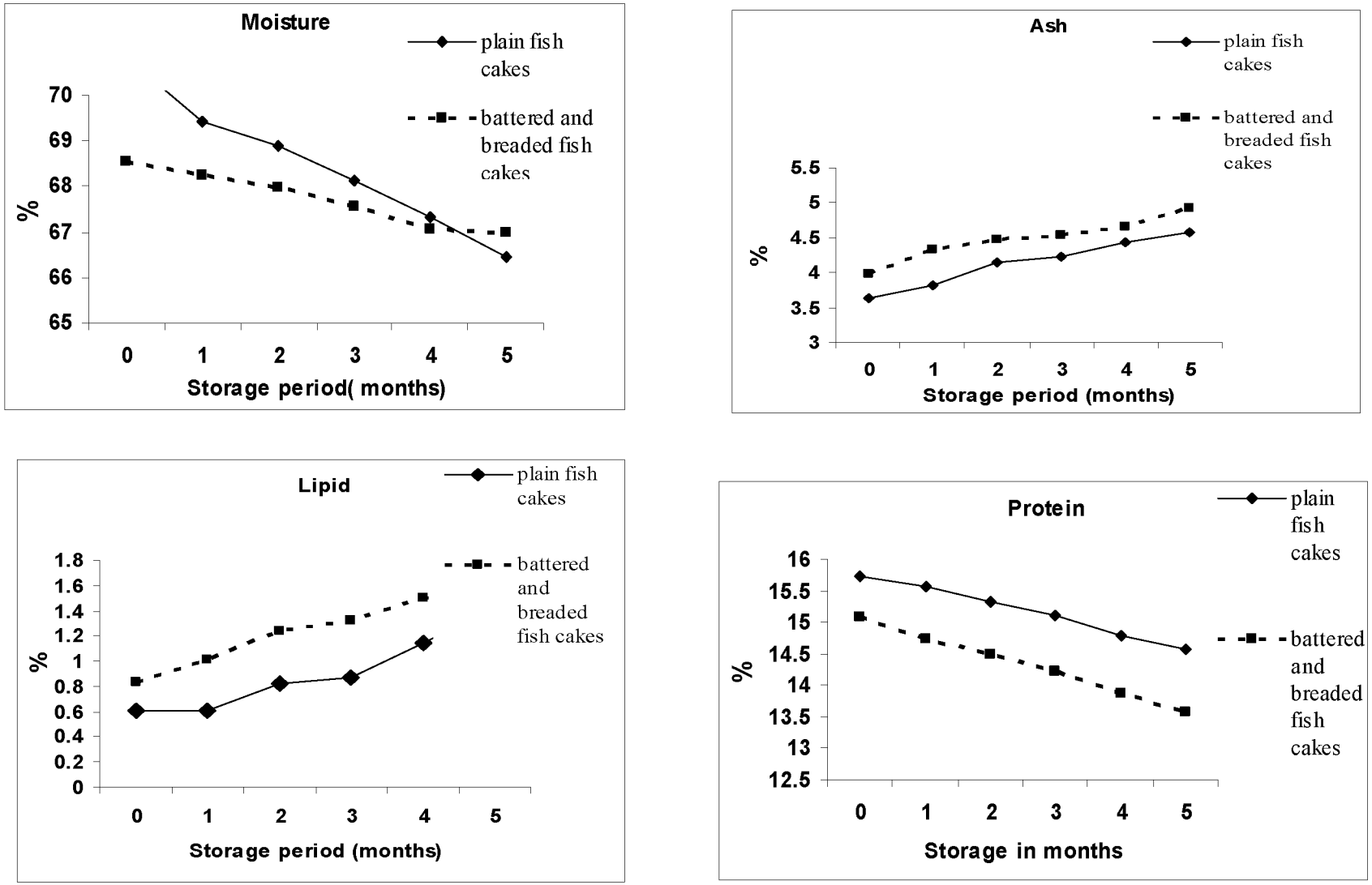
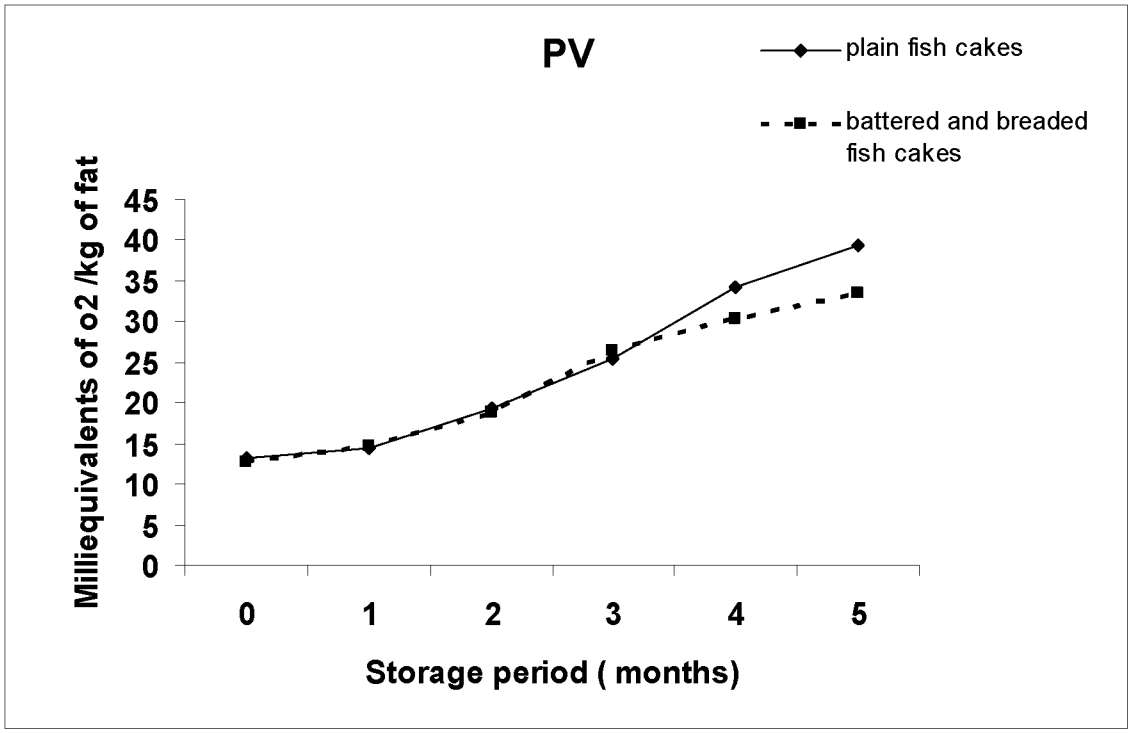


FIG. 3 Proximate composition of fish cakes during frozen storage



