MICROBIAL RISK ASSESSMENT AND PROCESS STANDARDIZATION FOR "COOK-CHILL FISH" AND "PARTIALLY PROCESSED VALUE ADDED FISH".

By

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THESIS

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PANANGAD, COCHIN

DECLARATION

I hereby declare that this thesis entitled - **MICROBIAL RISK ASSESSMENT AND PROCESS STANDARDIZATION FOR "COOK-CHILL FISH" AND "PARTIALLY PROCESSED VALUE ADDED FISH"** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, association, or other similar title, of any other University or society.

Panangad,
19 / 10/ 2007

Anju
ANJU

Dedicated To

My Loving Parents and Brother

DECLARATION

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CERTIFICATE

Certified that this thesis entitled - **MICROBIAL RISK ASSESSMENT AND PROCESS STANDARDIZATION FOR "COOK-CHILL FISH" AND "PARTIALLY PROCESSED VALUE ADDED FISH"** is a record of research work done independently by **Miss ANJU** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or association to her.

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INTRODUCTION

1. INTRODUCTION

Seafood is a common ingredient in the diet of many people. Today more and more people are turning towards seafood for scientific reasons like better protein quality, digestibility and beneficial effects on the cardio vascular system of man. However, unlike other food sources, the species diversity and location specific properties of fish are numerous. Species and location specific hazards and their associated risks for consumers are a major problem for fish and fishery products. Majority of fish stock harvested is from the wild. Fish and shellfish tend to accumulate different kinds of microorganisms and chemicals from water bodies as they filter large quantities of water for collecting oxygen and water. A thorough analysis of the risks involved and streamlining the processing procedures to suitably address the risks are essential steps in ensuring steps for safety of seafood.

We are constantly subjected to risks or hazards during our life times. There is no absolute safety in anything we do, including the food we consume. Food should be safer today than it was before due to increased knowledge of microbiology and sanitation, as well as increased regulations. However, due to large scale, high speed food processing, alteration of traditional processing methods resulting in less control of microorganisms, proliferation of ready to cook and ready to eat products etc. there has been an increase in the risk of food borne illness.

Over the last twenty years, there has been increased activity in the developments of concepts for production of safe foods to protect the consumers. The record of seafoods as vehicles of infection or intoxication is extremely good. Seafoods generally rank low in the list of foods involved in food borne infections or intoxications in most countries (Shewan, 1962).

The presence of highly resistant faecal streptococci and coliforms in fish indicates faecal contamination of fish and probable presence of enteric pathogen (Frazier and Westhoff, 1978). The bacteriological quality of freshly landed and

retail level seafood sold in different markets of the country has been reported by previous workers (Comar *et al.*, 1979; Lakshmanan *et al.*, 1984; Iyer *et al.*, 1986).

In those countries that maintain useful record of food borne diseases, fishery products account for a significant proportion of outbreaks reported. Compared to gram positive pathogens, gram negative intestinal pathogens such as *Salmonella* spp., *Vibrio* spp., *Campylobacter* spp. and *Yersinia* spp. are most frequently implicated in outbreaks of food borne illness (Bean and Griffin, 1990). Nambiar and Iyer (1990) reported that quality of fish sold in domestic market was poor compared to that of export trade and they were mostly contaminated with pathogenic organisms. Microbial food safety issues involve interdisciplinary research in both basic and applied sciences (Archer, 1988; Archer and Young, 1988; Mossel and Struijik, 1993).

Innovative developments from research have advanced and scientific basis for HACCP approach to food safety, furthered the understanding of microbial adaptation and survival in both food processing and infected host environments, and suggested new methods for controlling food contamination (Archer and Young, 1988; Mossel and Struijik, 1993).

Fish, shellfish and other marine organisms are responsible for at least one in six food poisoning outbreaks with known etiology in United States. In other parts of the world, the impact of seafood poisoning is even greater. In the period 1971- 1990 seafood was single most important vehicle in food poisoning outbreaks in Korea (32%) and Japan (22%) where seafood was responsible for 43% and 62%, respectively of outbreaks related fatalities (Chan 1995; Lee *et al.*, 1996).

Risk assessment is a quantitative estimation of the probability of occurrence of a hazard. This information is important for managerial decisions (Notermans *et al.*, 1997). Several researches have been conducted to study microbiological quality of fish and fishery products. Biochemical and microbiological qualities of ice- stored *Labeo gonius* sold in Imphal market of Manipur were studied over a period of three

months from September to November(1994). Fishery products for human consumption should be free from pathogenic microorganisms. Several new preservation techniques are useful to control microbial growth. Low temperature preservation is the most common practice in all parts of the world. Hatha and Lakshmanperumalswamy (1997) isolated a number of serotypes of *Salmonella* from retail fish markets. Coliforms, *Staphylococus aureus* and faecal streptococci were detected in samples whereas *Escherichia coli* and *Salmonella* were absent (Lilabathi and Vishwanath, 1999).

Risk assessment consists of four steps (a) identification of hazard in food (b) exposure assessment (c) nature of adverse effect of hazard on consumer (d) risk characterization (Mukundan, 2003). (Nambiar and Surendran, 2003) reported presence of number of microorganisms in fish harvested from natural water bodies including farms. Fish can be contaminated during handling, transportation and processing. These microorganisms include both pathogenic and non pathogenic bacteria. Most important pathogens which gain entry into fish during handling, transportation and processing are *Salmonella, Vibrio cholerae, Staphylococcus aureus*, and *Listeria monocytogenes*. In addition other pathogenic organisms also gain entry.

For the past several years, health risks have been recorded as a result of eating various kinds of food materials. Such records are many in developed countries; the same is scanty in developing and underdeveloped countries due to poor documentation and also due to the fact that less importance is attributed to human health hazards. Use of good manufacturing practices followed by process control, based on HACCP was seen to make food safe but not absolutely safe. Therefore there is growing interest in risk assessment or at least elements of it.

Contamination of seafood by harmful microorganisms is of great concern from public health viewpoint. Studies show that most of the increase in cross contamination in developing countries like India is due to temperature abuse of fish.

Minimum prescribed standards of fish markets to be implemented with immediate effect and constant monitoring and enforcement is required to ensure safety of food.

Contamination due to unhygienic handling procedures entails the risk of spreading the bacterial, viral or pathogenic agents of communicable enteric diseases. Seafood related diseases caused by bacterial pathogens or their toxins are associated with improper food preparation, handling and storage (Lalitha and Thampuran, 2006). To reduce the number of seafood related outbreaks, coordinated efforts by several agencies is required. Absence of illness and pathogen does not guarantee safety of consumers.

This work consists of preparation of two new products i.e. cook-chill fish and partially processed value added fish and then carrying out their microbial risk assessment. Risk assessment is estimation of probability of occurrence of a hazard. Main objectives of the study are to carry out microbiological tests for detection of some commonly occurring pathogens and also to conduct some biochemical tests to estimate the quality of the products.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 COOK CHILL PROCESS:

Cook chilling may be defined as a catering system based on the full cooking of food followed by fast chilling and storage in controlled low temperature conditions above freezing point (0 to 3°C) and reheating before consumption. It can be used for five days including the day of cooking (Department of Health, 1989).

Microwave heats food by dielectric heating mechanism, which is an entirely different way of heating compared to conventional heating. Microwave cooked food hardly gets an internal temperature of about 80°C. It is still a controversy if there is some non thermal killing effect of microwave on the micro organisms, because of the very small heat exposure time at lower internal temperature. Scientists have been giving theories about destruction of food poisoning and pathogenic organisms in microwave cooked food (Brown and Morrison, 1954; Campell *et al.,* 1958; Dessel *et al.,* 1960; Greez *et al.*, 1964; Baldwin *et al.*, 1968; Delaney *et al.*, 1968).

Earlier studies indicates that destruction of red halophiles which cause spoilage in salted or brined fish could be more easily killed by steam under pressure than by dry heat treatment(Castell, 1950). White and Hobbs (1963) have observed that vegetative cells of *Escherichia coli* and *Salmonella* were completely destroyed in beef stew after microwave heat treatment, but *Bacillus subtilis* was not affected.

Attraction of prepared and precooked frozen foods is largely based on convenience and on retention of the quality of freshly prepared foods. Problems of maintaining the texture and structure of precooked or prepared foods after freezing and storage primarily occur in the case of starch or egg thickened case (Palmer, 1972). Mathen *et al.* (1979) developed a method for preparing cook- peeled- frozen prawns. Cooking of raw prawns resulted in very low TPC.

The introduction of the cook-chill system in the year 1967 was catalyzed by requirement to overcome some of the economic and quality problems associated with conventional catering practices. Despite a lack of initial interest and several limiting factors in terms of potential application the system has become well established (Collison, 1979; Andrews, 1982). In 1988 there were over 300 cook chill units operating in the U.K (Walker and light 1988; Wood *et al.*, 1989). These were used for production of meals or meal components for a wide range of institutional and commercial catering situations (Light, 1988). Mason *et al.* (1990) reported that cook-chill produces a product that is generally inferior in sensory quality to that produced by conventional methods in ideal situations, but superior to that produced by conventional methods in non ideal situation that are often found with catering industries. In package pasteurization of vacuum packaged Vienna sausages the treatment increased the shelf life but did not eliminate microbial spoilage (von Holy *et al,* 1991). Venugopal (1993) developed cook-chill process for peeled and deveined shrimp and white pomfret.

Heating salt at a temperature of 80°C for 30 min eliminates red halophilic cocci, responsible for red discolouration in salt cured fish (Prasad and Rao, 1995). Processing can have a considerable effect on microbial contamination of raw materials. Heating will destroy pathogens present in raw materials. Steam treatment given to beheaded and eviscerated catfish reduced aerobic plate count (APC) to below limit of detection. Increasing the time of steam pasteurization increases antimicrobial effect (Bala *et al.*, 1999).

Pasteurization, the term given to heat processes typically in range of the 60-80°C and applied for few minutes, is used for two purposes. First is the elimination of specific pathogen or pathogens associated with a product. This type of pasteurization is often a legal requirement introduced as a public health measure when the product has been implicated as a vehicle of illness. The second reason for pasteurization of a product is to eliminate a large proportion of potential spoilage organisms, thus extending its shelf life. Microwave cooking has the capacity to kill

vegetative cells of certain pathogens effectively but microwave heating is more food dependant than conventional heating (Muzaddadi and Nayak, 2000).

On its own the contribution of pasteurization to the extension of shelf life can be quite small, particularly if pasteurized food lacks other contributing preservative fractions like low temperature, pH, and water activity etc. Thermoduric organisms such as spore formers survive pasteurizing temperature and hence, refrigeration of the product is a must (Khaterpaul, 2006).

2.2 MICROBIAL RISK ASSESSMENT

Problem of bacterial counts in processed fishery products is one of the most perplexing things that a processor faces. Bacterial contamination can occur in more than one way. Organisms present in slime, gill, and gut find entry in to flesh of fish. Meat may become contaminated with heavy bacterial load (Velankar *et al.*, 1961). Survey of the microbiological quality of commercial frozen prawn products was carried out by Pillai *et al.* (1965). Govindan (1966) reported that personal hygiene was very important. People who suffered from any sort of disease or infected wounds and cuts should not be allowed to work in food processing industries.

Major single factor that causes spoilage in fish products is bacteria. Apart from organisms in raw meat it may pick up bacteria from surrounding during different stages of preservation and processing. Contamination can take place from boat decks, preprocess centre, utensils, water, ice etc. and at processing factories until raw material is processed into finished form (Iyer *et al.*, 1966).

In 1977, the food and agricultural organization (FAO) published its code for fresh fish which states fish as an extremely perishable food and should be handled at all times with great care in such a way so as to inhibit the growth of microorganisms. Fish quality deteriorates rapidly and potential keeping time is shortened if they are not handled and stored properly. Much of all fish landed for human consumption is

subjected to rough treatment. Fish should not be exposed to direct sunlight or to drying effect of wind but should be carefully cleaned and cooled down to temperature of melting ice, 0°C as quickly as possible . Changes during storage of sardine in ice with particular reference to bacterial load have been carried out by Krishnakumar (1985).

Bryon (1980) reported that different factors affect the microbial quality of fish. Purity of ice and water is very important as washing and ice during melting will carry away with it the surface bacteria and slime thereby improving quality. But if bacterial quality of ice and water is poor it will increase microbial load on contact. The presence of faecal coliforms is not only objectionable but also will be regarded as not fit for human consumption. There are about 107 cases of *Staphylococcus* intoxication outbreaks in USA due to consumption of contaminated fish. Surendran and Gopakumar (1982) studied bacteriology of oil sardine and mackerel caught from tropical waters off Cochin. In Japan, where dishes based on raw seafood are extremely popular, about 70% of food poisonings that occur in summer months is due to bacterial pathogens (Joseph *et al.*, 1982). Valsan *et al.* (1985) isolated *Salmonella* from shrimps sold in Bombay markets. In India, reports of *Salmonella* isolation from fishery products were limited to those by Iyer *et al.* (1986) in the samples from Bombay markets, Iyer and Srivastava (1989) from shrimp, lobsters, cuttle fish, seer fish and catfish. Sanjeev and Iyer (1988) isolated Staphylococcus aureus from palms and throats of workers in processing plant.