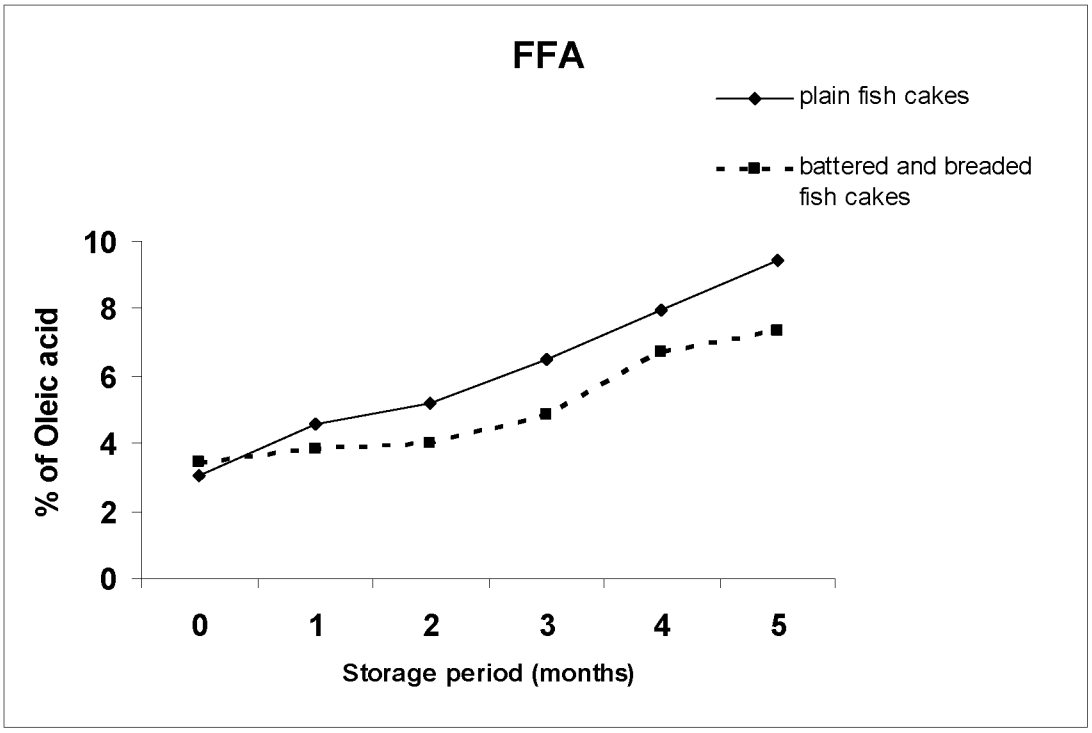


FIG.4 Changes in lipid quality of fish cakes during frozen storage

FIG. 5a Changes in pH of fish cakes during fozen storage at -20°C

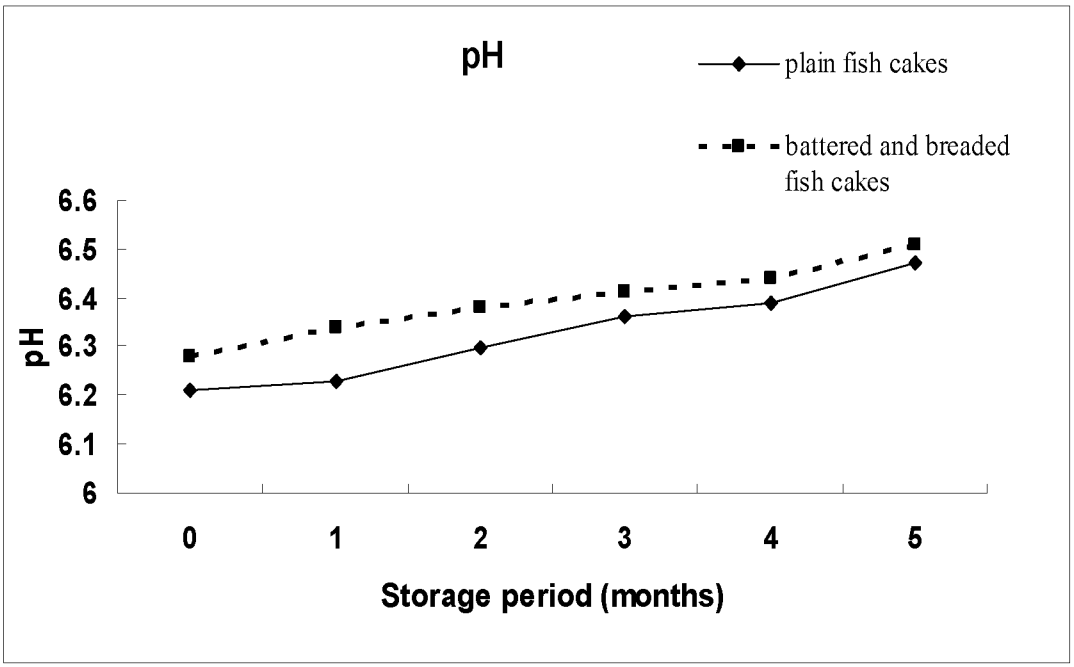
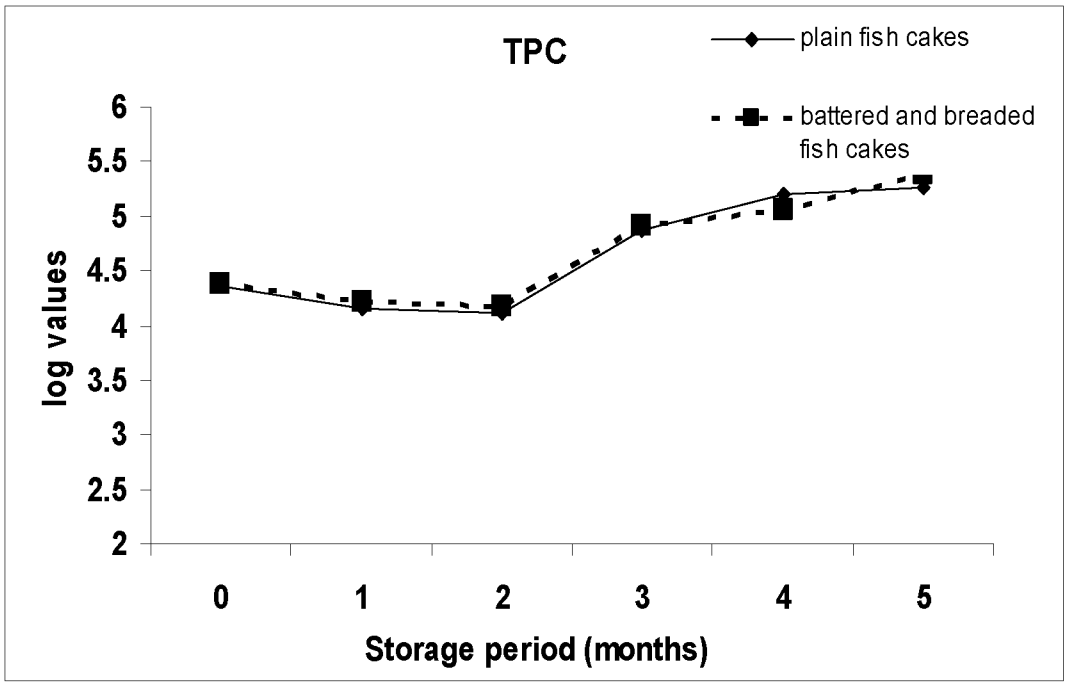