Salmonellosis is an important food borne disease and accounts for all outbreaks of such disease where the causative agent is identified (Anon, 1988). Over 2000 *Salmonella* serotypes are known today and in India more than 160 serotypes have been isolated from various sources. Occurrence of *Salmonella* in meat has been reported by Bacchil and Jaiswal (1988) and in fishery products by Iyer and Srivastava (1989). In Ireland thermophilic *Campylobacter spp.* were found in 42% of 380 shellfish (Arumugaswamy and Proudford, 1987). Food poisoning may occur if product is handled carelessly during processing, resulting in high multiplication ($>1\times 10^5$cfu/ g) (Varnam and Evans, 1991; Vishwanath *et al.*, 1998).

In USA, Salmonellosis accounts for about 60% of all bacterial disease outbreaks (Bean *et al.*, 1990). In a study conducted by Nambiar and Iyer (1990) incidence of *Salmonella* was more in grey mullet, pink perch, tilapia, sardine and pearl spot found in retail trade in Kochi. All the serotypes isolated were pathogenic to human beings and it stressed on great significance from point of view of health hazards. *Salmonella* is sensitive to heat; hence cross contamination can occur in case of food items not subjected to heat treatment.

Hatha and Lakshmanaperumalsamy (1997) reported that in a developing country like India, there is no such constant monitoring system and the numbers of exact cases are not known. Fish, molluscs and marine crustaceans were implicated as causes of food borne outbreaks. Quality standards for fish in domestic trade require detailed background and in depth studies. Fishery products have been recognized as a major carrier of food borne pathogens like *Salmonella, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Yersinia enterocolitica, Listeria monocytogenes, Campylobacter jejumi,* and *Escherichia coli* (Venugopal *et al.*, 1999).

Anandavally (2000) reported that fish harvested from natural water bodies including farms harbours large number of microorganisms. These microorganisms can be pathogenic or non pathogenic. To safeguard human health it is necessary to identify appropriate interventions to prevent, reduce or eliminate risks. HACCP is widely used to control contaminants in food. But the challenge is to control pathogens or toxins produced by them. Microbiological risk assessment has evolved within food regulatory agencies and academia of a few developed countries.

2.3 PACKAGING

Years ago seafood packaging consisted of little more than paper bags and plastic wraps. But the last five years have seen a transformation in seafood industry.

New merchandising methods and better packaging suited to the supermarket trade, have been introduced for nearly all other types of food (Robinson, 2000).

Castell (1958) carried out an experiment, in which wrapped and unwrapped cod fillets were stored at temperature between -1 and 0°C. It was found that on the 5th day of storage, wrapped fillets had very offensive odour as compared with unwrapped lot. British research workers (Anon, 1962) claim that shelf life of prepackaged cod and a haddock fillet is markedly dependant on packaging materials. Anon (1963) experimented with vacuum packed haddock fillets and found that lesser the permeability of the package to carbon dioxide and oxygen, longer the shelf life.

Investigations suggest that efficacy of vacuum packaging or any other processing treatment may vary with composition of fish. Combination procedures integrating irradiation with vacuum packaging have been developed for controlling rancidity in radiation pasteurized *Petrale sole* (Spinelli *et al.*, 1965). Learson *et al.* (1969) observed that the development of rancidity in radiation sterilized cod, halibut, ocean perch and flounder fillets could be suppressed by vacuum packaging , browning of these foods at ambient temperature however could not be arrested.

The shelf life of products can be considerably extended by vacuum packaging in a film of low gas permeability, and the product, method of packing and storage conditions all affect the storage life and type of spoilage that eventually occurs (Newton, 1977). Fish being a perishable food item, has relatively very low shelf life. There is currently considerable interest in shelf life of chilled, packed fresh fish for supermarket/ retail market (Meekin *et al.*, 1982). However vacuum packaging can deform the products by film tightening and cause temporary discolouration (Gram, 1983).

Vacuum packaging can delay the growth of aerobic spoilage microorganisms in meat products (Genigeorgis, 1985). Vacuum packaging Vienna sausages, treated by a combination of in package pasteurization and surface application of an organic acid combination improved shelf life of the product (Dykes *et al.*, 1996).

Vacuum packaging, in fact, greatly delays any deterioration caused by ambient air; it is an obstacle to the development of bacteria and moulds and markedly reduces negative effects of temperature changes. Packaging in airless space also gives a slow ripening effect, thus protecting the organoleptic qualities of the meat. As with any packaging method, the success of vacuum packaging depends on quality of raw material and methods of storage (Robinson, 2000). Prafulla *et al.* (2000) observed that indirect icing (samples separated by thin polyethene sheets) preserved most of the nutrients in squid and cuttle fish but a mixture of salt and ice gave better quality products.

Combination of different factors *viz,* vacuum packaging treatment with preservative (sodium acetate) and storage at refrigerated temperature could be used to prolong the shelf life of fish fillets to a great extent (Shalini *et al.*, 2000). Seafoods like all food commodities have their own distinctive microbiology. Growth of *Listeria monocytogenes* inoculated into trout was suppressed by 2 log cycles after irradiation (2kGy) and vacuum package storage at 4°C (Ioannis *et al.*, 2002).

Two common types of packaging used by seafood processors are air tight vacuum packs and windowed tray packs. Vacuum packs are used to extend shelf life by retarding oxidation, hydration and rancidity. This packaging method is more useful for boneless product, as spines may puncture the film and destroy vacuum (Gopal, 2002). Study by Padmakumar *et al.* (2003) indicates that vacuum packaging of tandoori chicken and storage at low temperature considerably improves the shelf life of product without significantly affecting the sensory quality of product.

Kamat *et al.* (1972) found that vacuum packed fatty fishes did not show appreciable rise in indices of oxidative changes as function of radiation dose and remained free from development of rancid odour and yellow discolouration. Studies conducted by Shenoy and James (1974) showed that fillets and chunks of seer had a longer shelf life when packed and then stored in ice compared to seer fillets, chunks stored in direct contact with ice.

2.4 CHILLING:

All species of fish when properly chilled will stay fresh for longer periods than those not preserved in any way. The use of chilling techniques such as ice prolongs the length of time available for fishing trips. Properly chilled fish will prevent spoilage bacteria from multiplying (Gopal, 2002).

Icing is accompanied by loss of nutrients from the fish and flavour and textural changes to meat, ultimately rendering the raw material unsuitable for further processing (Govindan, 1962, 1964; Velankar *et al.*, 1961). Venkataraman *et al.* (1968) have studied the storage characteristics of freshly caught silver pomfrets stored in ice and found that they remained in good condition for a period of 14 days in properly iced conditions. The results on the storage characteristics of the transported fish stored in ice reveals that the samples were in good conditions only for 2 days, as judged by physical, chemical, organoleptic tests.

Fluctuating temperatures and icing have considerably affected the keeping quality of fish in storage. Susamma and Nair (1969) found fall in nucleotide concentration in samples at a slow rate up to 2 days of ice storage and thereafter it proceeded at much faster rate. According to Flores and Crawford (1973) chemical changes in ice stored seafoods were mediated by a combination of bacterial action and endogenous enzymatic activity.

Solanki and Venkataraman (1978) reported that brining of fillets prior to ice storage improved the quality and enhanced shelf life of fillets considerably, especially under packaged condition. Packaging of control fillets with polythene bag did not improve their storage life; instead these samples showed early spoilage within five days of storage. Lower bacterial counts was found in ice stored fresh and brined fillets which might be due to the washing effect of ice melt water on microbial flora. In a developing tropical country like India where frozen fish industry for internal market might not flourish in near future due to lack of facility of

cold chain; the preservation of fish by ice would play an important role in the distribution of fish. The fish held in ice for different periods ranging from 0 to14 days and then frozen, showed shelf life of less than 2 to 30 weeks at -18°C in an inverse relation to the period of storage of fresh fish in ice (Joseph *et al.*, 1980).

Jensen and Hansen (1983) found that chilled seawater system storage of fish helped in reducing belly bursting in fish. It is necessary to know various changes taking place in fish during handling and chill storage to improve quality of fish when it reaches customer. In general the transported fish can be effectively stored in ice for two days.

Govindan (1985) reported that an average consumer prefers fresh fish to iced fish showing some chemical indices of spoilage. This is probably due to leaching of some constituents by ice melt water. Leaching may remove components for good organoleptic characteristics.

2.5 STORAGE STUDIES:

Govindan (1985) found that icing of fish was an integral part of fish processing industry; the quantity of ice to be used depended on period of preservation. Such icing procedures were invariably accompanied by several changes in the composition of the edible muscle. Ice lowered the temperature of fish to its melting point and destroyed mesophilic microbial load. Partmann (1965) reported that fish stored in ice underwent various organoleptic changes. Fish stored in ice for 19 days was found to be very soft and due to pressure of ice there was fall in moisture level. Water holding capacity of fish muscle decreased during rigor development hence there was increase in amount of water exuded even at low temperature.

Volatile base nitrogen (VBN) and Total Volatile Base - nitrogen (TVB-N) increased to spoilage level were found to be high in ice when compared to chilled sea water (Connell, 1975). Native flora of mackerel underwent significant changes

during storage in ice. After storage for 21 days, the flora was constituted by 74% Pseudomonas and 15% *Achromobacter* (Surendran *et al.*, 1976).

During a study done on iced and frozen storage of squid it was found that total bacterial count throughout 6 days of storage indicated no bacterial spoilage. But significant changes were observed in organoleptic tests of cooked meats. Based on biochemical and organoleptic changes it could be seen that squid meat could not be kept in ice for more than 2 days (Verma and Venkataraman, 1977).

Solanki *et al.* (1977) found chilled storage using ice to be very important aspect in case of fishes used for further processing. The freshness of fish, being related to the period of ice storage prior to subsequent processing, determined to a large extent the quality of the processed fish reaching the customer. In case of Perch stored in ice used for canning, it was found that initial bacterial load of fish was very less. Pathogens were absent. Total plate count increased as storage period increased, revealing advancement of spoilage of fish. The rise in bacterial count was observed to be accompanied by rise in TMA content of muscle which was very significant. Fish kept in ice for 7 days was not found suitable for canning process, due to higher loss of nitrogenous material and organoleptic quality. Similar work has been carried out in case of mackerel and sardine by Madhavan *et al.* (1970).

Garg *et al.* (1982) reported that bacterial changes during iced storage of *Kati* were less till 9^{th} day but there was large increase during the 11^{th} and 13^{th} day. Total bacterial load which was 4.18×10^4 initially, decreased to 1.5×10^3 by 5^{th} day. Thereafter it gradually increased to 5.45×10^5 by 13^{th} day. Texture of fish was firm up to 7 days. Beyond this period it indicated perceptible sign of softening and by 13^{th} day it became very soft.

Subrata *et al.* (1985) reported that packed samples showed gradual increase in TVBN content. This high initial value and very slow increase of volatile bases indicated leaching of volatile nitrogen materials by ice water. TVB-N values in packed sample were attributed to species characteristics of the brackish water fish.

Since rate of percentage leaching of total volatile nitrogen material was very high, it could not be considered as an index of spoilage in the case of fish stored in ice in direct contact. In unpacked samples most of the volatile nitrogenous compounds were leached out while in case of the packed samples all the volatile nitrogenous compounds produced during ice storage were present in the muscle as leaching was absent. From organoleptic evaluation it was seen that packed sample was more acceptable than unpacked ones. In both packed and unpacked samples, initially a definite decrease in total count was observed due to chilling effect of ice on bacteria, thereafter the count decreased. Several studies on iced storage of fish have been carried out. Quality changes in sardine in ice were studied by Ababouch *et al.* (1996).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. COLLECTION OF FISH

Market fresh *Selar crumenophthalmus* (Bigeye scad) and *Oreochromis mossambicus* (Tilapia) was purchased from local market and transported in iced condition to the laboratory.

3.2. PREPARATION OF COOK- CHILL FISH:

The whole fish (*Selar crumenophthalmus*) were beheaded, eviscerated, and filleted. The fillets were immersed in potable water, drained and then immersed in equal amount of solution of common salt for flavour development at room temperature. The fillets were then drained. Salted fillets were blanched at 75°C for 90 sec. After cooling the fillets were packed in polythene packets, heat sealed using electric heat sealer and chilled stored at 4°C.

3.2.1 STANDARDIZATION OF METHOD:

The method of cook-chill process was standardized based on following parameters; brine concentration and brining time, blanching time. Sensory evaluation was carried out by a panel consisting of ten judges. Quality was evaluated on the basis of colour, taste, texture, appearance and overall score using a ten point scale. Total plate count was also carried out.

3.2.2 BRINING CONDITIONS:

In the standardization of brining conditions, both brine concentration and brining time were standardized. The treatment combinations were T_1 (3% for 5 min), T_2 (3% for 10min), T_3 (3% for 15 min), T_4 (5% for 5min), T_5 (5% for 10min), T_6 (5% for 15min), T_7 (6% for 5min), T_8 (6% for 10min), T_9 (6% for 15min). Samples

were steam cooked and subjected to sensory evaluations. The samples that were found most acceptable to taste panel were selected for further studies.

3.2.3 BLANCHING CONDITIONS:

After salting different lots of fillets were subjected to following blanching conditions- 75°C for 60 sec, 75°C for 90 sec and 75°C for 120 sec. Samples were subjected to both sensory evaluation as well as TPC.

3.2.4 PREPARATION OF STANDARD PRODUCT:

Standardization of brining, blanching were done as described above. The whole fish were beheaded, eviscerated, and filleted. The fillets were washed in potable water, drained and then immersed in 5% salt solution for 10 min. The fillets were drained and then blanched at 75°C for 90 secs. After cooling fillets were packed in polyethene bag, heat sealed using electric heat sealer and chilled stored using ice.

3.3 PREPARATION OF PARTIALLY PROCESSED VALUE ADDED FISH:

Fresh fish (*Oreochromis mossambicus*) were beheaded, eviscerated and steaked. Steaks were washed in potable water, drained, immersed in common salt solution at ambient temperature. The salted steaks were then vacuum packed and chilled stored at 4°C.

3.3.1 STANDARDIZATION OF METHOD:

The method for preparation of partially processed value added fish was standardized based on brining concentration and time. Sensory evaluation was conducted by a panel of ten judges.

3.3.2 BRINING CONDITIONS:

In the standardization of brining conditions both brine concentrations and brining time were standardized. The treatment combinations were A_1 (1% for 5min), A_2 (1% 10min), A_3 (1% 15min), A_4 (2% for 5min), A_5 (2% for 10min), A_6 (2% for 15min), A_7 (3% for 5min), A_8 (3% for 10min), A_9 (3% for 15min). Samples were steam cooked and subjected to sensory evaluation. The samples that were found most acceptable to taste panel were selected for further studies.

3.3.3 PREPARATION OF STANDARD PRODUCT:

Fresh fish were beheaded, eviscerated and steaked. Steaks were washed in potable water, drained, immersed in common salt solution of 2% for 10min at ambient temperature. The salted steaks were then vacuum packed in 12μ polyester laminated with polyethylene 200 gauge with a vacuum of 650mm Hg using vacuum heat seal (Sevena make) and chilled stored in ice at 4°C.

Controls for both the products were not given any treatment given to the test. In case of cook-chill fish control used was not given any brine, heat treatment. Partially processed value added fish, control used was fish steaks packed in polyethylene and sealed using electric heat sealer. Before carrying out storage study the proximate composition of fish used for product preparation was carried out. Test for specific microorganism was also carried out.

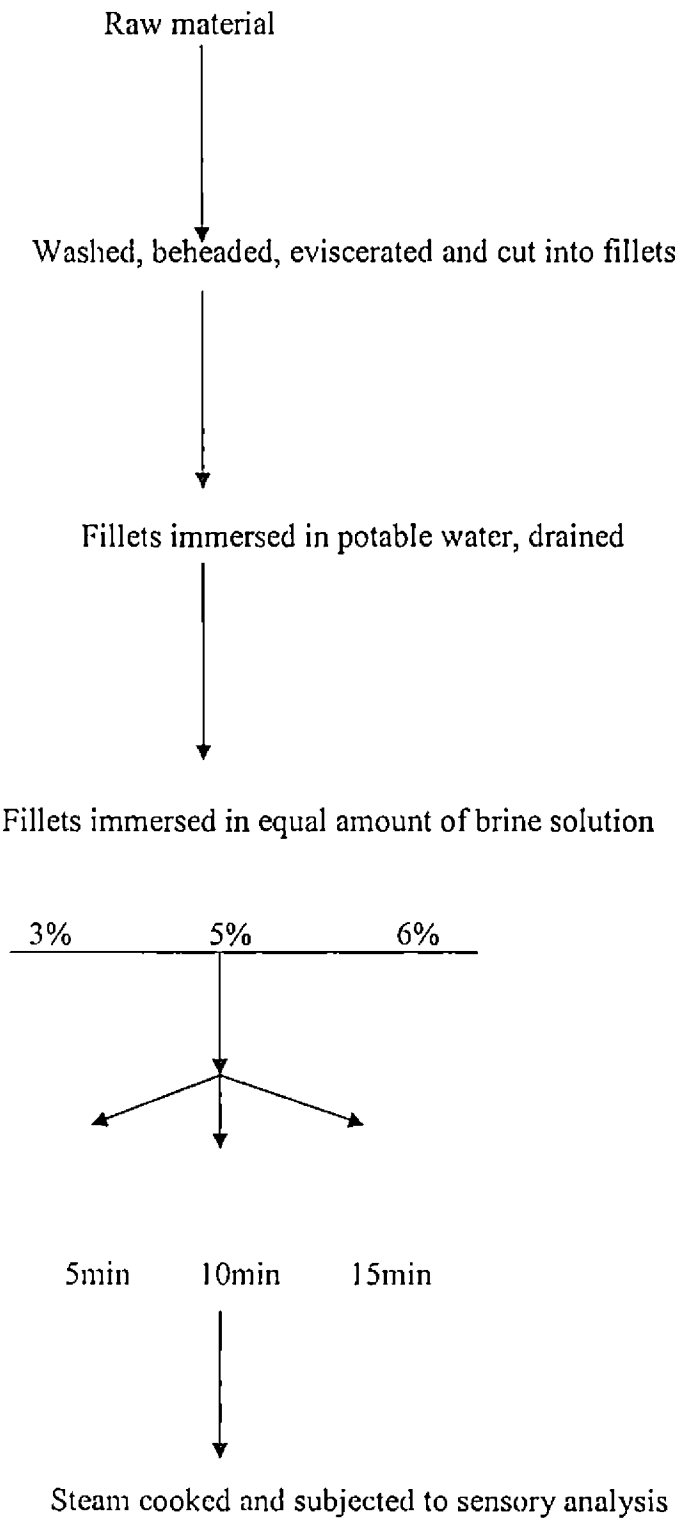

Fig1. Flow chart for standardization of brining for cook-chill fish.

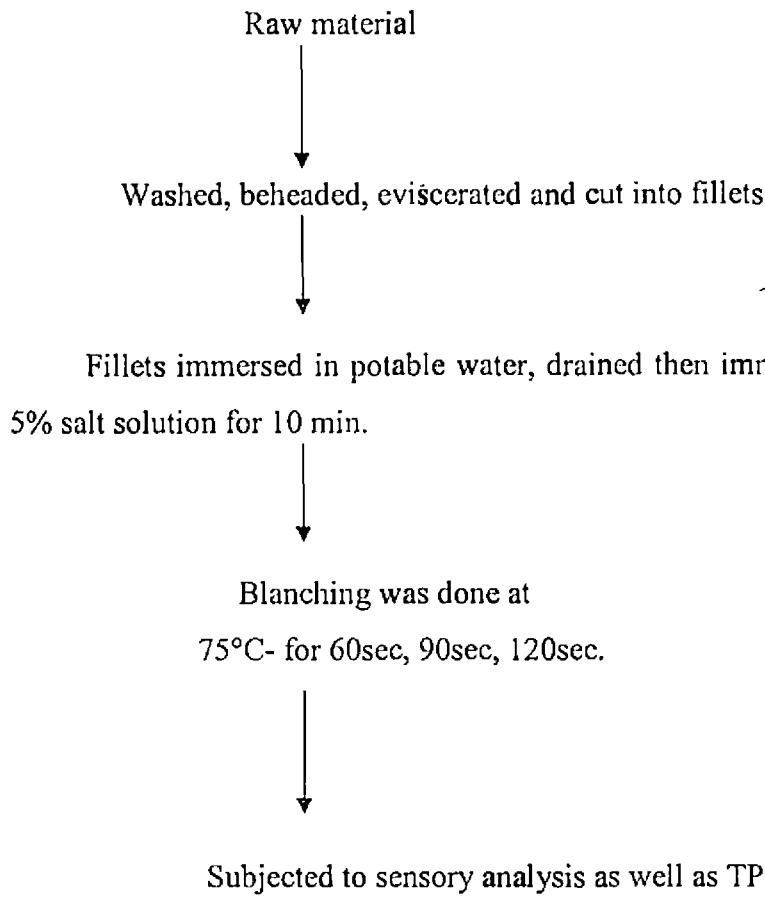

Fig 2. Flow chart for standardization of blanching time for cook-chill fish.

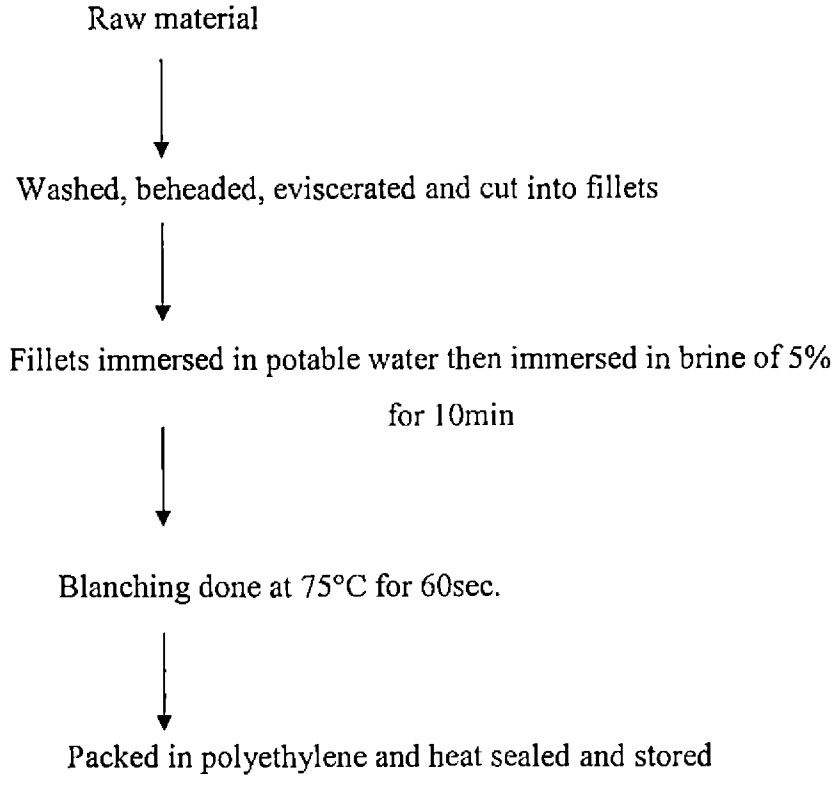

Fig 3. Flow chart for preparation of cook-chill fish (standard product).

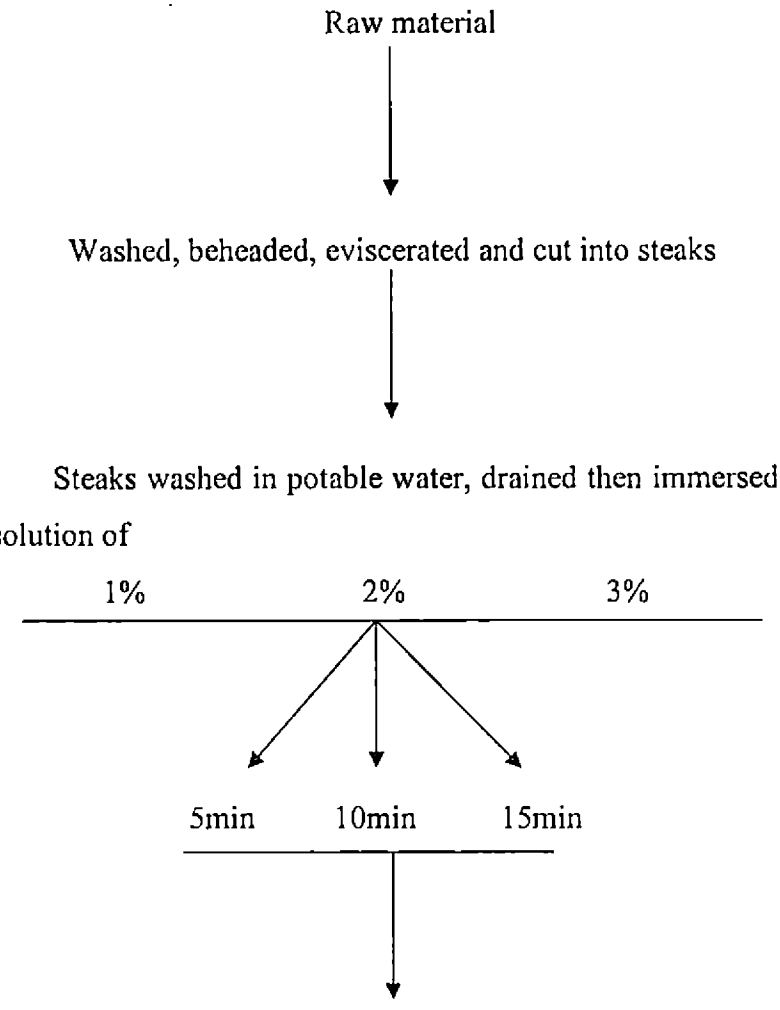

Fig 4. Flow chart for standardization of brining for partially processed value added fish.