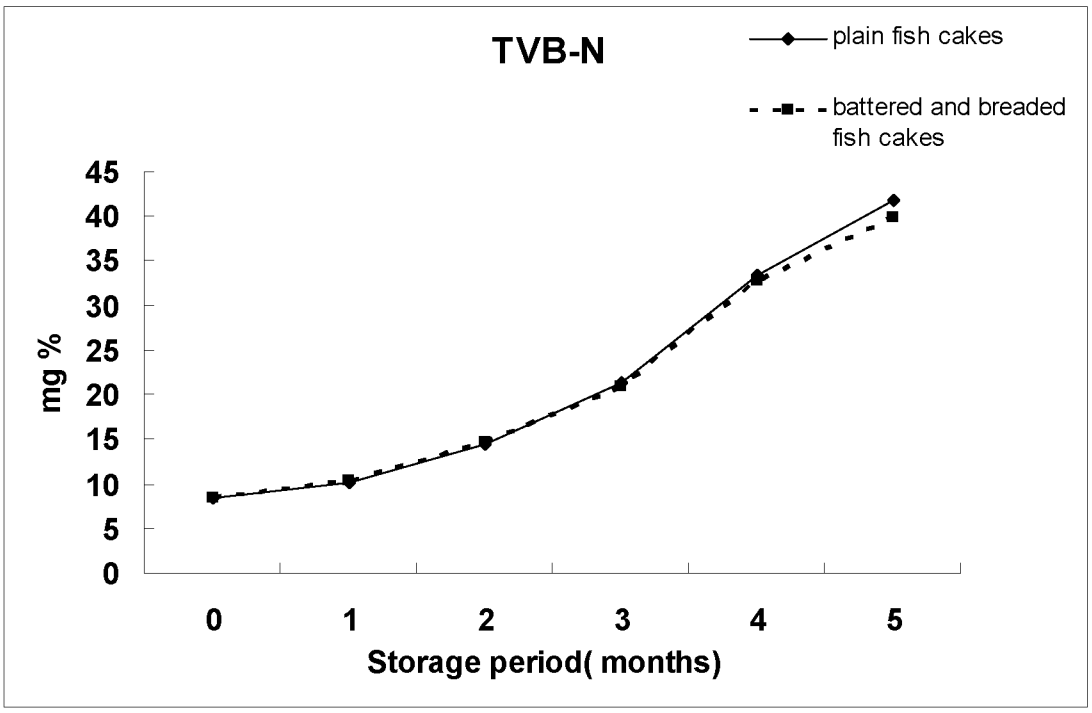


FIG. 5b Changes in TPC of fish cakes during fozen storage at -20°C





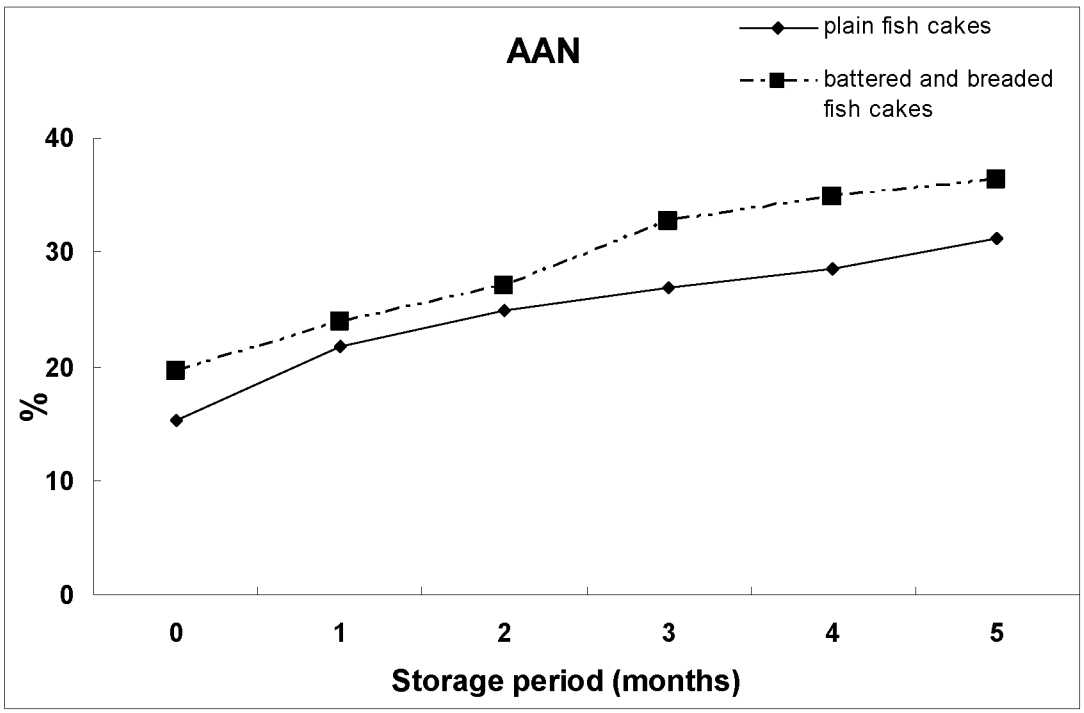