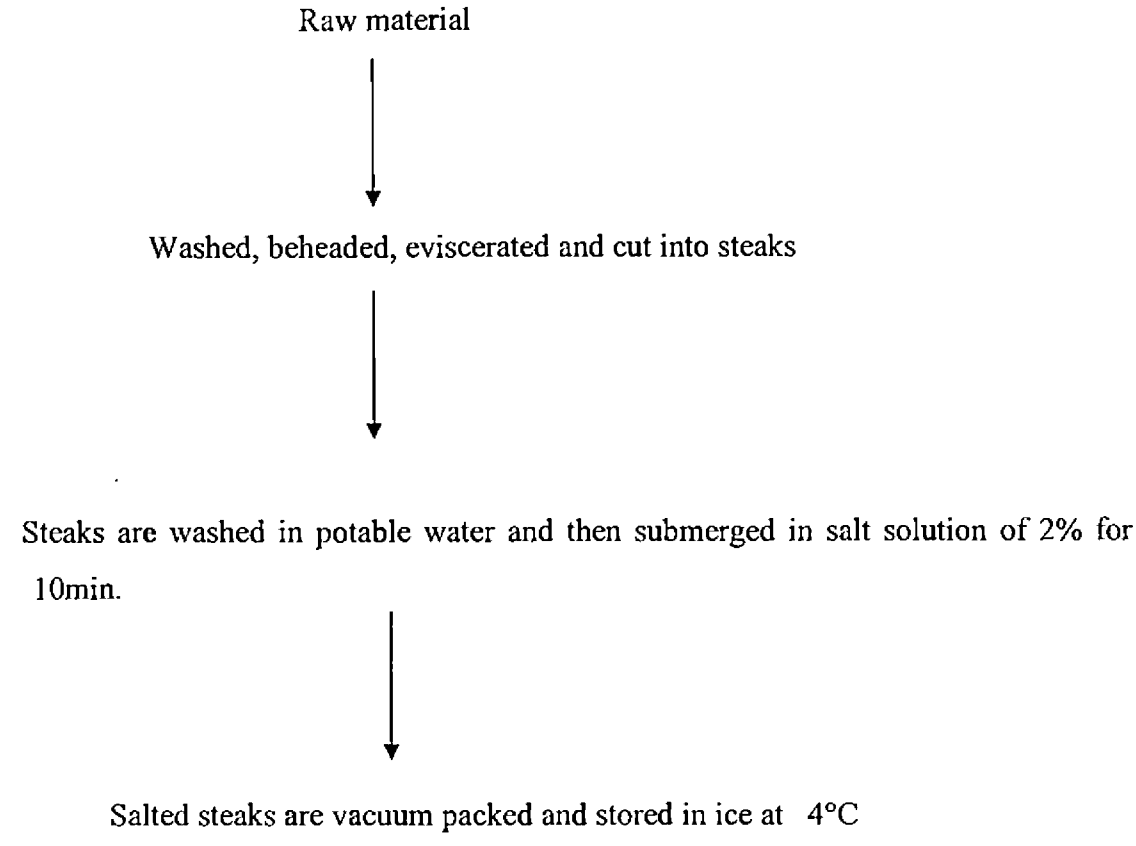

Fig5. Flow chart for preparation of partially processed value added fish

3.4 PROXIMATE COMPOSITION OF FISH USED FOR PREPARATION OF PRODUCTS

3.4.1 Moisture content

Moisture content of products was determined by the method of AOAC (1975). About 5gm of sample was accurately weighed in a clean dry petridish using electric balance and was dried to a constant weight at a temperature of 100°C in a hot air oven. The dried material was cooled in a dessicator. The moisture content was calculated as the percentage loss of weight of the sample upon drying.

3.4.2 Protein content

Protein content of the samples was estimated by the Microkjeldahl's method (AOAC, 1984).

One gram of sample was accurately weighed and transferred to a Kjeldahl's flask. A pinch of digestion mixture ($CuSO_4$: K_2 SO_4 = 1 : 8) and 10ml of concentrated H_2SO_4 were added and digested by heating at a temperature of 100°C for 12hrs over a heating mantle. About 25ml of distilled water was then carefully poured into the flask along the side. The flask was swirled to dissipate off the heat evolved. When the solution attained room temperature, it was quantitatively transferred to a 50ml standard flask with distilled water washings. The solution was then made up to 50ml using distilled water and mixed thoroughly. Five ml of this solution was subjected to distillation using a distillation unit ('Kjelplus' make). 10ml 10N NaOH solution was added to the sample solution for distillation. The vapors were collected in 5ml of 2% boric acid that was previously mixed with 2 drops of Tashiro's indicator. The boric acid was titrated against standard N/70 H_2SO_4 to the pink end point.

$$\text{Protein\%} = \frac{V \times 14 \times 100 \times 50 \times 6.25}{1000 \times 70 \times 5 \times W}$$

Where,

V= volume of N/70H_2SO_4

W= weight of sample.

3.4.3 Ash content

Ash content of fresh fish was estimated by the method of AOAC (1984). About 3g of dried sample was weighed accurately in a silica crucible. It was then ignited in a muffle furnace at a temperature of 550°C until sample was free of carbon. It was then allowed to cool in a dessicator and weighed. The difference in weight was expressed as a percentage of sample weight to denote total ash.

3.4.4 Fat content

The Soxhlet method of fat content estimation was followed (AOAC, 1990). A sample of about 2g of moisture free minced meat was taken into an extraction thimble. The electric heating unit was adjusted so that the solvent, petroleum ether (60°C-80°C), siphons over 5-6 times per hour. Heating was done in a water bath. The extraction was carried out for 16-20hrs. The solution was then transferred to a pre weighed beaker and evaporated off on a boiling water bath, then cooled to room temperature in a dessicator and weighed. The difference in weight was expressed as percentage of sample weight to denote fat content.

3.4.5 Salt content

Salt content of product was estimated by the method of AOAC (1970). A sample of 2g of brined fish product was weighed accurately, disintegrated well and transferred into 250ml conical flask. Added 15ml of concentrated nitric acid, 25ml of 0.1N silver nitrate. Digested the sample in a sand bath until the solution is clear. After digestion the solution was cooled. 50ml of distilled water, 2ml of nitrobenzene and 1ml of ferric alum indicator were added. The contents were titrated against 0.1 std ammonium thiocyanate solution until solution becomes brick red.

0.1ml of 0.1N $AgNO_3$ is equivalent to 0.00585g NaCl

The results are expressed as

$$\% \text{ NaCl} = 5.85 \times \text{Volume of } NH_4 \text{ CNS} \times \frac{\text{Normality of } NH_4 \text{ CNS equivalent to reacted } AgNO_3}{\text{Weight of the sample}}$$

3.5 STORAGE STUDIES:

Both the products were prepared, according to the standard process. Cook-chill fish was packed in polyethylene bags and partially processed value added fish was vacuum packed. Both products were chilled stored using ice and temperature was maintained at 4°C.

Different tests were carried out during storage. Before carrying out storage study the proximate composition of fish used for product preparation was also carried out. Test for specific microorganism was carried out.

3.5.1 Total Volatile Base Nitrogen- (TVB-N)

TVB-N content was determined by Conway's micro-diffusion method (Conway, 1947). A sample of 10g of the product was extracted in 10% trichloroacetic acid (TCA) solution. One ml of this extract was pipetted out into outer chamber of Conway's unit and 1 ml of standard 0.02N H_2SO_4 solution was taken in the inner chamber. Then 1 ml of saturated sodium carbonate solution was added to the outer chamber. The unit was closed immediately. The solution in outer chamber was mixed by slow rotation of the apparatus. It was then kept at room temperature overnight. The content of inner chamber was titrated against standard 0.02N NaOH solution using Tashiro's indicator. A blank was also run using 1ml of 10% TCA solution in outer chamber instead of sample extract.

$$\text{TVB-N (mg \%)} = \frac{(A-B) \times .28 \times 50 \times 100}{W}$$

Where A= volume of NaOH solution required for control.

B= volume of NaOH solution required for test.

W= weight of sample.

3.5.2 Trimethyl Amine (TMA) content

TMA content was determined by Conway's micro-diffusion method (Conway, 1947). The TCA extract prepared for determination of TVBN was used for TMA determination also. The procedure followed was similar to that of TVBN, except that 1ml of formaldehyde was added to TCA extract in the outer chamber of the micro-diffusion unit before adding sodium carbonate solution.

$$\text{TMB-N (mg \%)} = \frac{(A-B) \times .28 \times 50 \times 100}{W}$$

Where A= volume of NaOH solution required for control.

B= volume of NaOH solution required for test.

W= weight of sample.

3.5.3 Total Plate Count

TPC was determined by the method of Maturin and Peeler (1995). A sample of 10g of product was aseptically weighed and transferred to sterile sample dish. The weighed sample was transferred to sterile mortar and homogenized with 90ml of sterile normal saline. Serial decimal dilution was carried out to get appropriate dilution for plating so that the number of colonies in a petridish is between 30 and 300. 1ml each from the required dilutions was transferred to separate sterile Petri dishes. 10-15ml of sterile plate count agar cooled to 40°C was added to each

petridish. Plates were incubated at 37°C for48 hrs and colonies were counted as follows

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

3.5.4 Test for specific organisms

Test for specific microorganisms i.e. ***Salmonella***, ***Vibrio cholerae*** ***Staphylococcus aureus***, Total coliforms and ***Escherichia coli*** was carried out by the method of Surendran *et al.* (2006).

3.5.4.1 *Salmonella*

25g of the fish sample was macerated with 225ml of pre-enrichment medium lactose broth. Incubate at 36±1°C for 18-24hr. 1ml of culture from the pre-enrichment medium was pipetted out into 10ml of selective enrichment medium tetrathionate broth and incubated at 36+1°C for 18-24hrs.

One loopful from selective enrichment medium was streaked onto pre-dried selective plating medium, Bismuth Sulphite Agar (BSA) and incubated at 36+1°C for 24hrs.

Plates were examined for typical Salmonella colonies. On BSA these appear as brown, grey with metallic sheen, surrounding medium brown to black.

3.5.4.2 *Vibrio cholerae*

25g of sample was blended with 225ml Alkaline Peptone Water(APW); and transferred aseptically to a sterile 500ml conical flask and incubated at 36±1°C.

After it is incubated for 6-8hrs and 16-24hrs (do not shake the flask) Loopful from surface growth (pellicle) is streaked on to pre set Thiosulphate Citrate Bile salt Sucrose agar (TCBS)and Incubated at 36±1°C for 18-24hrs. The plates were

examined for typical *V. cholerae* colonies. Typical *V. cholerae* colonies are large (2-3mm dia), smooth, yellow slightly flattened with opaque centres and translucent peripheries (*Vibrio* spp. do not form tiny, creamy yellow colonies on TCBS).

3.5.4.3 *Staphylococcus aureus*

About 25g of fish sample was taken aseptically and homogenised with 225ml of 0.85% saline diluent. 0.1ml of the inoculum was plated onto the Baird Parker agar plates and spreaded using bent glass rods. The plates were incubated at 37°C for 24-48hrs. The colonies of Staphylococcus aureus appears as black, convex, 1-1.5mm diameter, narrow white entire margin and surrounded by a mark of clearing2-5mm in width.

3.5.4.4 Total coliforms and *Escherichia coli*

15 tubes were taken and Durham tubes were placed inside each test tube in the inverted position. 5 tubes of 10ml of double strength and 10 tubes of 10ml single strength Lauryl Sulphate Tryptone broth were prepared and sterilized at 110°C for 10min. The broth were cooled and used for inoculation of samples. 25g of fish sample was taken and homogenized with 225ml of physiological saline. 10ml of 1in 10ml dilution of sample was inoculated in to 5 tubes of double strength medium, 1ml of 1in 10mldilution of sample was inoculated in to 5 tubes of single strength medium, 0.1ml of 1in 10mldilution of sample was inoculated in to 5 tubes of single strength medium. All tubes were incubated at 37°C for 24hr. The tubes were observed for gas formation and acid production. This test is presumptive test, which gives total coliforms.

E. coli

About 25g of fish sample was taken aseptically and homogenised with 225ml of physiological saline, which gave 10^{-1} dilution. 0.1ml of inoculum from the

dilution was spread onto Tergitol-7 agar plates. The plates were incubated at 37°C for 48hrs. Positive E. coli appear as circular, non mucoid, flat yellow with pinkish tinge.

3.5.5 Sensory evaluation

A taste panel consisting of ten judges carried out sensory evaluation of the product sample. The quality characteristics assessed were colour, odour, taste, texture, appearance. Scoring was done on the basis of 10 point scale. Score points given were 9-10, 7-8, 6-5, 4-3, and 0 for excellent, very good, good, borderline of acceptability and poor. The format of sensory evaluation score sheet is given in Appendix I and Appendix II.

3.6 STATISTICAL ANALYSIS:

The experiments were carried out using Completely Randomised Design (CRD). Data obtained during chilled storage of products were analyzed using two ways Analysis of Variance (ANOVA) with observations per cell technique. Pair wise comparison of treatments done wherever necessary using least significant difference. Sensory evaluation results were analyzed using Friedman test (Sprent, 1989).

RESULTS

4. RESULTS

4.1 PROXIMATE COMPOSITION:

Proximate composition of both species was carried out and is given in Table 1.

Table1. Results of proximate composition of *Selar crumenophthalmus* and *Oreochromis mossambicus*

Components	*Selar crumenophthalmus*	*Oreochromis mossambicus*
Moisture (%)	67.37	75.35
Protein (%)	25.14	20.70
Fat (%)	5.82	1.8
Ash (%)	0.84	1.27

4.2. STANDARDIZATION OF COOK-CHILL FISH

4.2.1 Brining concentration and brining time:

Standardization of brining time and brining concentration of cook-chill fish was carried out by using the following treatment combinations 3%, 5%, 6% for 5min, 10min, and 15mins. Proportion of brine to fillets used was 1:1 (for 1kg of fish fillets 1litre brine was used). Sensory analysis carried out showed the best treatment as 5% for 10min. Statistical analysis by Friedman's test showed significant difference between treatments. Salt content of product was analyzed and is given in Table 2. Average sensory evaluation scores for different brining conditions of fish fillets for cook-chill fish is given in Table 3.