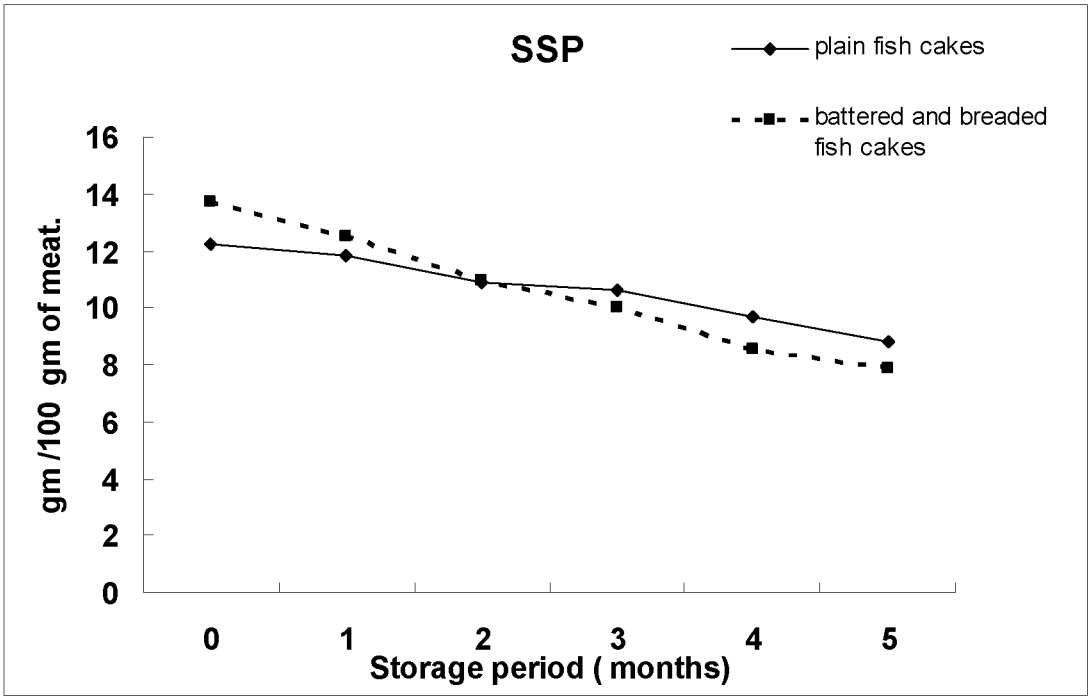


FIG. 6 Changes in nitrogenous constituents of fish cakes during frozen storage period



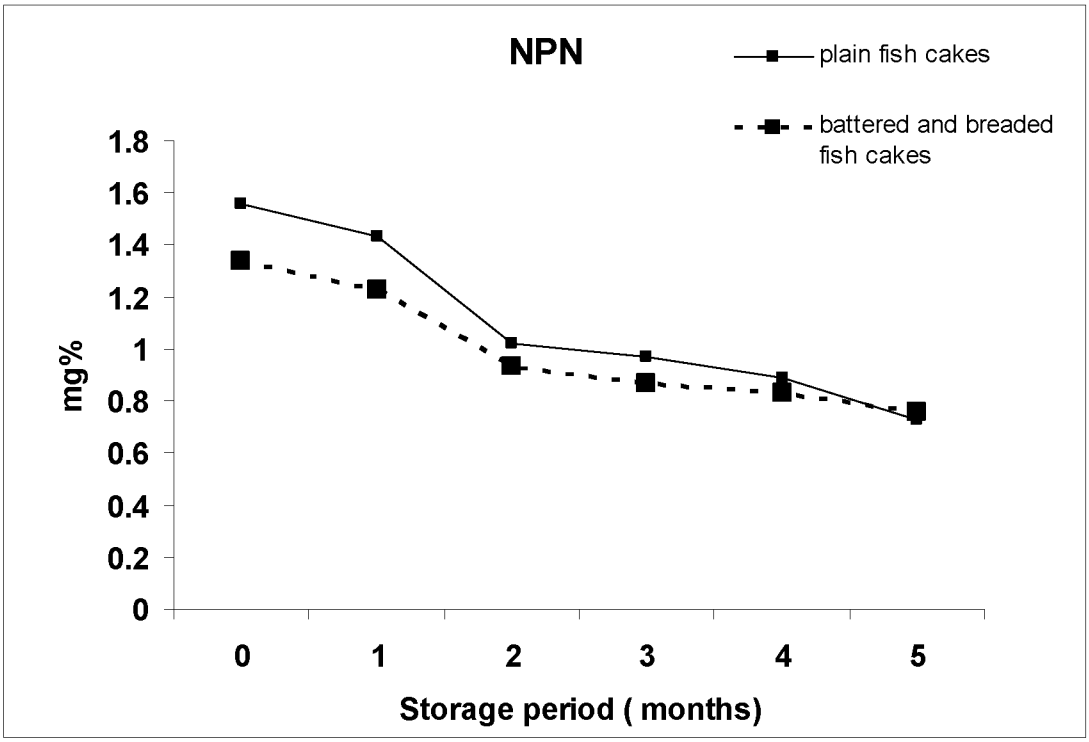


FIG. 7 Changes in protein quality of the fish cakes during frozen storage period