Table 2. Salt content (absorbed by product) of cook-chill fish (*Selar crumenophthalmus*) for different brining concentration and brining time.

	Salt content (%) at different brining concentration		
Time in(min)	3%	5%	6%
5	0.263	0.585	0.555
10	0.409	0.789	0.936
15	0.812	1.220	1.55

Table 3. Average sensory evaluation scores for different brining condition of fish fillets (*Selar crumenophthalmus*) for cook-chill fish.

Brine concentration in (%)	Time in (min)	Colour	Odour	Taste	Texture	Appearance
3	5	7.28	7.67	6.92	6.97	6.5
3	10	7.59	7.21	6.72	7.27	6.4
3	15	7.66	7.66	7.03	7.17	6.2
5	5	7.02	7.45	7.42	7.32	6.3
5	10	7.56	7.82	8.01	7.75	6.5
5	15	7.47	7.05	7.14	7.57	6.1
6	5	7.3	7.13	7.68	7.34	6.2
6	10	6.85	7.01	7.52	7.39	6.3
6	15	7.77	7.2	7.32	7.18	6.3

4.2.2. COOKING TIME

Standardization of cooking time in the case of cook-chill fish was carried out. The treatment combinations given were blanching at 75°C for 60sec, 90sec, and 120sec. Both sensory evaluation as well as TPC was carried out to decide on the best treatment. It was observed that 120sec gave lowest TPC but the texture of the product was affected greatly hence blanching time of 90sec was selected as it gave lower TPC compared to raw fillets and had good sensory score. Statistical analysis by Friedman's test showed significant difference between treatments. The TPC of fish fillets after various blanching conditions are shown in Table 4. ANOVA for TPC of products after different cooking time is given in Table 5. Average sensory evaluation score is given in Table 6.

Table 4. Total Plate Count of fish fillets (*Selar crumenophthalmus*) for different cooking time.

Treatments	TPC (cfu/ml)				
Control	$3.55x10^4$	$5.07x10^4$	$5.2x10^4$	$5.11x10^4$	$5.55x10^4$
60sec	$4.0x10^3$	$4.12x10^3$	$5.00x10^3$	$4.50x10^3$	$6.00x10^3$
90sec	$2.11x10^3$	$2.22x10^3$	$4.11x10^3$	$2.6x10^3$	$2.29x10^3$
120sec	$5.5x10^1$	$2.2x10^2$	$2.1x10^2$	$2.5x10^2$	$4.0x10^1$

Table 5. ANOVA of TPC of fish fillets after different cooking time.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	17.22267	3	5.740891	138.1011*
Error	0.665123	16	0.04157	
Total	17.8878	19		

*Significant at 5% level
Critical Difference = 3.238

Table 6. Average sensory evaluation scores of fish fillets (*Selar crumenophthalmus*) for different cooking time

Treatments	Colour	Odour	Taste	Texture
75°C for 60sec	7.10	6.54	7.28	7.14
75°C for 90sec	7.66	7.64	7.67	7.70
75C for 120sec	7.28	7.5	7.38	7.18

4.3. STANDARDIZATION OF PARTIALLY PROCESSED VALUE ADDED FISH

4.3.1. Brining concentration and brining time:

Standardization for brining time and brining concentration was carried out in the case of partially processed value added fish. Treatment combinations used were 1%, 2%, 3% for 5min, 10min and 15mins and proportion of brine to fillets was 1:1 (for 1kg of fish fillets 1litre brine was used). Sensory analysis carried out showed the best treatment as 2% for 10min. Salt content of product was estimated and given in Table 7. Average sensory evaluation scores for different brining conditions of fish steaks are given in Table 8. Friedman's test showed significant difference between treatments.

Table 7. Salt content (absorbed by product) of partially processed value added fish (*Oreochromis mossambicus*) for different brining concentration and brining time.

	Salt content (%) at different brining concentration		
Time in(min)	1%	2%	3%
5	0.05	0.17	0.22
10	0.08	0.24	0.38
15	0.09	0.50	0.84

Table 8. Average sensory evaluation scores for different brining condition of fish steaks (*Oreochromis mossambicus*) for partially processed value added fish.

Brine concentration in (%)	Time in (min)	Colour	Odour	Taste	Texture	Appearance
1	5	7.06	8.06	7.43	7.43	6.23
1	10	7.43	8.13	7.43	7.43	6.53
1	15	7.23	7.86	7.53	7.53	6.8
2	5	7.53	7.5	7.2	7.2	6.86
2	10	7.8	8.06	7.96	7.96	7.1
2	15	7.7	7.6	7.4	7.4	7.06
3	5	7.5	7.8	7.16	7.16	7.0
3	10	7.7	7.73	7.06	7.06	7.06
3	15	7.8	7.26	7.03	7.03	7.03

4.4. STORAGE STUDIES:

4.4.1Total volatile base- nitrogen (TVB-N):

The TVB-N content showed a steady increase in the case of cook-chill fish. Compared to the test, control (sample without any treatment) showed greater values. In the case of cook-chill fish the value of TVB-N ranged from 3.54mg% to 10.7mg% in control and 2.64mg% to 10.45mg% in test. Table 9 shows the TVB-N content in cook-chill fish during chilled storage. Observation statistically analyzed showed significant difference between treatments and between storage periods. Interaction also showed significance. ANOVA for TVB-N content for cook-chill fish is given in Table 10. Changes in TVB-N content during storage period are given in Fig. 1.

Table 9. TVB-N content in cook-chill fish (*Selar crumenophthalmus*) during chilled storage.

TVB-N (mg%)

Treatments	Days				
	0	5	10	15	20
Control	3.54	7.28	9.99	10.45	10.69
Test	2.64	3.45	5.72	7.0	10.45

Table 10. ANOVA for TVB-N content in cook-chill fish.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	41.34828	1	41.34828	341.2888*
Between days (B)	183.8775	4	45.96939	379.4315*
Interaction (AxB)	26.02779	4	6.506947	53.70836*
Error	2.423067	20	0.121153	
Total	253.6767	29		

Significant at 5%
Critical Difference (days) = 0.452

Days:	0	5	10	15	20
Means:	3.59	5.86	7.86	8.72	10.59

Treatments	Control	Test
Means	8.402	6.054

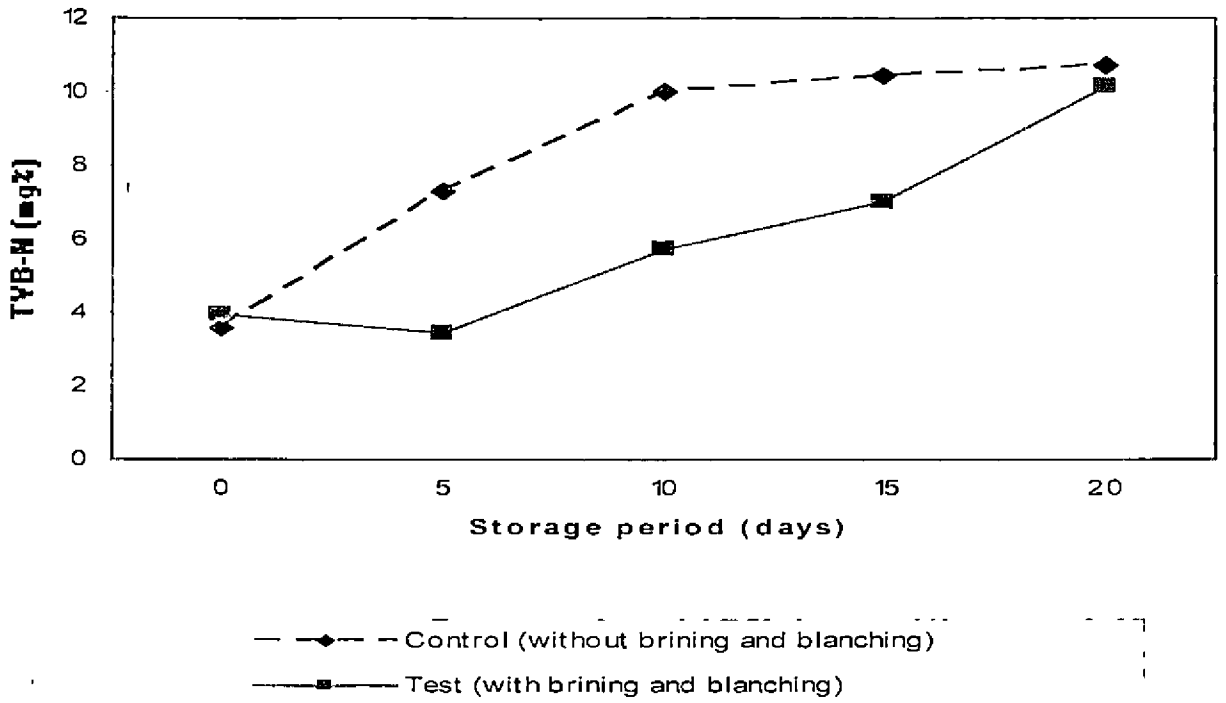

Fig 1: Changes in TVB-N content of cook-chill fish during chilled storage

TVB-N content in the case of partially processed value added fish also showed steady increase throughout the storage period. For partially processed value added fish TVB-N content was 2.24 mg% to 12.48 mg% in control and 2.24 mg% to 10.36 mg% in test. Test showed lower values for TVB-N content. Observation statistically analyzed showed significant difference between treatments and between storage periods. TVB-N content of the product during storage is shown in Table 11. ANOVA for TVB-N content of partially processed value added fish is given in Table 12. Changes in TVB-N content for partially processed value added fish during storage has been shown in Fig 2.

Table 11. TVB-N content of partially processed value added fish (*Oreochromis mossambicus*) during chilled storage.

TVB-N (mg %)

Treatments	Days				
	0	5	10	15	20
Test	2.24	2.8	5.6	8.4	10.36
Control	2.24	5.32	9.8	10.36	12.48

Table 12. ANOVA for TVB-N content in partially processed value added fish during chilled storage.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	13.27104	1	13.27104	7.90218*
Between days (B)	162.4351	4	40.60879	24.18032*
Error	6.71766	4	1.679415	
Total	182.4238	9		

*Significant at 5%
Critical Difference (days) = 3.59

Days:	0	5	10	15	20
Means:	2.24	4.06	7.7	9.38	11.42

Treatments	Control	Test
Means	8.04	5.88

Underlined means are not significantly different

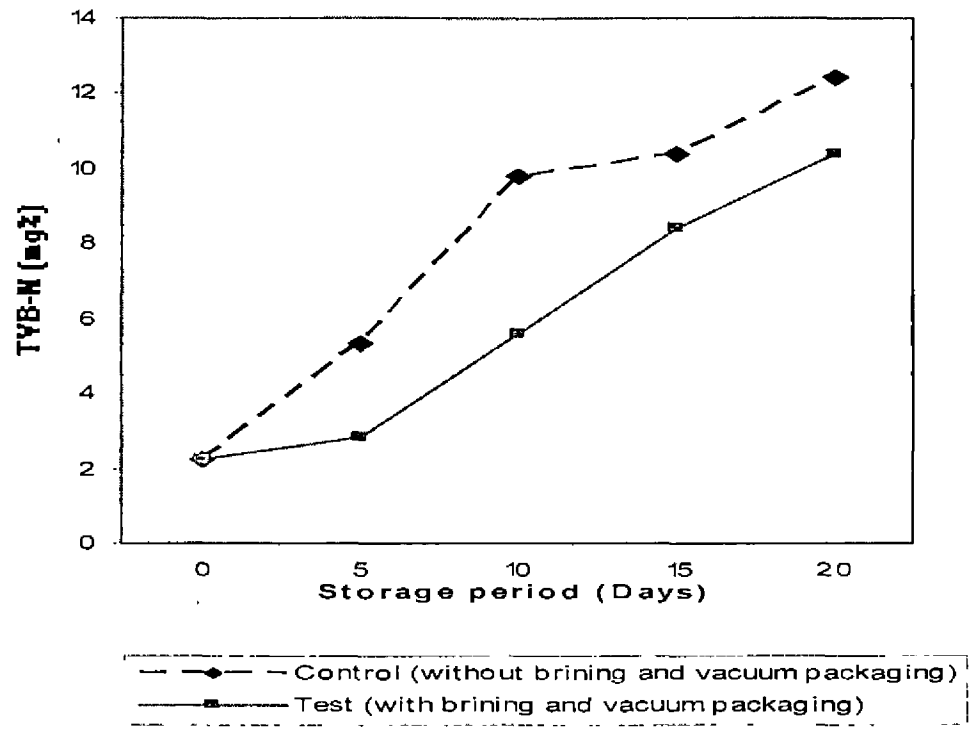

Fig 2:Changes in TVB-N content of partially processed value added fish during chilled storage

4.4.2 TRIMEMETHYL AMINE (TMA)

TMA content also showed steady increase in both the products. Compared to control, test showed lower values for TMA content. Control in the case of cook-chill fish ranged from 2.52 mg% to 6.25 mg% Test showed comparatively lower values i.e., 2.52mg% to 5.97mg%. TMA content of cook-chill fish during storage are given in Table 13. ANOVA for TMA content is shown in Table 14. Significant difference was observed between treatments and between storage days. Change in TMA content during storage is shown in Fig. 3.

Table 13. TMA content of cook-chill fish (*Selar crumenophthalmus*) during chilled storage.

TMA (mg%)

Treatments	Days				
	0	5	10	15	20
Test	2.52	2.61	3.26	5.04	5.97
Control	2.52	2.98	5.69	6.25	6.25

Table 14. ANOVA for TMA content in cook-chill fish.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	10.34881	1	10.34881	26.88097*
Between days (B)	132.5788	4	33.1447	86.09311*
Interaction (AxB)	11.01532	4	2.75383	7.153053*
Error	7.699733	20	0.384987	
Total	161.6427	29		

*Significant at 5%

Critical Difference (days) = 0.746

Days:	0	5	10	15	20
Means:	2.52	2.8	4.48	5.64	6.11

Treatments	Control	Test
Means	4.74	3.88

Underlined means are not significantly different

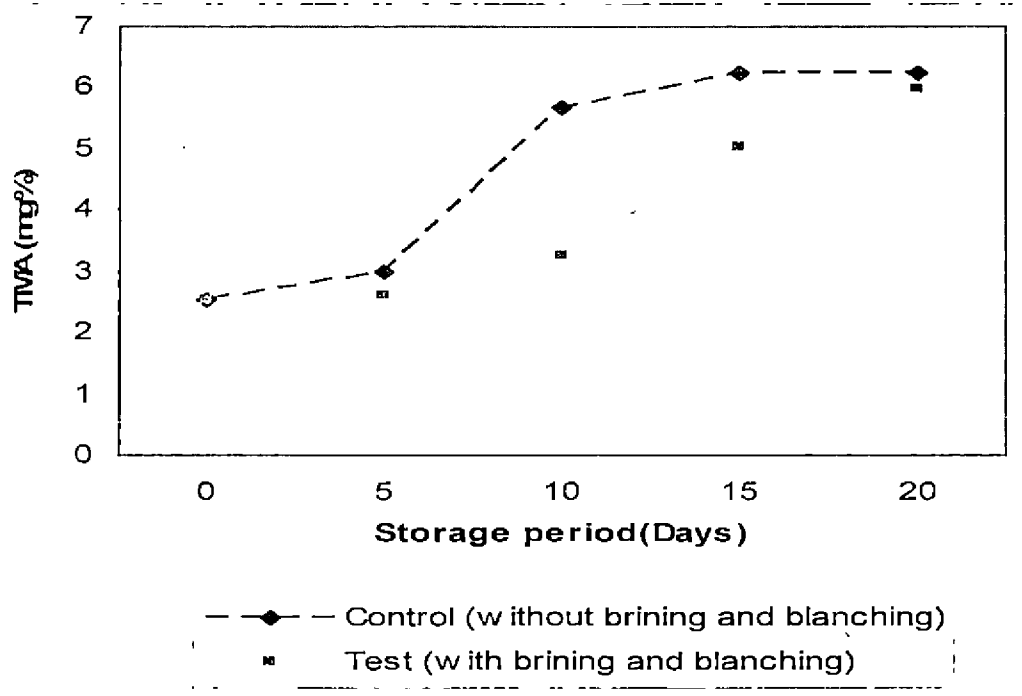

Fig 3. Changes in TMA content of cook-chill fish during chilled storage

TMA content in the case of partially processed value added fish also increased from 2.8mg% to 8.68mg% in the case of control and 2.8mg% to 5.6mg% in the case of test. ANOVA for TMA content showed significant difference between treatments and between storage days. Changes in value of TMA content during storage in case of partially processed fish is given in Table 15. ANOVA for TMA content is given in Table 16. Significant difference between treatments is observed. Graphical representation in Fig 4. also shows significant difference.

Table 15. TMA content of partially processed value added fish during chilled storage.

TMA (mg %)

Treatments	Days				
	0	5	10	15	20
Test	2.8	2.94	3.5	5.6	5.6
Control	2.8	5.6	6.44	8.4	8.68

Table 16. ANOVA for TMA content in partially processed value added fish.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	20.56356	1	20.56356	15.456*
Between days (B)	47.61906	4	11.90477	8.9480*
Error	5.32174	4	1.330435	
Total	73.50436			

*Significant at 5%
Critical Difference = 3.2

Days:	0	5	10	15	20
Means:	2.8	4.27	4.97	7.0	7.14

Treatments	Control	Test
Means	6.38	4.08

Underlined means are not significantly different

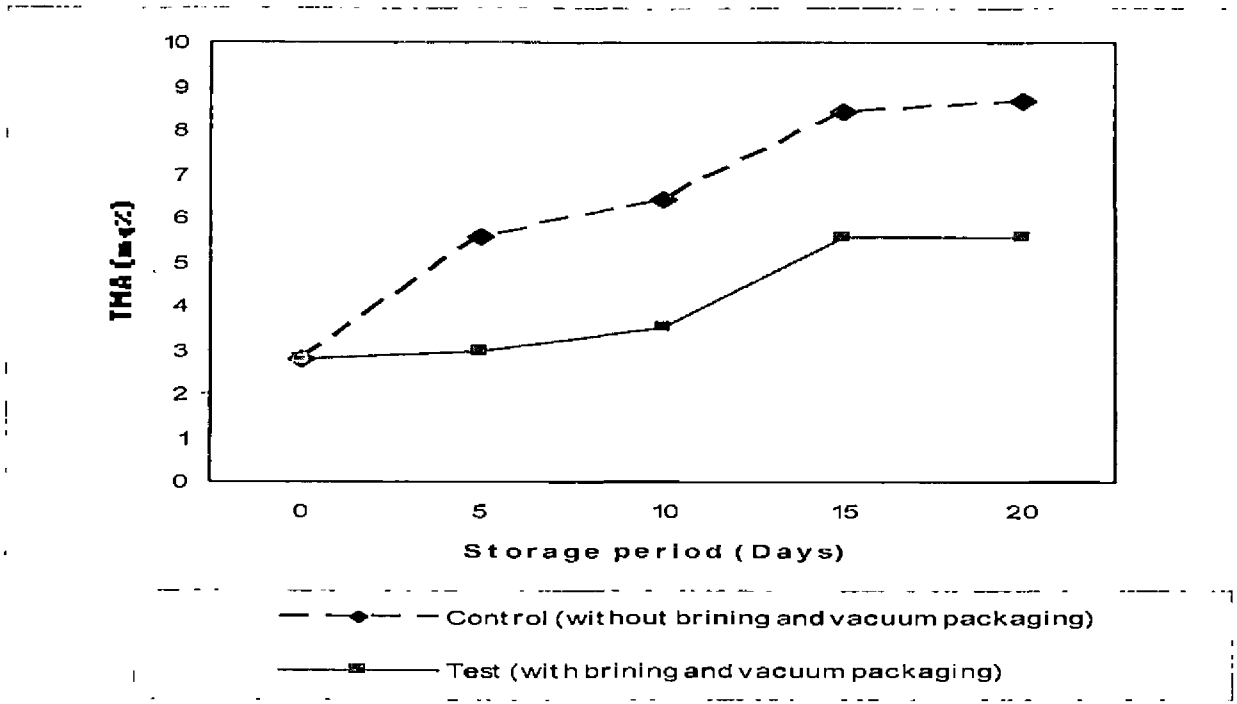

Fig 4. Change in TMA content of partially processed value added fish during chilled storage

4.4.3 TOTAL PLATE COUNT:

TPC of control for cook-chill fish ranged from $5.55x10^4$cfu/gm to $1.28x10^8$cfu/gm and for test it was from $2.15x10^3$cfu/gm to $2.55x10^7$cfu/gm. TPC of control was comparatively higher than TPC of test. TPC of cook-chill fish during storage has been given in Table 17. ANOVA for TPC of cook-chill fish given in Table 18 shows significant difference between treatments and also between storage days. Graphical representation given in Fig 5 shows significant difference between treatments.

Table17. TPC of cook-chill fish (*Selar crumenophthalmus*) during chilled storage.

TPC (cfu/ml)

Treatments	Days				
	0	5	10	15	20
Control	$5.55x10^4$	$5.52x10^5$	$2.94x10^6$	$1.10x10^7$	$1.28x10^8$
Test	$2.15x10^3$	$1.81x10^4$	$2.25x10^5$	$2.6x10^6$	$2.55x10^7$

Table18. ANOVA of TPC of cook-chill fish during chilled storage

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	6.061	1	6.061005	12496.92*
Between days (B)	33.830	4	8.457718	17438.59*
Interaction (AxB)	0.49117	4	0.122793	253.1804*
Error	0.0048	10	0.000485	
Total	40.3879	19		

*Significant at 5%
Critical Difference (days) = 0.069

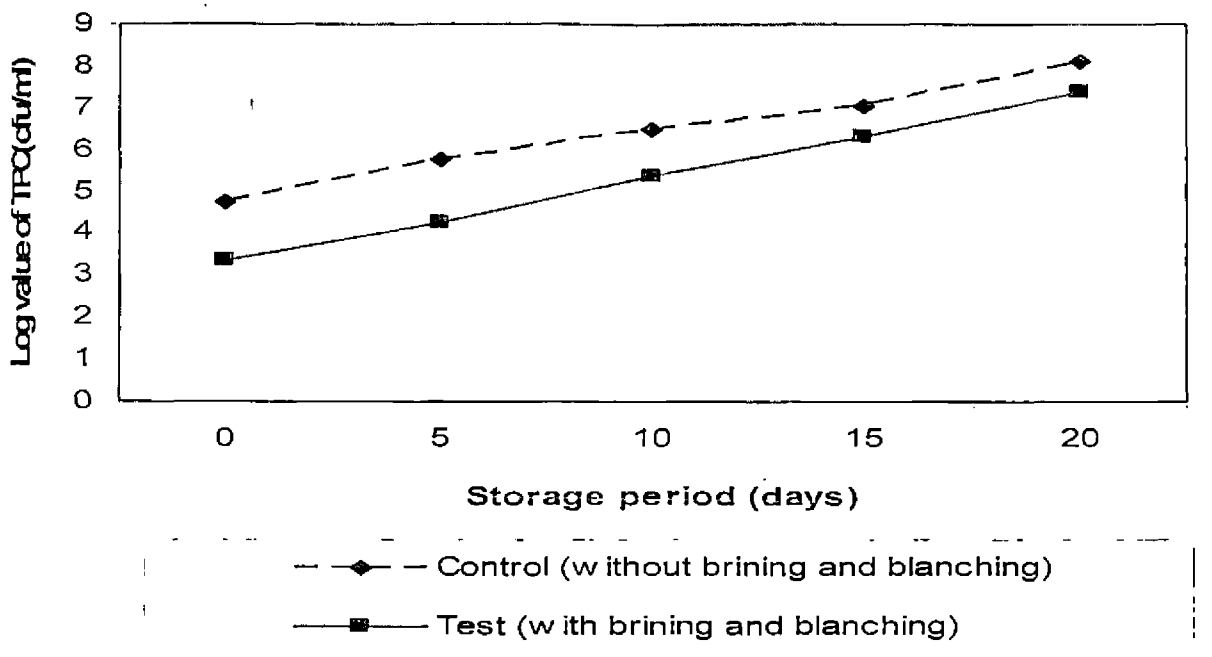

Fig. 5.Change in TPC of cook-chill fish during chilled storage

TPC in the case of partially processed value added fish showed a continuous increase during the storage period. Compared to the control, the test sample had lower TPC. TPC of control for partially processed value added fish ranged from $5.0x10^3$cfu/gm to $2.5x10^8$cfu/gm and for test it was from $5.2x10^3$cfu/gm to $4.3x10^7$cfu/gm .TPC during the storage period is given in Table 19. ANOVA of TPC for partially processed value added fish is given in Table 20. Statistical analysis showed significant difference between treatments and between storage days. Graphical representation is given in Fig. 5.

Table 19. Average TPC of partially processed value added fish (*Oreochromis mossambicus*) during chilled storage.

TPC (cfu/ml)

Treatments	Days				
	0	5	10	15	20
Control	$5.0x10^3$	$5.1x10^4$	$4.2x10^5$	$3.20x10^6$	$2.5x10^8$
Test	$5.2x10^3$	$2.67x10^4$	$3.25x10^4$	$5.0x10^5$	$4.3x10^7$

Table 20. ANOVA of TPC of partially processed value added fish during chilled storage.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- values
Between treatments (A)	1.722845	1	1.722845	863.5815*
Between days (B)	43.84883	4	10.96221	5494.841*
Interaction (AxB)	0.80243	4	0.200607	100.5551*
Error	0.01995	10	0.001995	
Total	46.39406	19		

*Significant at 5%
Critical Difference (days) = 0.043

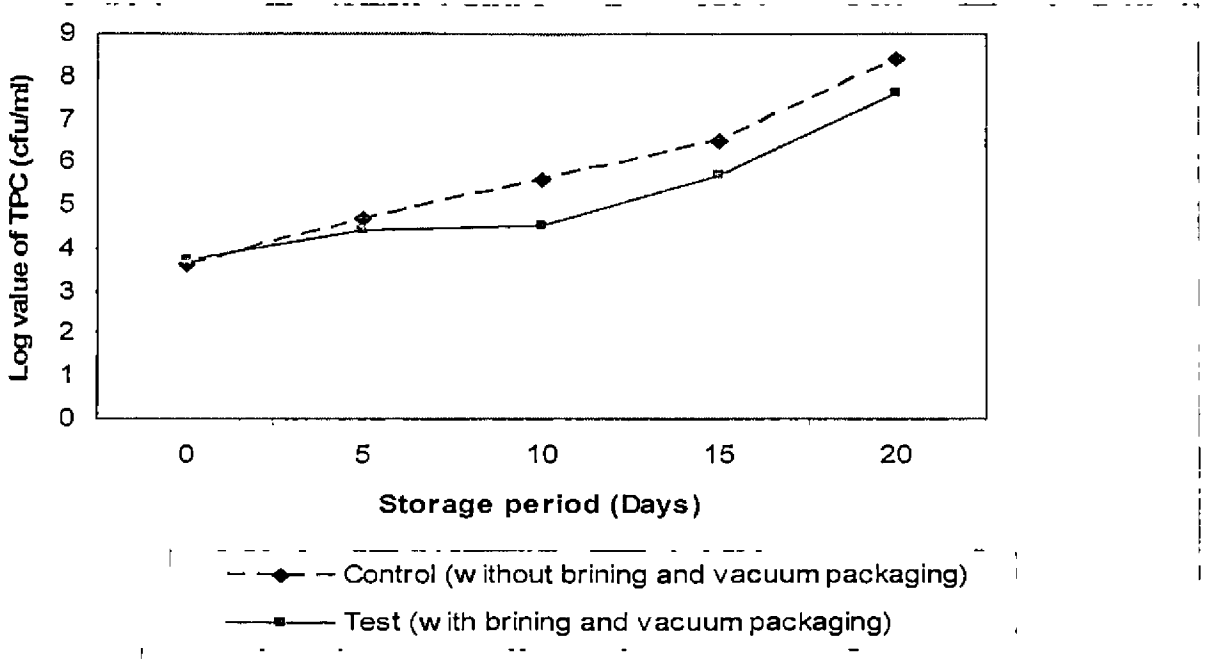

Fig 6. Change inTPC of partially processed value added fish during chilled storage

4.4.4 Test for specific microorganisms

Both the species (*Oreochromis mossambicus and Selar crumenophthalmus*) were subjected to test for specific microorganisms. Initially as well as throughout the storage period there was no presence of *Salmonella, Staphylococcus aureus, E.coli, Vibrio cholerae*, Coliforms detected. Result is shown below in Table 21.

Table 21. Result of test for specific microorganisms presence in the fresh fish

Type of microorganisms	Fish species	
	Selar crumenophthalmus	*Oreochromis mossambicus*
Salmonella	NIL	
Staphylococcus aureus		
Vibrio cholerae		
Coliforms		
E. coli		

4.4.5 Sensory evaluation :

Sensory evaluation conducted showed significant difference between test and control. Average sensory score during storage are given in Table 22. In the case of cook-chill fish, the control sample had a shelf life of 10 days whereas in case of test it had a shelf life of 15 days. Fig 7 shows sensory evaluation scores during storage period.

Table 22. Average sensory evaluation scores for cook-chill fish (*Selar crumenophthalmus*) during chilled storage.

TEST					
Days	Colour	Odour	Texture	Flavour	Appearance
0	7.66	7.63	7.67	7.06	8.0
5	7.5	7.5	7.21	7.03	7.35
10	7.42	7.21	7.0	7.28	7.28
15	6.01	6.55	6.5	6.0	7.5
20	ND	ND	ND	ND	ND

CONTROL					
Days	Colour	Odour	Texture	Flavour	Appearance
0	7.5	7.2	7.7	7.5	7.7
5	7.0	7.1	7.0	6.9	7.0
10	6.08	6.75	7.0	6.7	6.9
15	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND

ND- not done as the sample crossed borderline of acceptability.

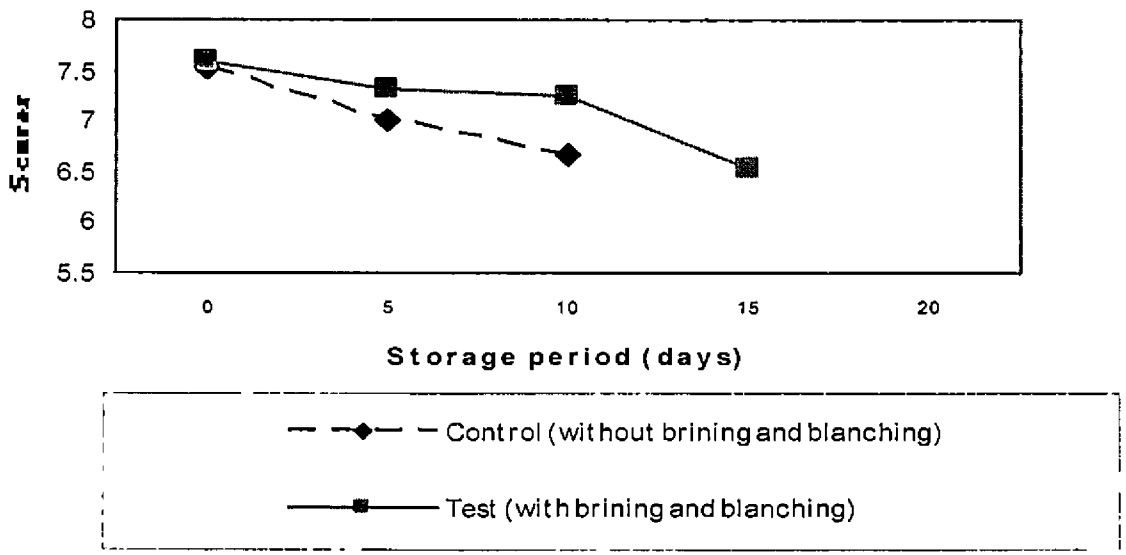

Fig 7: Sensory evaluation scores of cook-chill fish during chilled storage.

Sensory evaluation of partially processed value added fish was also carried out during chilled storage. In the case of the control sample the organoleptic quality was maintained for 10 days whereas for the test sample it was 15 days. Average sensory score during the storage period is given in Table 23. Fig 8 shows sensory evaluation score during storage period.

Table 23. Average sensory evaluation scores for partially processed value added fish (*Oreochromis mossambicus*) during chilled storage

TEST					
Days	Colour	Odour	Texture	Flavour	Appearance
0	8.2	7.4	8.0	8.1	8.0
5	7.4	7.5	7.5	7.8	7.8
10	7.1	7.0	7.1	7.2	7.7
15	7.05	6.7	7.2	6.1	7.2
20	ND	ND	ND	ND	ND

CONTROL					
Days	Colour	Odour	Texture	Flavour	Appearance
0	8.2	7.4	8.0	8.1	8.0
5	7.1	7.0	7.1	7.2	7.6
10	6.9	6.5	6.3	6.5	7.2
15	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND

ND- not done as the sample crossed borderline of acceptability.

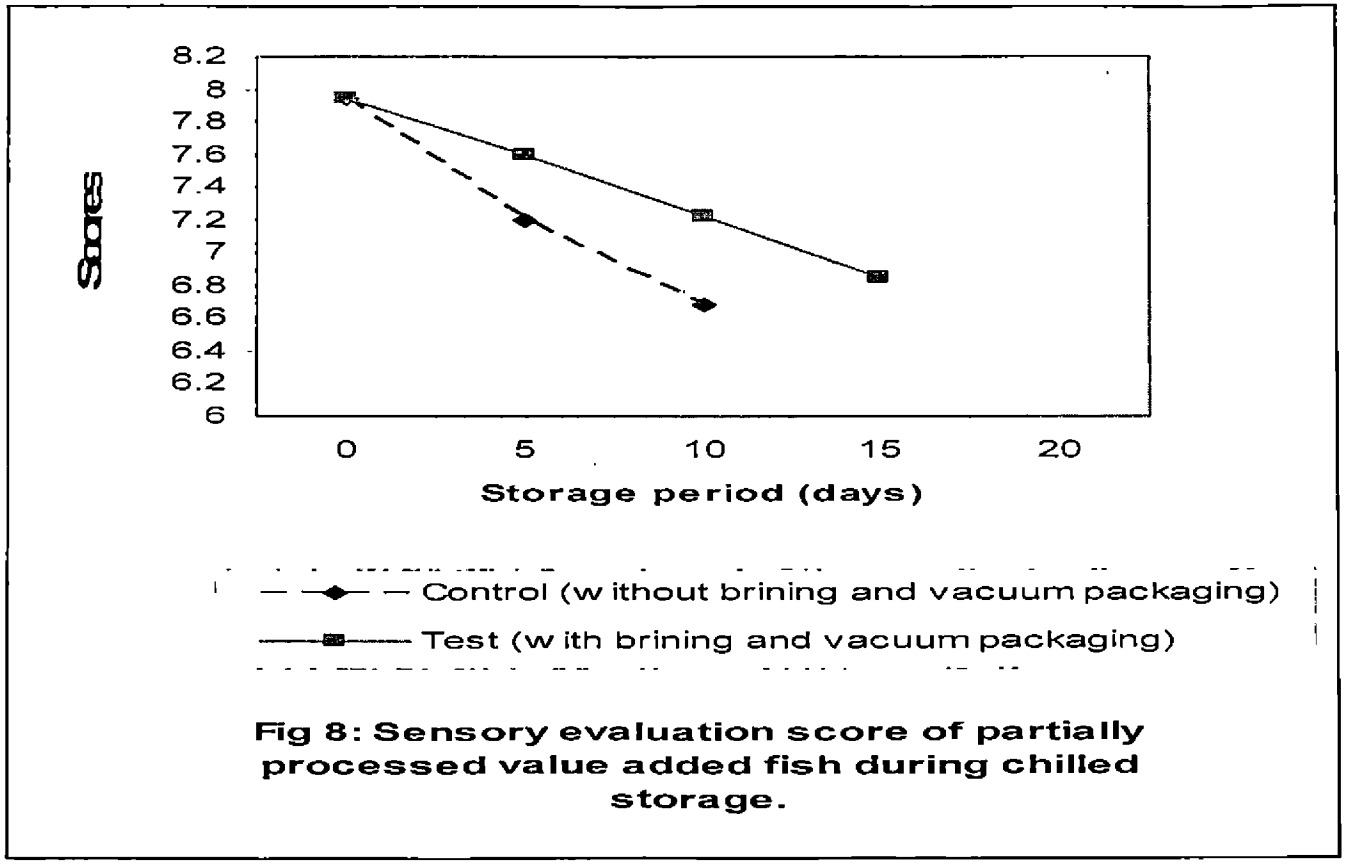

Fig 8: Sensory evaluation score of partially processed value added fish during chilled storage.

DISCUSSION

6. DISCUSSION

Microbiological safety and quality of muscle foods have great health and economic consequences to the general public, health officials and food processors (Parmley, 1988; Lambert *et al.*, 1991; Jay, 1992; Hofstra *et al.*, 1994.). Present study has been carried out to study the risk associated with two products cook-chill fish prepared from *Selar crumenophthalmus* and partially processed value added fish from *Oreochromis mossambicus*. More emphasis was given on maintenance of hygienic condition and proper handling of raw material as well as the prepared product.

Mukundan (2003) expained that risk and hazard are widely used terms in food quality assurance programmes. Risk is defined as probability of likely occurrence of an illness/injury due to hazard in food material. Risk evaluation and risk assessment are essential for identification of any hazard in food material and is a precondition for HACCP implementation.

Food borne diseases are of major concern to consumers, producers and authorities alike. Despite an increased awareness, the number of causes and outbreaks doesn't appear to be decreasing (Todd, 1977). Seafood borne disease may be caused by variety of agents, including aquatic toxins, biogenic amines, bacteria, virus and parasites (Gram and Huss, 2000).

There are a number of methods that can be used for conducting microbial risk assessment. These methods generally fall into two categories qualitative and quantitative. This is a scientifically based programme (Anandavally, 2000). Microbial safety and stability has been the prime consideration in food processing operations (Roberts *et al*; 1983). The bacterial count of frozen product reflects the bacteriological quality of the raw material or its combination during processing, but reduction in bacterial count resulting from frozen state is highly variable making any prediction of quality unreliable (Anon, 1980).

5.1. PROXIMATE COMPOSITION

In the present study proximate composition for both the species (*Oreochromis mossambicus*, *Selar crumenophthalmus*) was carried out. Protein content of *Selar crumenophthalmus* (25.14%) was found to be higher than *Oreochromis mossambicus* (20.70%). Fat content was higher in *Selar crumenophthalmus* (5.82%) hence rancid flavour was observed during the final days of storage.

5.2. BRINING

In the following study brining has been done to improve the flavor of the product. Salt content absorbed by the final product was found to be 0.789% in the case of cook-chill fish and 0.24% in partially processed value added fish. Solanki *et al.* (1970) observed that to serve other purposes of salting like firm texture, formation of pellicle and above all to exert pronounced preservative effects against mould and bacteria, a level of 8-10% salt in fish is essential.

5.3. COOK-CHILL FISH

Foods are subjected to thermal processes in a number of different contexts. When pasteurization is introduced to improve safety, its effect can be doubly beneficial. The process cannot discriminate between target pathogens and others hence improve shelf life of the product (Khaterpaul, 2006).

Cooking is an important step for reduction of bacterial load. In the present study steam treatment was given but this resulted in curd like formation due to coagulation of protein in the product and gave a bad appearance. To overcome this problem the products were blanched. For standardization of the blanching time 3 treatments were given i.e. 75°C for 60sec, 90sec, and 120sec. Best treatment was selected based on sensory evaluation and TPC. Temperature of 75°C was selected based on earlier research done. Anon (1966, 1967) reported that a quick dip of fish in

hot water (70°C to 85°C) prior to packing eliminated harmful bacteria, particularly haemolytic micrococci in cooked and peeled shrimps before freezing. Kamat and Kumta (1972), Kumta *et al.* (1970) found that steaming reduced total plate count in shrimp and pomfrets and extended their shelf life when compared with fresh ones kept in ice.

In this study, among the 3 treatments carried out the best one was 75°C for 90sec because it had maximum sensory score. In the case of TPC, 120sec had lowest TPC but texture of product was not firm and hence could easily disintegrate. Blanching at 75°C for 90sec was ideal for the product. Combining the sensory evaluation and result of ANOVA for TPC, 75°C for 90sec was selected.

On its own, contribution of cooking to extension of shelf life can be very small, particularly if the pasteurized food lacks other preservative factors (Khaterpaul, 2006). In the present study main treatment given was cooking but during storage period there was steady increase in the TPC. Cook-chill product being not sterile, harbor microorganisms. Product is unprotected due to negligible content of salt and no preservatives. Therefore utmost importance was given to raw material, its handling and freshness at the time of purchase. Thomas and Mathen (1995) found that maintenance of proper hygiene and immediate chilling considerably enhanced the shelf life of fish.

4. PARTIALLY PROCESSED VALUE ADDED FISH

In the present study partially processed value added fish was vacuum packed in 12μ polyester laminated with polyethylene of 200 gauge. It was observed that when compared to non vacuum packed control this showed better shelf life and lower microbial load. This could be attributed to the packaging material which has great barrier properties against moisture and oxygen. In case of control polyethylene was used which was heat sealed using electric heater. Polyethylene lacks good barrier properties and because the product was not vacuum packed, it was exposed to both moisture and oxygen. Anon (1969) reported that using polyethylene as

packaging material, no difference in bacterial growth was observed when the fish were packed with or without vacuum, but a small extension was seen in vacuum packed plaice. British research workers (Anon, 1962) claim that shelf life of prepackaged cod and haddock fillet is markedly dependant on packaging material. An outstanding feature was the extension of shelf life in many of the vacuum packs.

In the present study vacuum packed product had better shelf life but during storage period there was increase in TPC, TMA, TVB-N and sensory characteristics. This could be due to anaerobic bacterial growth. Gopal (2002) reported similar findings in case of vacuum packed seafoods.

5.5. STORAGE STUDIES

5.5.1. Total plate count (TPC)

The best, simple, standard and common test for bacterial quality of fish is the determination of total bacterial population (Farber, 1965). TPC for both control and treatment showed significant difference in cook-chill fish and partially processed value added fish. There was an increase in TPC during storage period. Compared to the control, the test samples (treated samples) of both the products showed lower TPC.

In case of cook-chill fish, cooking reduced the initial microbial load. Heat treatment given destroyed most of the microorganism but the product was not completely free of microorganisms hence it had to be chilled stored to prevent microbial growth. It was observed that chilling of the test product which was given heat treatment had lower microbial load. Rahkoen and Kaitala (1993) carried out similar work in sausages and reported that low bacterial count corresponded with high cooking temperature and low storage temperature.

In the present study cooking of fish at 75°C for 90sec destroyed most of the microorganisms. Huss (1970) observed that plaice stored in ice for varying periods,

have shown that up to 99.5% of initial flora could be eliminated by dipping the fish for 5sec at 80°C.

Cook-chill product is not sterile. In the present study the data given shows steady increase in TPC which could be due to microorganisms which survived the high temperature. Venugopal (2006) reported that in addition to microorganisms surviving the heat treatment, some viable but not culturable (VBNC) cells can pose problem during the course of chilled storage.

In the case of partially processed value added fish, initially the TPC of control and treatment differed significantly. Test showed lower TPC and better shelf life. Bala et al. (1999) reported that generally microorganisms in live fish are present in substantial number on skin, gill surface and in intestines, with muscle tissue of fish being sterile. In the present study initial TPC was lower for both control and test when compared with the raw material. This could be due to the immediate gutting and dressing of fish.

In the present study vacuum packed test samples showed comparatively lower TPC than non vacuum packed control because vacuum packed samples inhibited growth of aerobic microorganisms. Genigeorgis (1985) reported similar findings. It is believed that under vacuum packaging the microflora becomes dominated by gram positive bacteria (Ioannis, 2002).

ANOVA for TPC of both the products showed significance between treatments and between storage days.

5.5.2. Trimethylamine (TMA) and Total volatile base nitrogen (TVB-N)

Freshness may be considered synonymous with quality. Many methods are available for estimating the freshness and quality of fish (Uchiyama and Ehira, 1970). TMA is often used as an index to assess the keeping quality and shelf life of seafood products. Almanoos *et al.* (1984) believed Total Volatile Bases (TVB) as a

widely accepted index for evaluating changes in quality of fishery products and the variation of TVB in fresh muscle is due to formation of TMA from TMAO by bacterial activity. One of the most important characteristic features of the chemical spoilage is the production of volatile bases such as ammonia and lower amines. The precursor of TMA is TMAO, present in variable amount in marine species of fish but absent in freshwater species.

In the present study TMA and TVBN content of the test and control increased during the storage period for both the products, but compared to the control, test showed lower values in both cases. It was observed that increase in TMA and TVB-N corresponded with increase in TPC during storage. Initially the values obtained were within the acceptable range, this could be due to storage at low temperature. Hansen *et al.* (1974) found that increase in TPC correlated with increase in TMA and TVB-N values and holding temperature had a direct effect on the TVB-N production in fish.

ANOVA for TMA and TVB-N of both the products showed significant difference between treatments as well as between storage days.

5.5.3. Test for specific microorganisms

Test for specific microorganisms gave negative results. During storage also, microorganisms were not detected. Fresh fish when tested for specific organism showed negative results, this can be due to different reasons; one can be better handling like immediate icing of fish, proper handling, better storage etc. Another reason could be the small sampling done restricted to one place. Nambiar (1991) reported that nine out of 156 fresh samples and 11 out of the 127 frozen samples were found to be contaminated with *Salmonella* in a survey conducted on microbial quality of fish in retail trade in Cochin.

In the case of cook-chill fish the heat treatment could have destroyed most harmful microorganisms. In case of partially processed value added fish the vacuum packaging could have prevented growth of harmful aerobes.

In the present study the most important criterion was proper handling and maintenance of hygienic conditions throughout the process. Iyer *et al.* (1966) reported that microbiological conditions of frozen fish products is dependant upon various factors such as nature of raw material, its pre, post process treatments, the sanitary conditions of processing factories and rate of freezing. Since the chance of occurrence of some of the organisms is more after processing it is important to conduct microbiological examination of these organisms during storage also.

5.5.4. Sensory evaluation

The oldest and still most widespread means of evaluating the acceptability and edibility of fish are the senses smell and sight supplemented by taste and touch. The reasons for the preferential use of sensory tests are obvious, no special laboratory equipment is needed and fish can be examined very quickly and many samples can be evaluated in a relatively short time (Farber, 1965; Joseph and Iyer, 2002).

Ehrenberg and Shewan (1960) have described systems of grading and of numerical scoring of raw and cooked fish based on the evaluation of such factors as odour, general appearance, taste and texture of raw and cooked fish.

Friedman's test showed significance between treatments. There was significant difference between test and control during storage days. Major difference was observed in case of taste and texture. Control in both case developed unacceptable flavour and odour earlier compared to test samples. After the 10^{th} day odour and appearance of control in both cases deteriorated and could not be used for sensory evaluation whereas in case of tests the product had shelf life of 15 days.

Sensory analysis was carried out by panel of judges and scores were given for the various parameters. Scores for the sensory parameters show that the test products were acceptable for longer storage period when compared to their controls. Kramer

(1952) explained that sensory evaluation could be a precise tool for ascertaining the quality of fish if the tests are designed properly and trained personnel are selected.

SUMMARY

6. SUMMARY

1. The main objectives of the present investigations were preparation of cook-chill fish from *Selar crumenophthalmus*, partially processed value added fish from *Oreochromis mossambicus* and their microbial risk assessment. Study of quality changes during storage was another criterion.

2. Fresh *Selar crumenophthalmus* and *Oreochromis mossambicus* were transported from the fish market/ landing centre to laboratory in iced condition.

3. Fish were beheaded, eviscerated, and cut into fillets for cook-chill fish and into steaks for partially processed value added fish.

4. Effect of different blanching conditions, vacuum packaging and chilling were studied.

5. For evaluating quality of the products, salt content, TVB-N, TMA, TPC and sensory evaluation was carried out. Tests for specific microorganisms i.e. *Salmonella, Staphylococcus aureus, E.coli*, faecal streptococci, coliforms and *Vibrio cholera* were carried out.

6. Brine concentration of 5% for 10min and blanching at 75°C for 90sec were selected for the preparation of cook-chill fish. In case of partially processed value added fish brining concentration of 2% for 10min was selected. Final salt content in case of cook-chill fish was 0.789% and for partially processed fish it was 0.24%.

7. Both the products were packed. Cook-chill fish in polyethylene and partially processed value added fish in 12μ polyester laminated with polyethylene of 200 gauge. Then these products were chill stored using ice at 4°C.

8. Storage study was conducted for 20 days. TPC, TVB-N, TMA and Sensory evaluation by panel of judges was carried out.

9. During storage both the products showed steady increase in TPC. In case of cook-chill fish test sample (sample which was given brining, blanching treatment) showed lower microbial load when compared with the control which was not given any treatment. In case of partially processed value added fish test sample (sample which was vacuum packed) showed lower microbial load than control which was not vacuum packed.

10. Both the products showed absence of *Salmonella, Staphylococcus aureus, E.coli*, faecal streptococci, coliforms and *Vibrio cholera* throughout the storage period.

11. TMA and TVB-N increased during storage period. Increase in TMA and TVB-N corresponded with increase in TPC. For both the products control showed greater amount of TMA and TVB-N content when compared with the test sample.

12. Sensory evaluation carried out during the storage period showed progressive deteriorative changes in the product. Based on the scores and statistical analysis it was observed that both products were having better shelf life i.e. of 15 days than control with a shelf life of 10 days.

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6. REFERENCES

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MICROBIAL RISK ASSESSMENT AND PROCESS STANDARDIZATION FOR "COOK-CHILL FISH" AND "PARTIALLY PROCESSED VALUE ADDED FISH".

By ANJU, B.F.Sc

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree.

MASTER OF FISHERIES SCIENCE

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DEPARTMENT OF PROCESSING TECHNOLOGY

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ABSTRACT

A study was undertaken for the preparation of cook-chill fish from *Selar crumenophthalmus* and partially processed value added fish from *Oreochromis mossambicus* and their microbial risk analysis. Standardization of processing technique for both products was done.

Parameters standardized were brining conditions and blanching conditions for cook-chill fish. Standardization of brining condition was carried out for partially processed value added fish.

Various brining conditions studied were brine concentrations of 3%, 5%, 6% for 5min, 10min, 15min and blanching at 75°C for 60sec, 90sec, and 120sec for cook-chill fish. In the case of partially processed value added fish brining concentrations of 1%, 2%, 3% for 5min, 10min, and 15min.

Salt content and sensory evaluation were carried out, in case of cook-chill fish to select best blanching time. TPC values were determined. Based on the results the following treatments were selected. In the case of cook-chill fish brine concentration of 5% for 10min and blanching at 75°C for 90sec were selected. For partially processed value added fish the brine concentration of 2% for 10min was selected.

Both products were packed and chill stored using ice at 4°C. Cook-chill fish was packed in polyethylene and heat sealed. Partially processed value added fish was vacuum packed in 12μ polyester laminated with polyethylene 200 gauge. Control was used for comparative study. Control in the case of cook-chill fish (*Selar crumenophthalmus*) was fish fillets stored without any brining, cooking treatments. For partially processed value added fish (*Oreochromis mossambicus*), control was not given any brine treatment, was packed in polyethylene and heat sealed using electric heat sealer.

During storage TMA, TVB-N, TPC and test for specific microorganisms were carried out. Results of quality parameters showed a greater shelf life of five days for test samples compared to the control in both the products.

APPENDIX I

Score sheet for standardization of products

Samples of fish fillets/ fish steaks are given. Kindly evaluate the samples for the given sensory parameters based on the scale provided.

TREATMENTS	COLOUR	ODOUR	TASTE	TEXTURE	APPEARANCE
T_1					
T_2					
T_3					
T_4					
T_5					
T_6					
T_7					
T_8					
T_9					

Score- points

9-10: Excellent

7-8: Very good

6-5: Good

4-3: Borderline of acceptability

0: poor

Name:

Date:

Appendix II

Score sheet for sensory evaluation of storage studies

Kindly evaluate the given samples for the parameters given below and put appropriate score based on the scale provided.

Sample	Colour	Odour	Texture	Flavour	Appearance
A					
B					

Score- points

9-10: Excellent

7-8: Very good

6-5: Good

4-3: Borderline of acceptability

0: poor

Name:

Date: