INCORPORATION OF FISH PROTEIN GEL FROM SHARK MEAT INTO FISHERY PRODUCTS

By

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THESIS

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Dedicated To
My Beloved Parents

DECLARATION

I hereby declare that this thesis entitled " **INCORPORATION OF FISH PROTEIN GEL FROM SHARK MEAT INTO FISHERY PRODUCTS** " is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or society.

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Introduction

1. INTRODUCTION

Seafoods are gaining importance in human nutrition with increasing awareness of their nutritional significance. Although several hundreds of species of fish are caught annually throughout the world, all of them are not equally important for processing from a commercial point of view (Morissey, 1988).

Approximately 30% of the total landing can be considered underutilized. Therefore a need has been realized to shift emphasis from increased production to increased utilization (Morrissey 1988).

The annual global landing of elasmobranchs, which include sharks, rays and skates, contributes about 825000 metric tons (FAO, 2001) with a contribution of about one-tenth of the catch from India (MPEDA, 1994). These fishes are generally considered underutilized because of their peculiar characteristics such as appearance, meat odour, and taste. The putrid odour during spoilage of the fish has been attributed essentially to degradation of urea into ammonia (Ghadi and Ninjoor, 1989; Pastoriza and Sampedro, 1994). Therefore, more often these fish find use as a source of by-products such as gelatin, glue, fin, oil and leather (Molyneux, 1973; MPEDA, 1994).

Attempts have been made to use shark meat as human food (Koreeda *et al.*, 1982; Nakamura *et al.*, 1985; Venugopal and Shahidi, 1995; Diniz and Martin, 1997). Ideally efforts in this direction should take advantage of the physico-chemical properties of its meat protein for product development. Development of surimi and surimi-based foods from fish meat utilizes the gel forming property of myofibrillar protein in specific condition such as neutral pH, presence of salt and mild heat treatment (Asghar *et al.*, 1985; Lanier and Lee, 1992; Stone and Stanley, 1992). Venugopal *et al.* (1994) reported that gelation of washed shark meat could also be achieved under mild acidic condition brought about by acetic or lactic acid. The gelation, which was associated with increased apparent viscosity of the meat slurry, was dependent

upon protein concentration, temperature, pH and was inhibited by the presence of salts. There is a potential for the development of several edible products from the gel such as functionally active spray-dried protein powders, biodegradable film and sauces (Venugopal *et al.*, 1997; Venugopal, 1997).

On a global scale, more and more consumers are beginning to look at fishery products as health foods, since unlike red meat, fishery products are good sources of therapeutically important polyunsaturated fatty acids, easily digestible proteins and several micronutrients. Further, fishery products are the major commodities of international trade and sources of foreign exchange for several countries particularly those from Asia. Therefore, there is a need for total utilization of the catch to meet the increasing global requirements of the commodity (Venugopal, 2001).

Value addition of shark meat through development of surimi and seafood analogs has been attempted making use of its gel forming property at neutral or above neutral pH conditions (Nakumara *et al.*, 1985; Koreeda *et al.*, 1982; Suzuki, 1981).

The objectives of the present study are:

(a) to develop modified fishery products by incorporating gel extracted from shark meat.

(b) to assess the quality changes in the products during frozen storage.

Review of Literature

2. REVIEW OF LITERATURE

The demand for fish and fishery products are increasing throughout the world. But their availability is not keeping pace with the demand. Apart from rising population, the increase in awareness of the nutritional value of fish is also contributing to this demand. The high nutritional value of fish arises from the protein, lipids, vitamins and minerals. The protein from fish is easily digestible because of the low content of connective tissues (Love, 1974). Fish lipids are high in polyunsaturated fatty acids, which is known to reduce cardiovascular diseases (Kinsella, 1987). Apart from lipids and proteins fish meat is a rich source of vitamins and minerals.

The nature of protein in shark meat is found to exhibit excellent functional properties especially gelation (Kaminashi *et al.,* 1990; Venugopal *et al.*, 1994; and Kaminashi *et al.*, 1998).

2.1 UTILIZATION OF SHARK

Sharks are exploited for their meat (fresh, frozen, smoked, salted or in brine), fins (one of the most expensive fishery product), liver oil (for cosmetics and pharmaceuticals), skin (for leather and sand paper), teeth (in jewellery) and more recently cartilage (ground to powder and proposed as 'anticancer' cure). Discards are also used for production of fishmeal and fertilizer. It is not possible to make all these uses of each shark species as the method of preservation and preparation are often mutually exclusive and not all species of shark are suitable for every application nor have the same commercial value (Vannuccini, 2000)

Shark flesh is used for meat, which is highly favoured in some regions, most particularly in Europe, with northern Italy and France as the major consuming countries and Spain as the world's largest exporter of the shark meat (Vannuccini, 2000).

Possibility of preparation of acid induced gel has offered an effective alternate method to utilize shark meat for human consumption (Venugopal and Doke, 1998). Gel prepared from collagen-free meat is heat processed to different ready-to-eat products. Spray drying of acid induced gel dispersion could yield a stable product. The powder was colourless, odourless and nonhygroscopic with high protein content of 93% and low fat content of 0.7% (Venugopal and Doke, 1998). The product was stable at ambient temperature for one week and one month at refrigerated temperature.

Sharks are the major source of raw material for manufacturing surimi products in Taiwan (Chen, 1995). Few studies have been reported on the gel forming ability of shark muscle and its dynamic thermal stability (Shimizu *et al.*, 1981; Koreeda *et al;* 1982).

Utilization of shark meat for kamaboko and paste product manufacture is more prevalent in Japan (Kaminashi *et al.*, 1998). In India, utilization of shark meat for human consumption by preparing different products has been attempted by several workers (Chari and Sreenivasan, 1980; Sankar and Solanki, 1992; Kandodaran *et al.*, 1965; Venugopal *et al.*, 1994).

Venugopal and Doke (1998) have successfully developed edible film from shark meat. This film has the potential as a coating for frozen fishery products.

Chen (1995) found that the fish sausage prepared from surimi of different species of shark has given a highly acceptable product with high gel strength.

There is potential for the development of several edible products from the gel such as functionally active spray-dried protein powders, bio-degradable films and sauces (Venugopal *et al.*, 1997).

Development of restructured products has been recognised as a method to utilize processing wastes of red meats such as low value cuts and trimmings.

The process involves heat treatment of the edible portions of the waste with suitable binder to give products having desired texture and flavour that can satisfy specific consumer demands (Mandigo, 1986; Kuraishi *et al.*, 1997; Lee *et al.*, 1997; Quinton *et al.*, 1997).

While several methods are available for value addition of fish, surimi technology has been recognized as one of the most successful techniques for low cost fish utilization (Venugopal, 2003). Recently extrusion of proteins has received some attention and offers possibilities of developing texturised food products (Areas, 1992).

Venugopal, *et al.* (1994) have examined the feasibility of using the spray-dried shark powder as protein extender in extruded cooked foods.

Venugopal (1998) observed that the incorporation of shark meat gel in the feed might serve not only as a novel binding agent but also as good source of proteins. Such feasibility can alleviate the problem of water pollution by indigestible binder such as carbohydrates, besides helping to replace unhygienic fishmeal to yield good quality feed free from pathogenic microorganisms.

The gel could be applied for development of restructured products by incorporating suitable additives. There is potential for the application of the gel as a binder in sausage and aqua feeds. The gel dispersion could be used as a coating in order to enhance chilled storage life of fresh fish and to prevent quality loss during frozen storage of fishery products (Venugopal, 2003).

Acid induced gelation has been recently extended for development of low sodium surimi from Alaska Pollock (Lian *et. al.*, 2002).

Another application of shark gel is the preparation of microbiologically stable patties and sausages, that can have extended chilled storage life through

incorporation of acetic acid induced gel to the minced meat (Smruti *et al.*, 2004).

2.2 GELATION

Tanka (1981) has defined gel as a form of matter intermediate between a solid and liquid, consisting of strands or chains crosslinked to create a continuous network immersed in a liquid medium. Hence gels are essentially space filling three-dimensional structures.

Ziegler and Foegeding (1990) defined "gel as a continuous network of macroscopic dimension immersed in a liquid medium and exhibiting no steady state of flow"or gelation is the process of cross-linking of randomly dispersed polymer chain to form a three-dimensional network with entrapment of water in the matrix.

Gel forming ability is the key functional property of wide interest in kamoboko-type products. It is generally understood that to produce a gel product with the required elasticity and firmness, two basic requirements must be met 1. Myofibrilar protein must initially be solubilised in a salt solution. 2. On heating to forming a gel, the proteins must be denatured in such a way that they form a regular network structure capable of immobilizing the water present (Suzuki, 1981; Lee, 1984; Lanier, 1986).

According to Niwa (1992) the rheological characteristics of the gel depend on the properties of myofibrilar proteins, which are affected by the species and freshness of the fish, as well as on the processing parameters, mainly protein concentration, pH, ionic strength and temperature.

Venugopal *et al.* (1994) has reported that gelation of washed shark meat could also be achieved under mild acidic condition which was brought about by acetic or lactic acid.

Enzyme-assisted gelation of proteins has been observed to have scope to replace salting and heating steps, which are required in the conventional texturisation of restructrued products (Kuraishi *et al.*, 1997).

Gelation of myosin molecules involves partial denaturation followed by irreversible aggregation of myosin heads through formation of disulphide bonds and helix-coil transition of the tail part of the molecules resulting in three dimensional network (Niwa, 1992; Stone and Stanley, 1992).

According to Venugopal (1997) Gelation was found to be inhibited by NaCl, which could be due to the interference of salt with electrostatic interactions among the protein molecules. It was proposed that cross linking in acid induced gel could be due to acid induced shift in electrostatic equilibrium and resulting electrostatic repulsion of protein molecules (Venugopal, 1997).

Gelation involves essentially three steps, dissociation of myofibrillar structure by protein solubilisation in the presence of salt, partial unfolding of myosin structure caused by heating and irreversible aggregation of unfolded myosin to form a three dimensional structure (Lanier and Lee, 1992; Niwa, 1992; Sharp and Offer, 1992; Stone and Stanley, 1992).

Gelation resulted in 50% reduction in free sulphydryl contents as compared with unacidified, washed shark meat (Venugopal *et al.*, 2001).

Stone and Stanley (1992) reported that a decrease in free sulphydryl groups, presumably due to the formation of disulfide bonds also suggested network formation in the proteins during gelation. Formation of disulfide bonds has been recognised to be one of the major chemical changes during surimi gelation.

Surimi based products have an expressible water content generally not exceeding 10% (Lanier and Lee, 1992). The heat set shark gel, however, had

higher expressible water contents than conventional surimi, indicating the weaker nature of the gel network (Venugopal *et al.*, 2001).

Gelation of muscle proteins results from transformation of an amorphous viscous solution to a three-dimensional elastic network. Stress-strain relationship of gel could be monitored by rheological parameters (Egelandsdal *et. al.*, 1995; Hamann, 1987).

Chawla *et.al.* (1996) observed that mild acid induced gelation of fish meat is associated with decrease in sulphydryl contents and formation of disulphide bonds. This has been verified with respect to shark and threadfin bream gel.

Myofibrillar proteins undergo gelation under mild acidic conditions (Fretheim *et.al.*,1985). Gelation of fish proteins is the most important step in forming desired textures in many types of seafood. Functional properties, notably gel strength, of surimi gels can be affected by many physical conditions as well as chemical additives (Lee and Park, 1998).

2.3 UREA-PROTEIN INTERACTION

There is a possibility that urea has a small direct effect in weakening hydrophobic bonds. The insolubility of denatured proteins in water is undoubtedly caused by intermolecular hydrophobic bond formation as well as by intermolecular hydrogen bond and intermolecular disulfide bond formations. The fact that denatured proteins dissolve readily in strong urea solution might be taken as an indication that urea is able to weaken hydrophobic bonds as well as hydrogen bonds (Kauzmann, 1959).

High concentrations of urea are known to have a denaturing effect on many proteins (Tanford, 1968). The primary action of urea on proteins is unfolding the native structure. The addition of urea to the substrate liquid increases the area occupied by each molecule for lysozyme and synthetic polypeptides, indicative of unfolding action by urea (Hamaguchi, 1955).

According to Robinson and Jencks (1965) hydrophobic effect of urea will make relatively larger contribution to urea denaturation at elevated temperatures, while effect on amide and peptide groups are more important at lower temperature. The denaturation of many proteins by urea proceeds to a greater extent or more rapidly as the temperature is decreased (Kauzmann and Simpson, 1953).

Studies conducted by Harris (1956) on the effect of urea on trypsin showed that urea causes a reversible reaction in which trypsin is converted into an unfolded inactive form.

Simpson and Kauzmann (1953) found that the formation of intermolecular hydrogen bonds occurred between urea and exposed peptide linkages. A study of the stability of hydrogen bonded peptide structures in aqueous solutions has led to the view that random regions of the proteins are stabilised by binding urea at sites required for hydrogen bonding in the helix (Schellman, 1955). This does not account for the aggregation reaction that are so evident (Mc Kenzie *et al.,* 1955; Echols and Anderegg, 1960). Studies made on model compounds in urea solution do not support the view that urea facilitates the breaking of hydrogen bonded structures in proteins (Levy and Majoulas, 1961).

Various authors have studied the effect of urea on ribonuclease (Anfinsen *et al.,* 1955; Harrington and Schelleman, 1956). Studies on viscosity, optical rotation, sedimentation constant, hydrogen deuterium exchange and ultraviolet absorption have shown that ribonuclease is extensively unfolded in 8M urea.

Kinsella (1982) observed that the urea effectively disrupts the hydrogen-bonded structure of water and facilitates protein unfolding by weakening the hydrophobic interactions. Urea also increases the 'permitivity' of water (Franks and England, 1975) for the apolar residues causing loss of protein structure and heat stability.

Urea and chaotropic ions such as guanidine hydrochloride decrease protein-protein interaction and increase the protein solvation at concentration in the order of 4-8 M. It may cause protein denaturation by disruption of hydrogen bonds and interruption of the tertiary and even secondary structure of the protein to varying degrees, depending on the type of protein (Vojdani, 1996).

Bone *et al.* (1995) found that mammalian collagen, haemoglobin and many enzymes are denatured by 0.5 M urea. Either elasmobranchs have in some way modified their proteins to resist the effect of urea, or they protect their proteins otherwise. Some elasmobranch proteins, like hemoglobin and certain enzymes, are resistant to urea denaturation and indeed, the eye lens protein and the M4 lactate dehydrogenase (LDH) actually require urea to function properly.

The muscle of shark contains urea in the range of 250-350 mM (Nishimoto and Miki, 1980). Smith (1929) carried out extensive analysis of elasmobranch tissue to osmotic-balance function. The stability of proteins from sharks in the presence of urea has been reported by (Arai *et al.*, 1976; Nishimoto, 1981; Kanoh *et al.*, 1985) and they have found that they are more stable than those from teleosts.

Read (1968) observed that the urea is retained in many tissues of the body by the elasmobranchs, both in embryo and in adult with a concentration often exceeding 2% (w/w), and the osmotic balance being achieved with high concentration of sodium chloride and by the presence of other organic substances of low molecular weight such as trimethyl amine oxide and betaine.

Kanoh *et al.* (1985) have studied the urea resistability of shark myofibrillar protein and reported that urea did not affect the Ca^{2+} ATPase activity of requeim shark myosin upto about 0.6M and the activity tended to decrease at higher urea concentration resulting in an almost complete loss at 2M urea.

Niwa *et al.* (1989) observed that with the increase of urea concentration upto 2M, actomyosin from white flesh showed a marked increase of hydrophobicity. Actomyosin from red-fleshed fish and elasmobranch exhibited a lesser increase, whereas avian and mammalian actomyosin did not show any change.

Reduced viscosity of myosin B prepared from smooth dogfish muscle decreased gradually as the concentration of urea increased upto 0.7M, then decreased sharply until 1.5M (Kaminashi *et al.*, 1992).

In the presence of urea, myofibrillar Ca^{2+} ATPase of the blue shark was inactivated in two order reactions with an early fast rate and subsequently with a slow rate (Hashimoto *et al.*, 1984).

Kaminashi *et al.* (1998) studied the effect of urea on thermal gelation process of shark myosin B and reported that when heated at 40^0C the rigidity decreased with increasing urea concentration upto 1.0M and somewhat increased with further increasing of urea concentration. When heated at 70^0C, a decrease in gel rigidity accompanied the rise in urea concentration until 2.0M. It was suggested that the decrease in gel rigidity, which is prompted by the addition of less than 1.0M urea, is due to the instabilization of non covalent bonds, such as hydrogen and hydrophobic bonds. The increase in the rigidity by the addition of more than 1.0M of urea is due to the protein-protein interaction among myosin B molecules denatured by urea. It is evident that the myofibrillar proteins from elasmobranchs have the ability to resist higher urea concentration.

2.4. REMOVAL OF UREA FROM MUSCLE

In order to utilize shark meat effectively for human consumption several methods have been attempted to remove urea. Repeated water washing of shark meat could bring down urea concentration and meat becomes acceptable (Venugopal *et al.*, 1994). Use of urease, brine and calcium hydroxide is in

practice for production of marinades (Murtini *et al.*, 1985). Pirano *et al.* (1985) observed that the use of hot alkali, gradient salting and washing with cold water could remove more than 80% of urea.

Mathew *et al.* (2002). Observed that there was a reduction of 68% urea after the first wash and reached a value of 10.23mM at the end of third wash.

Chari and Sreenivasan (1980) found that all the urea from shark meat could be removed by boiling the meat twice in water and repeated washing.

The water washing of teleost fish meat for surimi production is a common practice to improve the frozen stability (Gomez Guillen, 1997, Sano *et al.*, 1988). The water washing of shark meat apart from removing sarcoplasmic protein fraction that may provide good frozen storage stability enable considerably reduction in urea content (Toyoda *et al.*, 1992).

2.5 WATER WASHING

Water washing of separated fish flesh is an essential unit operation in surimi production. The main objective of the water washing of fish is to remove soluble protein fractions to reduce the fat content and to remove colouring pigments in the muscle (Suzuki, 1981).

Generally with continued washing, the gel strength increases that reflect an increased myofibrillar protein content and decreased sarcoplasmic protein content (Toyoda *et al.* 1992).

Acton *et al.* (1983) observed that the increase in gel strength was mainly due to removal of sarcoplasmic protein fraction, which is known to hinder the gelling ability of myofibrillar proteins.

Water-soluble urea can easily be removed from shark meat during the water washing process. Urea as a potent denaturant may alter the conformation of protein leading to changes in the functional properties. The biological

concentration of urea in different species of shark is 250- 300mM (Nishimoto *et al.* 1980).

Mathew *et al.* (2002) observed that the washing of shark meat considerably reduced the ash content indicating that the minerals present in shark meat are water soluble. The reduction in fat content was found to be 23% of its initial value. The non-protein nitrogen fraction, which comprises free amino acids, urea and trimethyl amine oxide are water-soluble and a 62% reduction from the initial value could be seen. And he also explained that the water washing of shark meat improved the gel forming ability significantly with reduction in non-protein nitrogen and urea contents. And it could remove low molecular-weight protein fraction and concentrate high-molecular-weight protein fraction.

Joseph and Perigreen (1986) observed a reduction of 51.6% in the non-protein fraction during washing operation of catfish mince.

The washing treatment helps to remove soluble and odour bearing compounds as well as significant amount of lipids from minced meat (Venugopal *et. al* 1998). Suzuki (1981) reported that the washing of meat removes significant amount of the adhering compounds such as pigments, enzymes and lipids making polar sites available for interaction with water.

According to Chawla *et al.* (1996) washing of threadfin bream mince removed the carotenoids, blood pigments and soluble nitrogenous compounds which resulted in less colour and notable reduction in odour of the mince. About 25-30% of total nitrogen was leached out during washing. The washing procedure reduced lipid content and increased moisture content.

2.6 EFFECT OF ACID

The viscosity is drastically reduced by lowering the pH of the homogenates from 6.5-7.2 to 3.5- 4.0 followed by heating to 50^{0}C. The pH is reduced by adding a few drops of weak organic acids such as acetic acid or

lactic acid, since inorganic acids bring about precipitation of proteins (Venogopal *et al.*, 1998).

In the case of shark, the washed meat homogenate in water is initially passed through a nylon mesh to remove collagen and the collagen-free myofibrillar protein is treated with acetic acid to induce gelation (Venugopal *et al.*, 1994).

Fish muscle structural proteins are known to be sparingly soluble in water, requiring high ionic strength extractants for the dissolution (Sikorski, 1994; Sakaara and Regestein, 1990). Further, the proteins are highly labile to low pH. Lower the pH, higher the extent of denaturation of fish myosin (Suzuki, 1981).

Venugopal (1997) have found that unlike shark meat, the gelation of washed meat of Threadfin bream, Bombay duck, Indian mackerel, Atlantic mackerel and fresh water fish such as Rohu by acetic acid was not characterised by thickening or increase in viscosity of the meat slurry.

Venugopal, (2003) observed that, in presence of acetic acid, the product was stable against microbial growth when stored for 2 month at10^0C.

The relationship between pH and gel forming ability of fish muscle was reported by Shimizu *et al.* (1954). It was found that the myofibrillar fraction of protein in fish muscle is not stable below pH 6.5.

2.7 EFFECT OF STORAGE

2.7.1 Physicochemical changes

The structure of proteins determines the physicochemical, functional and rheological properties of the given food system. The changes in the properties of proteins from fish and shellfish have been documented for many varieties with more emphasis on spoilage microflora. The nonprotein nitrogen constituents in the muscle cell like TMAO, peptides, free aminoacids, urea and

various other inorganic compounds have important role in indicating the chemical nature of spoilage (Love, 1974). Quantifying the total volatile base nitrogen (TVBN) librated during ice storage of fish is routinely practiced to indicate chemical quality (Mathew *et al.*, 1999).

The changes in TVBN during ice storage is not uniform in all fishes and generally a value of 40-50mg/ 100g tissue is taken as unacceptable (Connell, 1995). Sakaguchi *et al.* (1984) observed that the increase in TVBN content mainly arise from degradation of nonprotein constituents and also deamination of nucleotides.

Kumar (2000) studied the biochemical, bacteriological and organoleptic changes in whale shark (*Rhincodon typus*; Smith) meat during storage in ice and found that there was a decrease in the proportion of soluble fractions of protein during storage. It was observed that meat could be stored in ice upto 12 days in acceptable condition.

Stability of proteins is an important criteria for many functional properties like gelation, emulsification capacity and viscosity (Cheftel *et al.*, 1985). According to Xiong (1997) Protein solubility can be defined as the amount of total muscle proteins that goes into solution under specified conditions and is sedimentable by specified centrifugal force. The factors responsible for solubility of proteins from fish are ionic strength, pH and temperature (Yang and Froning, 1990; Turgeon *et al.*, 1992; Monahan *et al.*, 1995).

Lin and Park (1998) investigated the relationship between solubility and conformation changes of myosin from salmon at various ionic strength and pH.

The myofibrillar proteins generally require higher ionic strength solutions usually more than 0.3M and solubility in such a solvent is taken as an index of protein conformation. Solubility of myofibrillar proteins gets altered

during many processing conditions including chilling by icing (Moorjani *et al.*, 1962; Devdasan and Nair, 1977; Reddy and Srikar, 1991).

Viscosity is one of the important functional properties of food proteins. It is important for providing physical stability to emulsion and other suspended particles in foods and contributes to the mouth feel of foods. Processing induced changes in proteins such as polymerization, aggregation and hydrolysis affect the viscosity of food products (Schenz and Morr, 1996).

According to Philips *et al.* (1994) protein aquire their three dimensional, biologically active structures by minimizing the contact between water and hydrophobic amino acid residues which form a hydrophobic nucleus in the protein interior, while the polar groups either form intermolecular hydrogen bonds or hydrogen bond with water molecules.

Venu (2002) found that the gel strength of lizardfish was decreasing during the ice storage and attained lowest gel strength of 10 after 15 days of storage in ice condition.

Tan Sen Min (1998) observed that Lizard fish after death spoil very quickly, if kept without proper icing, due to formation of formaldehyde and study indicated that the gel forming ability of Lizard fish decline drastically after 6 days in ice due to the higher level of formaldehyde.

According to Matsumoto and Noguchi (1992) there are two kinds of temperature tolerance noticed in the myofibrillar protein of fish. The first one is the thermal stability at temperature above the freezing point, which is affected by the raw material quality and the temperature maintained during the processing of surimi. The second is the stability during cold storage, which determine the shelflife of the surimi.

2.7.2 Functional changes

Any change in physiological property of proteins from fish lead to alteration in the functional properties. Gelation, emulsification and hydration are important functional properties, which get affected consequent to change in structure of protein.

The properties of the gels are the net result of the complex interactions between the solvent and the molecular network. For the production of gels, the important properties of proteins are their flexibility, including their ability to denature and give extended chains and especially, their ability to form extensive networks by cross linking (Oakenfull *et al.*, 1997).

Myosin and actomyosin are the principal proteins responsible for gelation in systems (Ziegler and Acton, 1984; Gordon and Barbut, 1992; Wang and Smith, 1994). Solubility and chromatographic profile of rabbit skeletal muscle myosin gels in various solvent systems implied that hydrophobic and hydrogen bonding were more important than SS bonds in gel formation (Foegeding *et al.*, 1987). Smyth *et al.* (1998) indicated that SS-bond formation by S-1 during heat-induced gelation of myosin is important in the general gelation of myosin.

When salted surimi paste is kept at 40^0C for 30 minutes to several hours prior to final heating at 80-90^0C, its rheological properties changes from sol to gel. Greater elasticity and higher water retention of the surimi gels are produced. This process is called as "setting" (Lee and Park, 1998). This setting process is mediated by transglutaminase enzyme, whereas MHC are cross-linked by covalent linkage (Kamath *et al.*, 1992).

Autio *et al.* (1989) studied that the rheological techniques have been successfully employed to understand behaviour of proteins from fish in gelation process. The effect of conditions such as temperature time profiles on rheological properties of myofibrillar proteins may enable the choice of processing condition to take full advantage of the structure forming properties of fish proteins.

Heat induced salt soluble protein (SSP) gels exhibited a regular, repeating, ordered network structure consisting of fine strands of uniform thickness, which contained small voids distributed throughout the matrix (Mc Cord *et al.,* 1998). Wang and Smith (1992) has reported a similar fibrous or thread like net work structure on heating chicken breast SSP to 80^0C in 0.6 M NaCl at pH 6.5.

Surfactant properties are related to the capacity of proteins to lower the interfacial tension between the hydrophobic and hydrophilic components in foods. Generally surfactant properties are related to solubility of proteins but there are some exceptions to this (Kinsella, 1976). Emulsifying and foaming properties are the two surfactant properties of proteins, which have received wide attention.

Colmenero, *at al.* (1988) have reported the correlation between the changes in viscosity, solubility and emulsifying capacity as a result of seasonal variation, time elapsed before freezing and type of freezing.

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted for three months from June to August, 2004

3.1. COLLECTION OF FISH

Market fresh Indian dog shark (*Scoliodon laticodus*) and bull's eye (*Priacanthus hamrur*) was purchased from the local market/ landing centre and transported in an insulated box in iced condition to the laboratory. Only fish that was iced immediately after catching was purchased.

3.2. PREPARATION OF PROTEIN GEL

The whole fish were beheaded, eviscerated, deskinned and filleted. The deboned meat was manually cut with a knife into small pieces (average weight 4 to5g) and washed in running tap water. The meat pieces were suspended in three times their weight of cold water (<10^0C) and held in a cold room (0 to 2^0C) for 18hours. The washed meat decanted through a nylon sieve and the meat pieces were washed again in cold water for one hour. For routine experiments the washed meat was homogenized in cold water at meat to water ratio 1:1.5 using a kitchen mixer for one minute. To homogenates 0.5% (v/w) glacial acetic acid was added dropwise while gently stirring to lower the pH from 6.8 to 3.5 to induce gelation. The acidified samples were left at ambient temperature for three hours for completion of gel formation.

3.3. PREPARATION OF FISH SAUSAGE

Fish sausage was prepared using standard procedure. Raw material either fresh or frozen fish was used and subjected to washing with chilled water. Low temperature (4^0C) was maintained throughout processing. Filleting and meat picking was done. Then mincing and mixing of additives were done in following order. Meat for two minutes, salt and sugar were added and mixing continued for few minutes. Then onion, spices mixture, phosphate, MSG (mono sodium glutamate) ascorbate, sorbate and colour solution were added and continued till uniform mixing. Starch or protein gel were added and mixed

for 2-3 minutes. Prior to completion of mixing, oil was added and mixed well. Finally ice was added in small amounts, mixed for 12-15 minutes. Stuffing of sausage using stuffer and sealing were done. Sausages were subjected to washing with chilled water and then with warm water. After that the sausages were boiled in water bath at 80-90^{0}C for 50 minutes, then cooled in chilled water of <15^0 for 10 minutes and subjected to reboiling in boiling water for ½-2 minutes. Sausages were dried under fan, labeled and stored either in chilled condition or frozen storage.

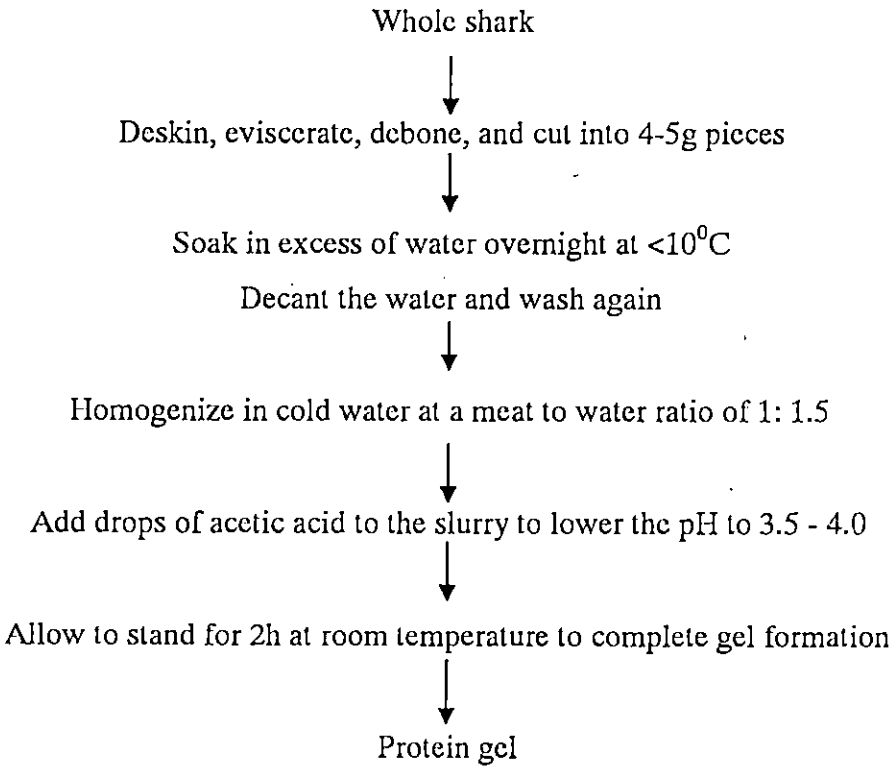

Fig. 1. Flow chart for preparation of fish protein gel

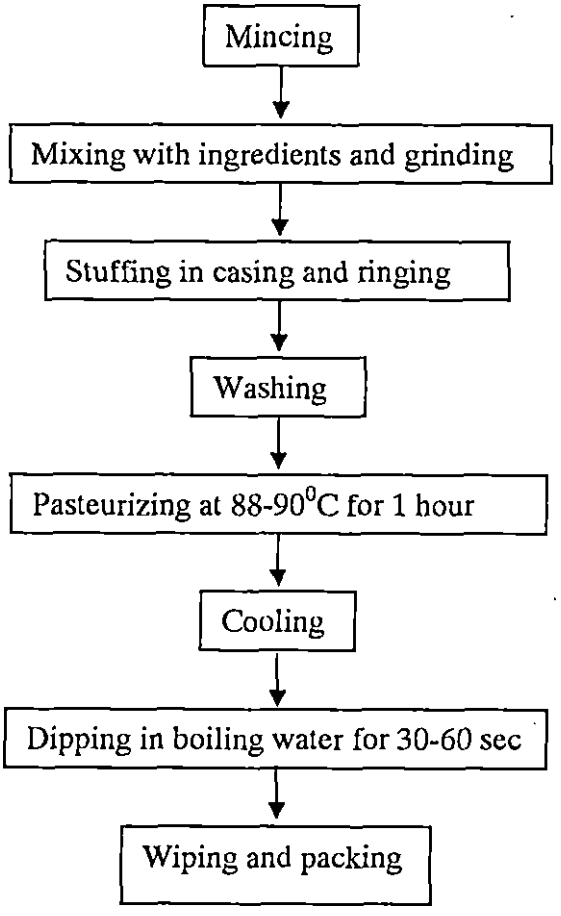

Fig. 2. Flow chart for preparation of sausage

Table1: Composition of Ingredients of paste used in preparation of Control

Sl.No.	Ingredients	Composition (%)
1.	Minced meat	70.00
2.	Starch	10.00
3.	Sugar	1.50
4.	Table salt	2.00
5.	Refined oil	4.00
6.	Ice water	10.00
7.	Spices	2.50

Table 2: Composition of Ingredients of paste used in preparation of Test

Sl.No.	Ingredients	Composition (%)
1.	Minced meat	70.0
2.	Starch	10.00
3.	Sugar	1.50
4.	Table salt	2.00
5.	Refined oil	4.00
6.	Protein gel	10.00
7.	Spices	2.50.

3.4. STANDARDIZATION OF PRODUCT

The product was standardized based on the use of different proportion of starch, protein gel, and ice water. Four sets of products were prepared and the acceptability of the products was evaluated by a taste panel. A taste panel consisting of 10 judges carried out sensory evaluation. Quality was evaluated

Plate 1: Indian dog shark (*Scoliodon laticaudus*)

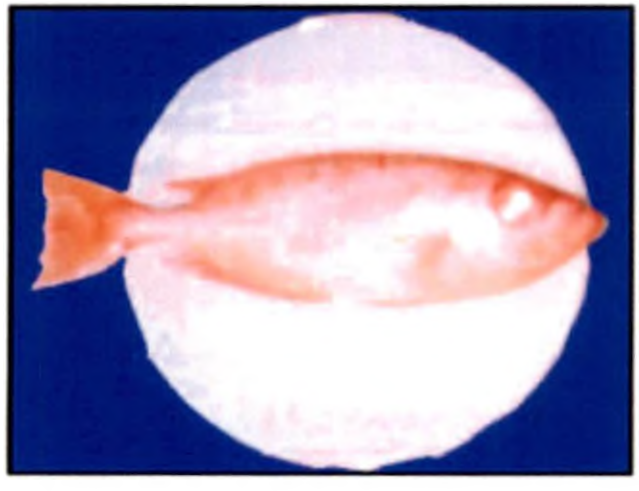

Plate 2 : Bull's Eye (*Priacanthus hamrur*)

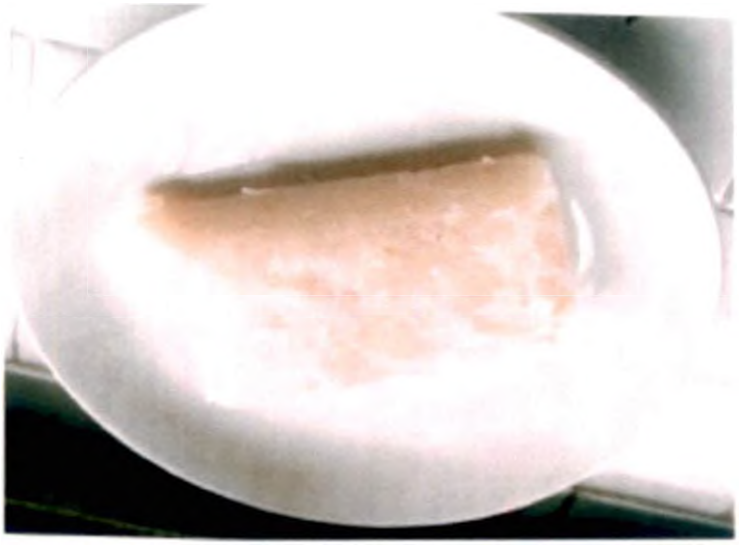

Plate3: Fish protein gel from shark meat

Plate 4: Minced meat

Plate 5: Silent cutter

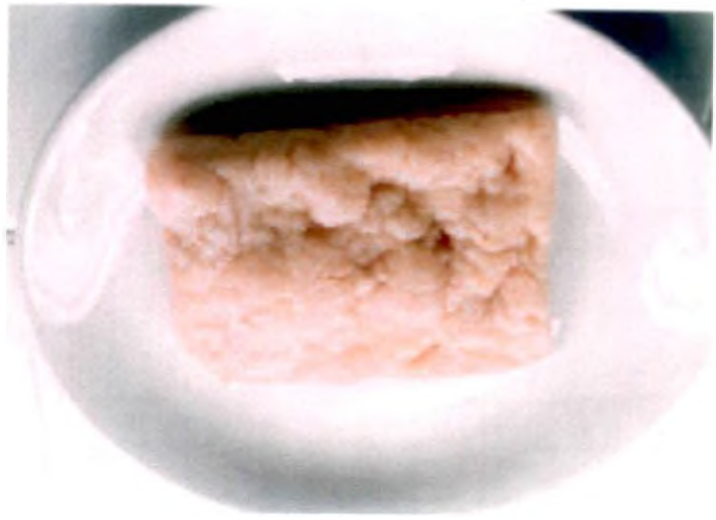

Plate 6: Surimi

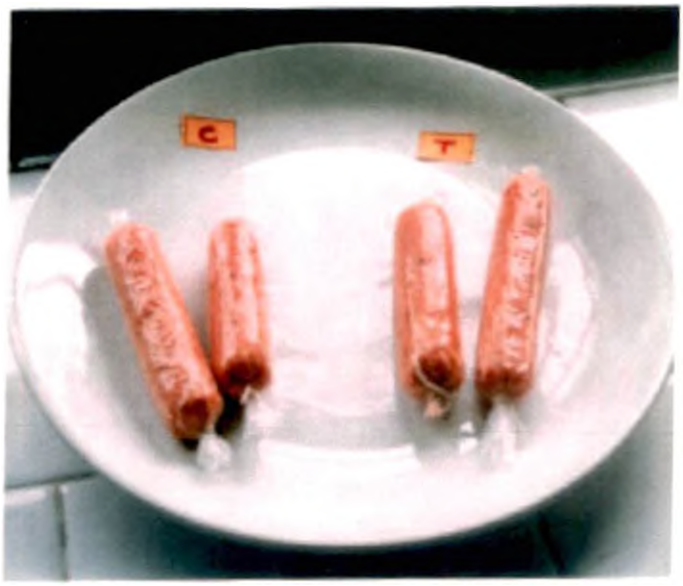

Plate 7: Sausage (C= Control, T=Test)

Plate 8: Instrument used for gel strength measurement

on the basis of texture and taste, using a 10-point scale. Products were also subjected to gel strength measurement and appropriate product was selected whose gel strength and sensory evaluation scores were higher.

3.5 STORAGE STUDIES

Fishery products were prepared using the standard method and packed in low-density polythene bag of thickness 300 guage. The bags were heat sealed using an electrical heat sealer. The products were stored in freezing condition at -20^0 C. Sampling was done every two weeks. Samples were subjected to various tests *viz*. moisture content, total volatile nitrogen content, peroxide value, total plate count, fungal count, gel strength, pH and sensory evaluation.

3.6. TEST

3.6.1. Moisture content

Moisture content of protein gel and products were determined by the method of AOAC (1975). About 5g of sample was accurately weighed in a clean dry petridish using an electronic balance and was dried to a constant weight at a temperature of 105°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.

3.6.2. Total volatile base nitrogen (TVBN) content

TVBN content was determined by conway's microdiffusion method (Conway, 1947).

A sample of 10g of fishery product was extracted in 10% trichloroacetic acid (TCA) solution. 1ml of this extract was pipetted out into the outer chamber of the Conway's unit and 1 ml of standard 0.02 N H_2So_4 solution was taken in the inner chamber. Then, 1ml of saturated Sodium carbonate solution was added to the outer chamber. The unit was closed immediately. The solutions in the outer chamber were mixed by slow rotation of the apparatus. It

was then incubated at room temperature overnight. The content of the inner chamber were titrated against standard 0.02 N NaOH solution using Tachirho's indicator. A blank was also run using 1 ml of 10 % TCA solution in the outer chamber instead of the sample extract.

$$\text{TVB-N content (mg\%)} = \frac{(A-B) \times 0.28 \times 50 \times 100}{W}$$

Where A =Volume of NaOH solution required for control

B = Volume of NaOH solution required for test

W = Weight of sample

3.6.3. Peroxide Value (PV)

The method of Connel (1975) was adopted for peroxide value determination. A sample of 10gms of the product was blended thoroughly with anhydrous sodium sulphate in a mortar. The blend was shaken with chloroform for 5 – 10 minutes and filtered using Whatman No. 42 filter paper. 10ml of the filtrate was taken in a pre weighed beaker and the solvent evaporated off. From this the fat content of the sample was determined. To another 10ml of the aliquot taken in a flask, 20 ml of glacial acetic acid and a pinch of potassium iodide were added. After closing the flask the reagents were mixed carefully and incubated for a period of 30 min. under dark at room temperature. Then the sides of the flask were washed with distilled water. A few ml of starch solution were added and titrated immediately against standard 0.002 N sodium thiosulphate solution till the blue colour disappeared. PV was expressed as the number of ml of 0.002N sodium thiosulphate required per gramme of sample.

3.6.4. Total plate count

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

Total plate count of the sample was determined according to the method of Maturin and Peeler (1995). A sample of 10gm was aseptically transferred to sterile blender and homogenized with 90ml phosphate buffer (0.1M KH_2PO_4 + K_2HPO_4, pH-7.2) as a diluent. Appropriate serial decimal dilutions of 1ml of the homogenate were made using phosphate buffer. Appropriate dilutions were plated, in duplicate, by pour plate technique using plate count agar. The composition of medium is given in Table 3. Plates were incubated at a temperature of 37^0C for 48hours. Plate showing 30 to 300 colonies were counted. Counts were expressed as colony forming units (c.f.u) per gram sample.

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

3.6.5. Fungal count

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

$$\text{TFC (cfu/gm of sample)} = \frac{\text{Number of colonies} \times \text{dilution}}{\text{Weight of sample}}$$

3.6.6. pH

Sample was blended with an equal quantity of water and pH of the blend was measured using a pH meter by immersing the electrodes well inside the blend. The instrument was set using a standard pH buffer (Global Electronics, Hydrabad).

3.6.7. Gel strength

Gel strength was determined as per the procedure of (Tan Sen Min *et al.*, 1987). 25mm length sample was cut out from the product prepared and gel strength measured by instrument (Push Pull scale, Japan). The spherical end of the probe punctured into the gel surface and force required to puncture the gel surface was taken as gel strength of the product.

3.6.8. Sensory evaluation

A taste panel consisting of ten judges carried out sensory evaluation of the product sample. The quality characteristics assessed were, texture and taste of the product on the basis of a 10 point scale as suggested by (Amerine *et al*; 1965). The format of sensory evaluation score sheet is given in Appendix I and Appendix II.

3.7. STATISTICAL ANALYSIS

The experiments were carried out using Completely Randomised Design (CRD). Data obtained during frozen storage of products were analyzed using Analysis of Variance (ANOVA) with two observations per cell technique (Kempthorne, 1952). Pairwise comparison of treatment means were done wherever necessary using least significant difference. Sensory evaluation results were analysed using the Friedman test (Sprent, 1989).

Table3: Composition of microbial media

Sl.No.	Ingredients	Composition (gm)	
		Plate count agar	Potato dextrose agar
1.	Hydrolysate	5.00	-----
2.	Yeast extract	2.50	-- ---
3.	Dextrose	1.00	20.00
4.	Agar	15.00	15.00
5.	Potato infusion	-----	200.00
6.	Tartaric acid (10%)ml	-----	1.00
7.	Distilled water	1000ml	1000ml
8.	PH	7.0±0.2	5.6±0.2

Results

4. RESULTS

4.1. STANDARDIZATION OF PRODUCT

Four types of products have been prepared using the different proportion of starch, protein gel and ice water. The product was selected based on gel strength and sensory evaluation for texture and taste. The ANOVA for the gel strength is given in Table 5. Significant difference was noticed between the treatments. However, result of pairwise comparison has shown that the three treatments namely A (10: 0: 10), B (10: 5: 5) and C (10: 10: 0) belong to the same homogenous group. Average scores for sensory evaluation are given in the Table 6.

Table 4. Proximate composition of fish protein gel, control and test (percentage by weight).

Components	Fish protein gel	Control	Test
Moisture	90.6	70.8	68.75
Protein	9.0	9.96	12.0
Fat	0.40	0.86	0.78
Ash	0.55	1.98	1.27
Carbohydrate	-----	16.4	17.2

Table 5: ANOVA for gel strength of different starch: protein gel: water proportion

Sources of variation	Sum of squares	Degrees of freedom	Mean sum squares	F value
Between treatments	2606.25	3	868.75	5.56*
Error	1250	8	156.25	
Total	3856.25	11		

* Significant at 5% level

CD (Critical difference) = 23.53

Treatments:	C	B	A	D
(Starch: protein gel: water)				
Means	120	110	105	80

Underscored means are not significantly different

Table.6: Average scores of sensory evaluation of starch: protein gel: water proportions

Quality characteristics	Starch: protein gel: water proportion			
	10:0:10(A)	10:5:5(B)	10:10:0(C)	5:15:0(D)
Texture	7.87	7.87	7.50	6.12
Taste	7.87	8.00	8.00	7.00

4.2 STORAGE STUDIES

4.2.1. Moisture content

The changes in moisture content of sausage are presented in Fig.3. The moisture content of control was 70.8% at start of storage studies but in the case of test moisture content was 68.75% initially. In the case of control moisture content decreased to 60.43% after 60 days. Whereas, in the case of test (10:10:0) moisture content decreased to 67.56%. Statistical analysis showed that there was no significant difference in moisture content of test and control. Also no significant difference in moisture content noticed with days of storage. But interaction means showed significant difference. The result of statistical analysis is given in Table7.

Table. 7: ANOVA for moisture content of control and test during frozen storage

Sources of variation	Sum of squares	Degrees of freedom	Mean sum squares	F value
Treatments	99.191	1	99.191	1.766
Days	230.124	4	57.531	1.024
Interaction	224.629	4	56.157	4.983*
Error	112.669	10	11.699	
Total	666.642	19		

* Significant at 5% level

Critical difference (Interaction) = 4.246

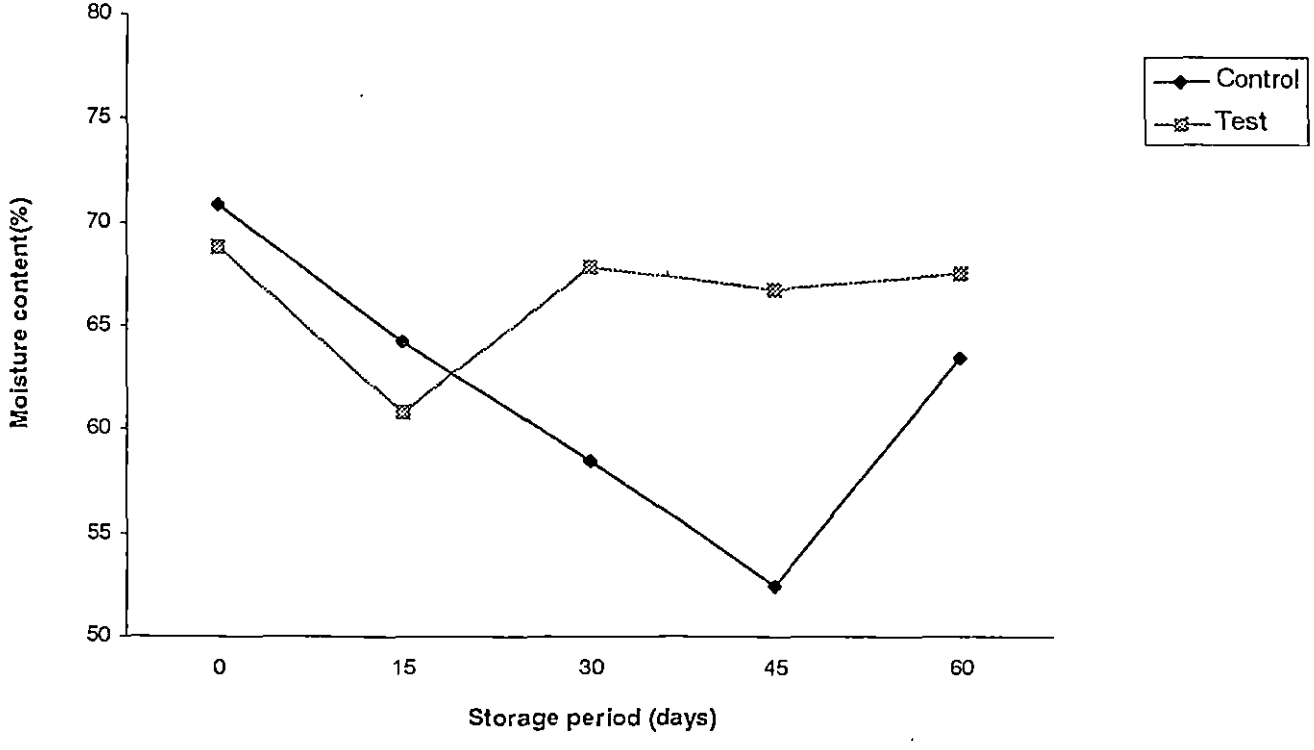

Fig. 3: Changes in moisture content of sausage during frozen storage

4.2.2. Total volatile base nitrogen content (TVBN)

The TVBN content increased steadily from an initial value of 2.8mg% to 18.18mg% till 45th day in the case of control and then decreased to 5.6mg% on 60thday. In the case of test TVBN increased from 4.9 mg% to17.37 mg% till 45th day and then reduced to 8.31mg%on 60th day. Observations were statistically analysed which showed significant difference between the two treatments and changes in TVBN with storage period (Table 8). Changes in TVBN content with days of storage are shown in Fig.4.

Table.8: ANOVA for TVB-N content of control and test during frozen storage.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum squares	F value
Treatments	13.122	1	13.122	5.66*
Days	489.421	4	122.355	52.78*
Error	32.452	14	2.318	
Total	534.996	19		

*Significant at 5% level

Critical difference (Days) = 2.34

Days:	0	15	30	45	60
Means	3.85	6.95	6.96	12.51	17.77

Treatments	Control	Test
Means	8.80	10.42

Underscored means are not significantly different

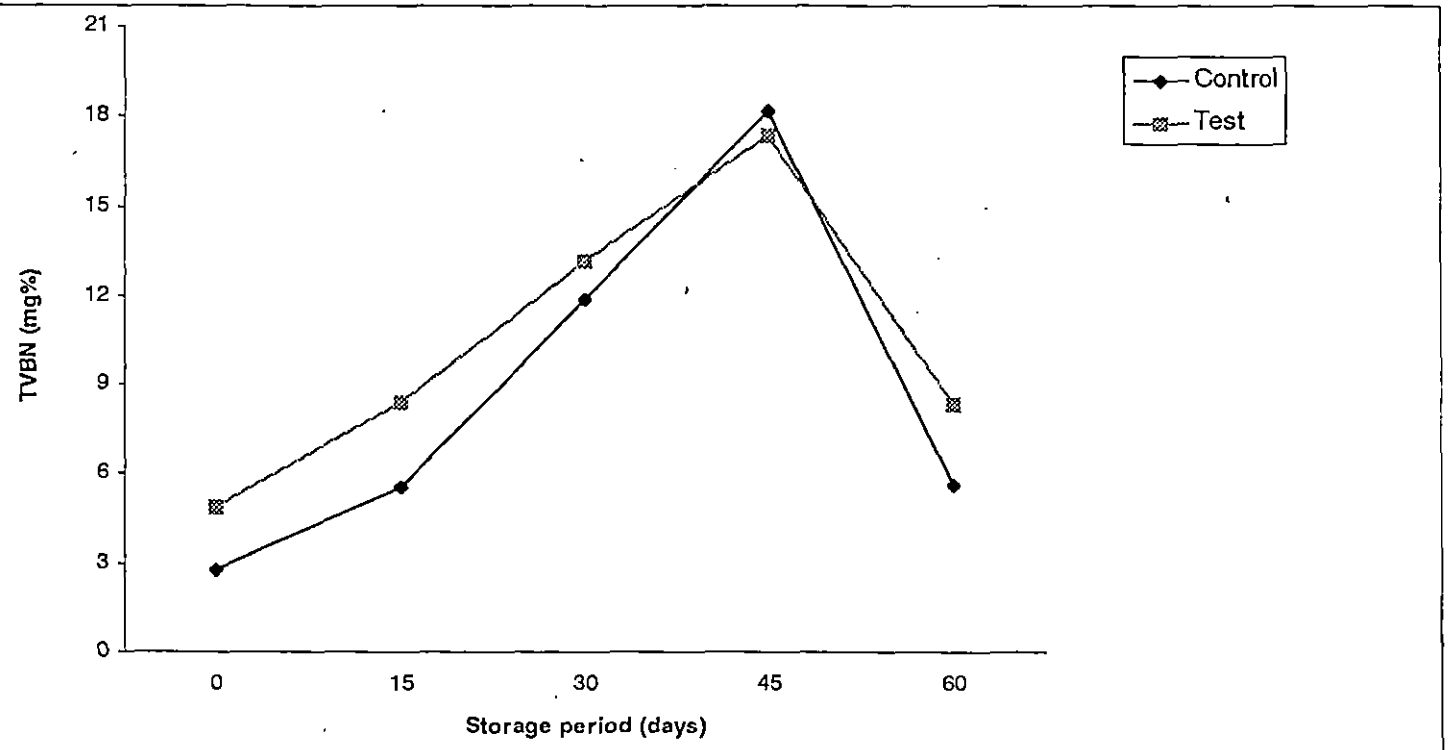

Fig. 4: Changes in TVBN content of sausage during frozen storage

4.2.3. Peroxide value

The peroxide value varied from 60 to 200 mM/gm of sample in the case of control and from 37.5 to 138.33 mM/gm in the case of test during the two months of frozen storage. Trend showed by control was increasing from 0^{th} day to 60^{th} day but test showed first an increase till 45^{th} day then a decrease (Fig.5). Statistical analysis (Table. 9) showed that there is no significant difference between test and control and also showed there is no significant changes in peroxide value with the storage period. But interaction means showed significant difference.

Table. 9: ANOVA for peroxide value of control and test during frozen storage

Sources of variation	Sum of squares	Degrees of freedom	Mean sum squares	F value
Treatments	42.137	1	42.137	0.013
Days	41478.680	4	10369.670	3.16
Interaction	13106.930	4	3276.733	330.970*
Error	99.004	10	9.900	
Total	54726.751	19		

* Significant at 5% level

Critical difference (Interaction) = 3.906

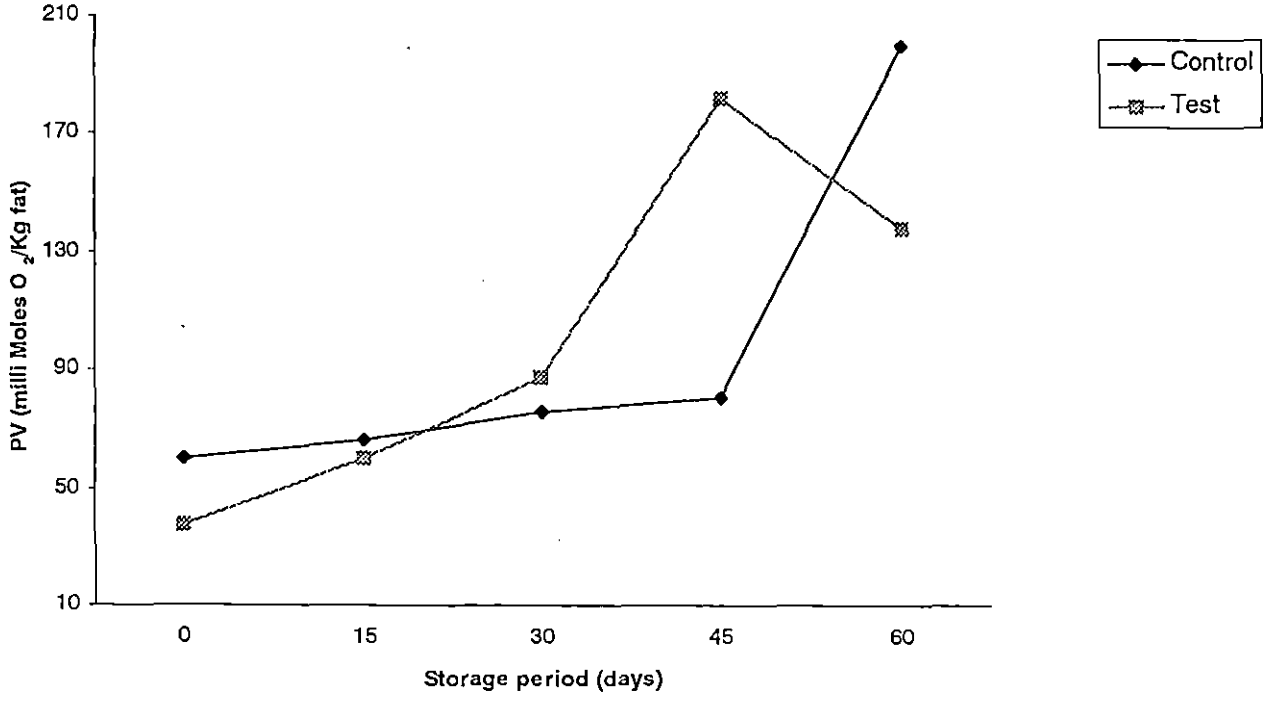

Fig. 5: Changes in Peroxide value of sausage during frozen storage

4.2.4. Gel strength

The gel strength varied from140gm to167.5gm in the case of control and from170gm to 202.5gm in case of test. Gel strength was measured using the instrument (Push Pull scale, Japan) shown in the Plate 8. Changes in gel strength with days of storage is given in Fig. 6. Statistical analysis showed that there is no significant difference between the two treatments and also there is no significant difference in values of gel strength with days of storage (Table 10). But interaction means are significantly different.

Table 10: ANOVA for gel strength of control and test during frozen storage.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum squares	F value
Treatments	4202.500	1	4202.500	3.73
Days	10440.000	4	2610.000	2.32
Interaction	4510.000	4	1127.500	3.833*
Error	8825.000	30	294.167	
Total	27977.500	39		

* Significant at 5% level

Critical difference (Interaction) = 21.29

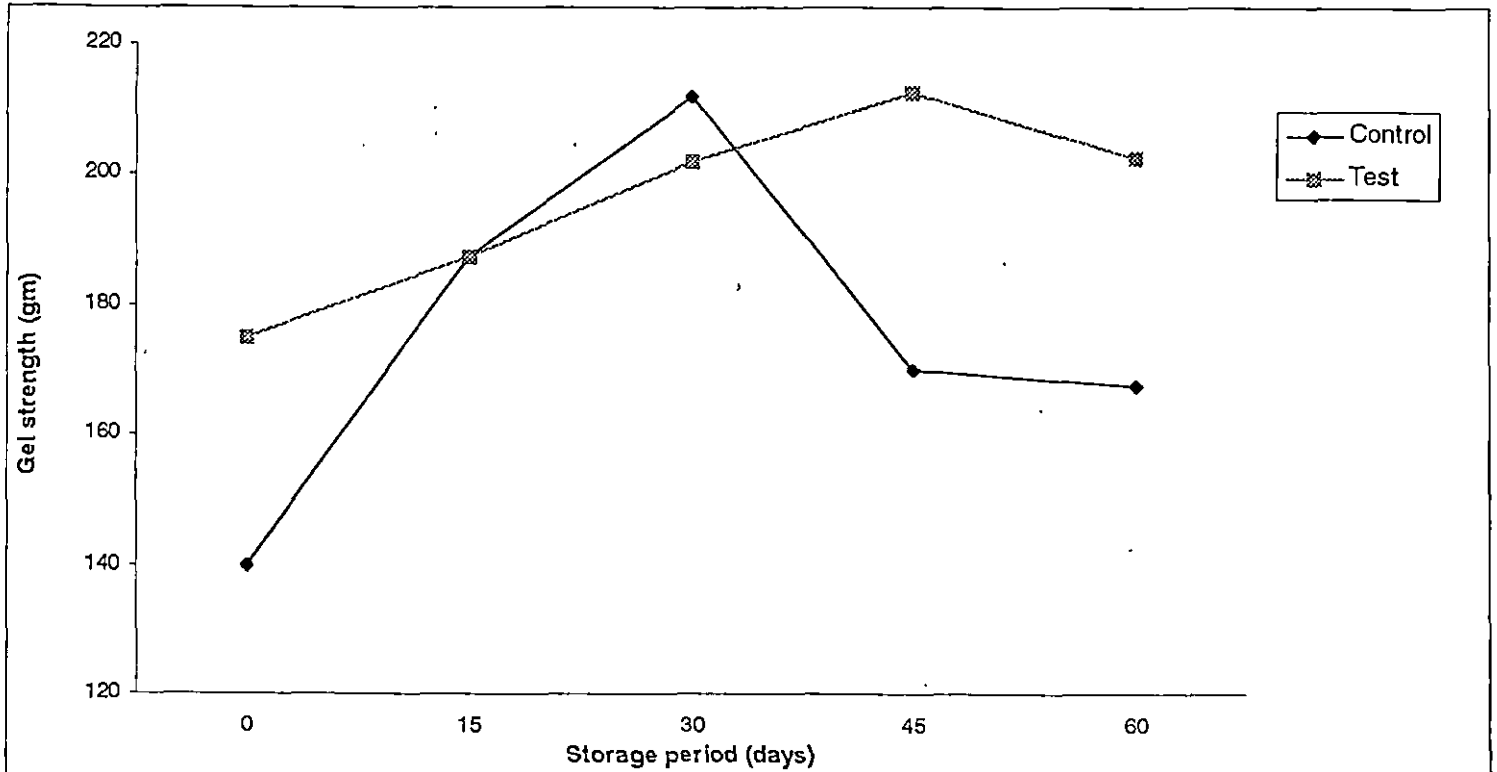

Fig. 6: Changes in gel strength of sausage during frozen storage

4.2.5. pH

The pH of samples taken from control varied from 6.51 to 6.88. But in the case of test, the pH varied from 6.45 to 6.68 during two months of frozen storage (Fig.7). The pH values for both control and test are given in Table. 11. The pH of both the samples have shown an increasing trend with days of storage.

Table 11: Changes in pH of control and test with storage period

Treatments	Days				
	0	15	30	45	60
Control	6.51	6.69	6.5	6.69	6.88
Test	6.45	6.56	6.58	6.63	6.68

4.2.6. Total plate count (TPC)

In both the cases number of colonies found was below 30 from the start of storage to the end of storage. This showed that bacterial growth was restricted during frozen storage. When the number of colonies were below 30 then result can be expressed as less than $3x10^2$ cfu/gm of sample. However, number of colonies were below 30 in both the cases, control showed comparatively higher number of colonies.

4.2.7. Fungal count

In the case of fungal count also the number of colonies were below 30 throughout the storage period. So fungal count during frozen storage can be expressed as less than $3x10^2$ cfu/ gm of sample. This showed that fungal growth was restricted during frozen storage.

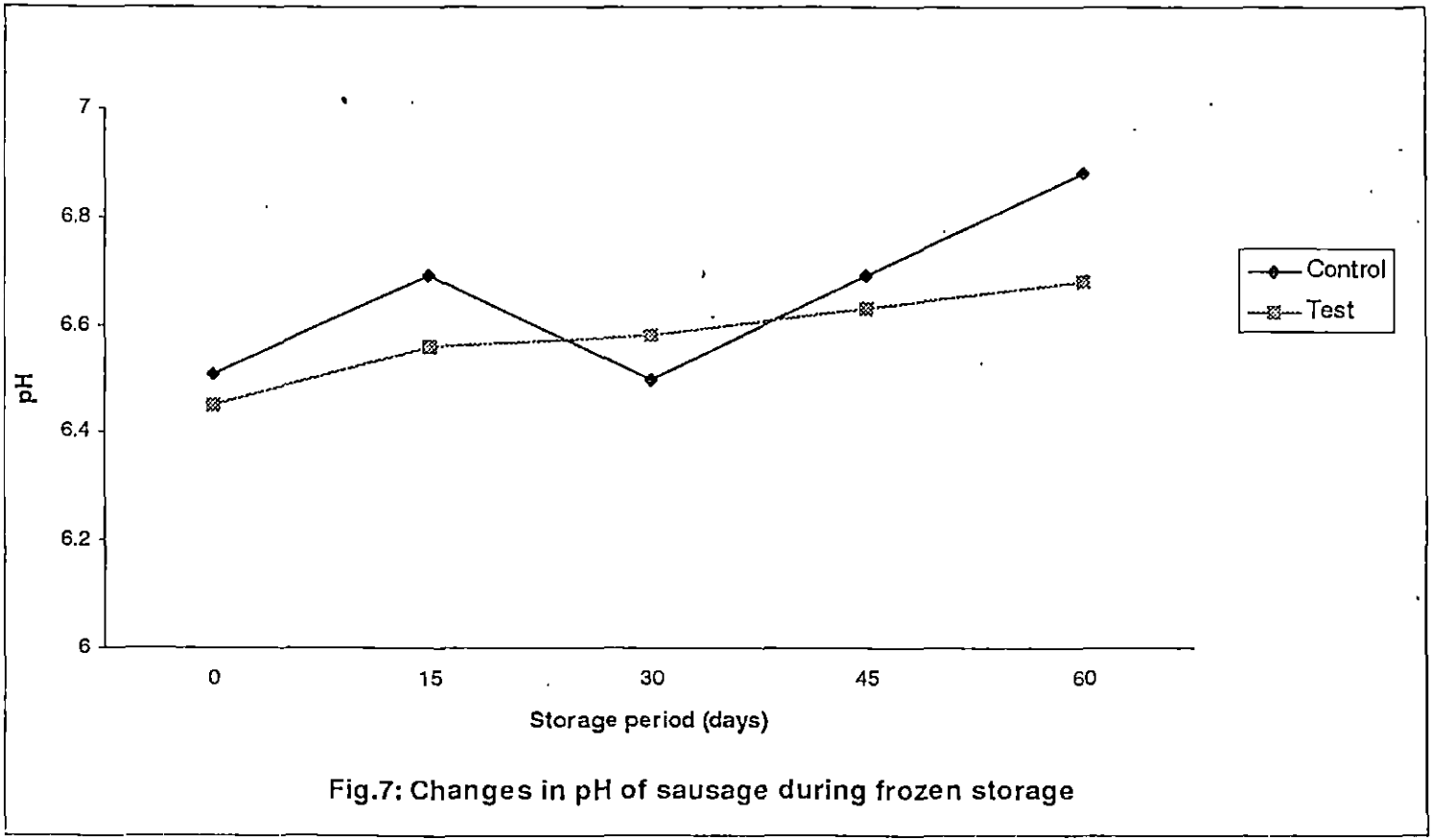

Fig.7: Changes in pH of sausage during frozen storage

4.2.8. Sensory evaluation

Sensory evaluations was done for both control and test samples every 15th day of frozen storage for texture and taste.

4.2.8.1. Texture

The mean score for texture decreased from 8.00 to 6.55 in the case of control. But test mean score was nearly same as on 0th day 8.00 and on 60th day 8.11(Fig. 10).

Friedman test showed no significant difference between control and test. But the graph showed variation in mean score of texture during storage (Fig. 8). Average scores for texture are given in the table 12.

Table 12: Average scores for texture during frozen storage

Treatments	Days				
	0	15	30	45	60
Control	8.00	7.00	8.30	7.80	6.55
Test	8.00	7.20	8.33	7.60	8.11

4.2.8.2. Taste

The mean score for taste decreased from 8.00 to 7.55 on 60th day in the case of control. In the case of test, the mean scores vary from 8.16 at start to 7.55 at the end of storage period (Fig.11).

Friedman test was carried out for the mean scores of taste of both samples. No significant difference was noticed between control and test after two months of storage period. Average scores for taste are given in the Table 13.

Table 13: Average scores for taste during frozen storage

Treatments	Days				
	0	15	30	45	60
Control	8.00	7.2	7.77	7.6	7.55
Test	8.16	7.5	7.77	8.4	7.55

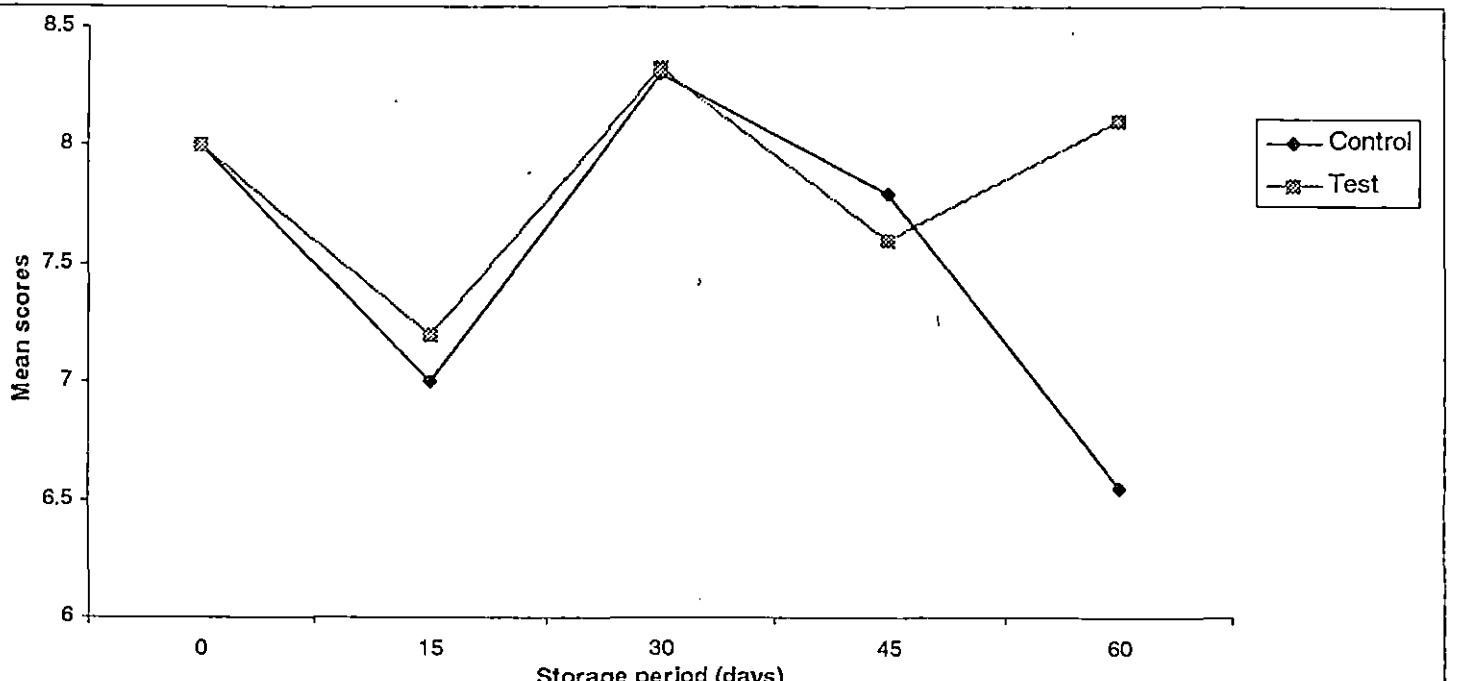

Fig.8: Changes in Texture of sausage during frozen storage

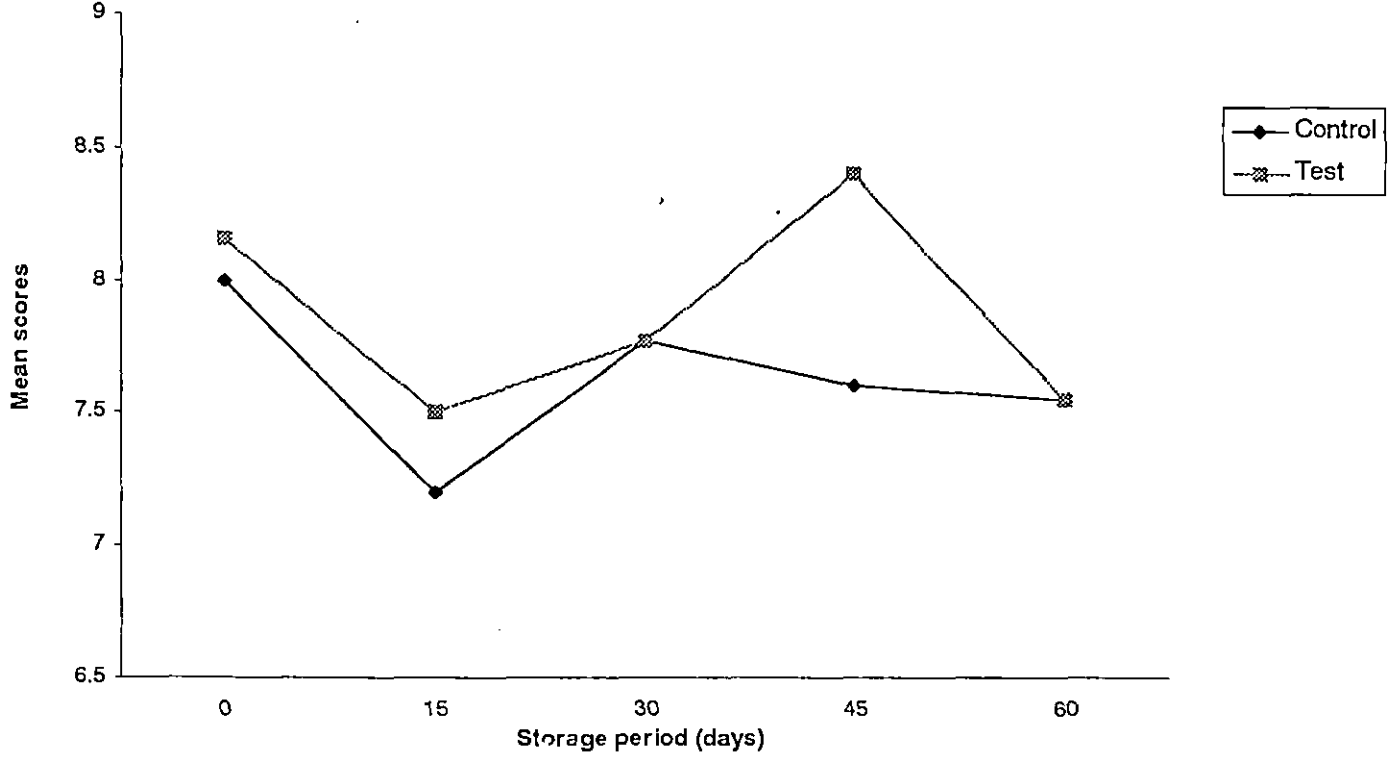

Fig.9: Changes in taste of sausage during frozen storage

Discussion

5. DISCUSSION

Utilization of underutilized fishes for product development is a great move by the scientists. Fish is a food rich in protein with an amino acid composition well suited to human dietary requirements. Fish is a cheap source of protein and ideal for combating protein malnutrition in the poor.

A number of techniques are available for making novel products from marine and freshwater aquatic resources. These include individual quick freezing, sous vide, cook-chill, high pressure processing, breading and battering, developing products from fish mince, surimi and sea food analogues, fish fillets and steaks, modified atmosphere packaging and products through improvement of traditional processing technique such as canning, curing, smoking etc.

Besides quality fishes like tuna, cuttlefish and squids, fishes which do not fetch high value such as catfishes, lizard fish, jewfish, shark, skates, rays, anchovy, threadfin, ribbon fish and silver belly, crabs and deep sea fishes can be utilized fully well for conversion into value added products for internal consumption and to cater to the needs of overseas markets (Shenoy, 1989).

5.1. STANDARDIZATION OF PRODUCT

Standardization of product was done based on the different proportions of starch, protein gel and ice water. Generally in sausage preparation starch is used as a binder. The present study was done based on incorporation of protein gel with starch as a binder and comparison of the quality changes during frozen storage with control, without protein gel. Four types of sausages with different proportions of starch, protein gel and ice water have been prepared. These proportions are A (10: 0: 10), B (10: 5: 5), C (10: 10: 0) and D (5:15: 0) where treatment A is treated as control. Products were subjected to sensory evaluation for texture and taste. Gel strength was measured using the instrument (Push and Pull scale, Japan). It has been found that product C

showed the highest gel strength. This may be due to the combined effect of starch and protein gel on the gel strength. Mean scores for texture and taste of product C was nearly equal to Control. Statistical analysis of gel strength showed that products A, B and C belonged to homogeneous group. Product D was different from the other three, this might be due to its higher content of protein gel. The protein gel had 90.6% water, which might have adversely affected the gel strength. Even treatments A, B and C belonged to a homogeneous group, treatment C showing highest mean for gel strength. So out of the four products, C has been selected as "test". Proximate composition of control and test were determined and it was found that "test" was having higher protein content (12%) than control (9.96%) (Table 4). This might be due to incorporation of protein gel. From sensory evaluation scores it was found that the sausage with 10: 10: 0 proportion of starch: protein gel: ice water was the best and hence, selected for further studies (Table 6).

5.2. STORAGE STUDIES

Changes during storage of fish sausage were studied based on various physical, chemical, microbiological and organoleptic tests. All the tests used are dependent on quality of the product and hence, are useful for determining their shelflife. In addition, sensory evaluation was used as a means of determining consumer acceptability of the products. It is well known that quality of fish products are most satisfactorily judged by organoleptic methods rather than by objective test (Gould and Peter, 1971). It may be noted that most of the parameters used in the present investigation are interrelated. For example TVBN is dependent on microbial activity (Beatty, 1938 and Castell, 1946, Connel, 1975). Peroxide value is the indice of the extent of fat oxidation (Tarr, 1944 and Melton, 1983). All these in turn, can influence the organoleptic quality of the product.

Fish sausages were stored under frozen condition at -20^0C for two months. Sausage prepared with proportion of starch: protein gel: water (10: 10:

0) showed lower rate of changes in quality parameters than control having proportion of (10: 0: 10) starch: protein gel: ice water.

5.2.1. Moisture content

The changes in moisture content of sausage during frozen storage are presented in Fig. 3.

Moisture content was showing decreasing trend with storage period. We could see that initial moisture content was high in the case of both control and test. This reduction in moisture content might be due to dehydration of product during frozen storage. In the case of control, variation in moisture content was higher in comparison to the test. In the case of control moisture content reduced from 70.8% to 60.43% on 60^{th} day, whereas, in the case of test it varied from 68.75% to 67.75% at the end of the storage studies.

However, the statistical analysis showed that there was no significant difference between treatments and days of storage but graph was showing some variation with storage period. Interaction means are significantly different so changes in moisture content might be due to combined effect of both treatments and days of storage.

Siddappaji (2002) found that the initial moisture content of sausage with cassava flour was 63.09%, this slightly less than that was observed in the case of the present study. This might be due to higher percentage of water used in the case of control and protein gel added to the test. Smruti *et al.* (2004) observed that patties prepared from hard head cat fish (*Aris felis*) mince showed moisture content 65.0±5% and patties, the fish mince when replaced with acid induced catfish meat gel at concentration of 10% (w/w) showed moisture content of 63.0± 0.64%. Here moisture content in case of gel incorporated patties showed lower value than observed in case of sausage with shark protein gel. This might be due to higher content of water in the case of shark protein gel.

As we could see that moisture content of acidified shark meat gel was 90.6% (Table 4), Venugopal (2002) observed 87.3% water for acidified shark protein gel, which is slightly lower than what was found in the present study. We could see that the protein content of shark protein gel was 9% (Table 4). Smruti *et. al.* (2004) found that protein content of gel from hardhead catfish was 14.56%, the remaining was water and traces of lipids. This higher protein content in the latter case might be due to difference in moisture contents.

5.2.2. Total volatile base nitrogen (TVBN)

The total volatile base nitrogen (TVBN) during frozen storage showed an increasing trend with storage period and started declining after 45th day. In the case of control, initial value was 2.8 mg% but in the case of test it was 4.9% this might be due to higher content of protein. Statistical analysis showed that there was significant difference between the control and test and also with days of storage.

Sini (2002) also noticed an increasing trend in TVBN at –20^{0}C in the case of sausage prepared with and without potassium sorbate. But after three months it started declining.

Siddappaji (2002) observed that the value of TVBN increased to 28 mg/ 100gm by 21 days in sausage prepared using corn flour and cassava flour and then decreased in both the products. Since bacteria mostly produce TVBN, the result gave clear indication of bacterial growth in the product at 10^{0}C. But present study was conducted in frozen condition so bacterial growth may not be the cause for it, because below biological zero temperature (-12^{0}C) bacteria cannot grow. So increasing trend of TVBN might be due to some chemical reaction during frozen storage.

The production of volatile base nitrogen is normally related to bacterial activity (Gokodulu et al., 1998). But that may be in the case of chilled storage. Present study was conducted under frozen condition, so that this may not be the

main reason for TVBN production. It might be due to chemical changes in the products during frozen storage.

5.2.3. Peroxide value

Fat oxidation is a free radical mechanism and is characterized by a slow initiation period followed by an accelerating rate of oxygen absorption.

However, statistical analysis showed that there was no significant difference in the peroxide value between control and test and with storage period. But the graph showed an increasing trend with days of storage (Fig.5). In case of control, variation in peroxide value was slightly higher than test with storage period which might be due entrapment of air during stuffing and higher content of fat.

In the case of test, peroxide value showed an increasing trend initially then started declining after 45th day. Initial increasing trend may be due to the accumulation of products of oxidation (peroxide and hydroperoxide). In due course, these products further undergo breaking down (aldehyde and ketones) and show a comparatively low value.

Although statistical analysis for peroxide value was not showing significant difference between treatments and days of storage, the interaction means showed significant difference. So, change in peroxide value might be due to combined effect of treatment and storage period.

5.2.4. Gel strength

Gel strength is one of important quality parameters for assessing the quality of sausage. The present study showed that gel strength of test was more than that of control. This might be due to binding properties of starch and protein gel. In both the cases gel strength increased initially and then showed declining trend during frozen storage. This might be due to denaturation of protein and reduction in water holding capacity of the product.

Although, statistical analysis showed that there was not significant difference in gel strength between control and test and days of storage, the graph showed an increasing trend initially and then declined with storage period (Fig.6). This might be due combined effect of treatments and days of storage, because interaction mean was showing significant difference (Table. 10).

Pacheco-Aguilar et al. (2000) found that decrease in gel forming ability could arise from different factors like pH, protein denaturation and species specificity. So changes in the gel strength in the case of the present study might be due to many of these factors.

5.2.5. pH

The pH is one of the important parameters to be tested for products, because pH is responsible for bacterial activity and protein denaturation during frozen storage. Test was prepared by incorporation of protein gel and preparation of protein gel required acetic acid to reduce the pH of fish meat to 3.5. Generation of gel networks from globular proteins often requires a degree of unfolding of the protein followed by aggregation of the partially denatured proteins. This can be brought about by either heating or changing the ionic strength or pH (Oakenfull et al., 1997).

Eventhough protein gel was added to the test, initial pH of the test was 6.45. This might be due to higher pH of other ingredients used for the preparation of sausage. Test showed slightly lower pH value than control. This might be due to incorporation of protein gel. Fig. 7 shows an increasing trend in pH with storage period. It could be justified by the increase in TVBN with days of storage.

5.2.6. Total plate count (TPC)

The best, simple and common test for bacterial quality of fish is the determination of total bacterial population (Tarr, 1940; Faber, 1965). The

present study showed that total plate count was stable with storage period. In both the cases number of colonies found were below 30 throughout the storage period. This can be expressed as total plate count less than $3x10^2$ cfu/gm of sample. So, we could say that products were safe microbiologically during storage. This might be due to the inhibitory effect of acetic acid operating in conjunction with freezing temperature, both synergistically providing a hurdle to microbial proliferation.

Microbial growth in such products during storage is controlled not only by refrigerated temperature but also by the inclusion of at least another additional microbial barrier (NFPA, 1988; Scott, 1989). The low pH in the case of test might have provided such a barrier, in addition to low storage temperature.

Sini (2002) also found not much changes in total plate count (TPC) in the sausage prepared with and without potassium sorbate stored at -10^0C and at refrigerated temperature (-20^0C).

5.2.7. Fungal count

Moulds are one of the important causes of spoilage of any kind of food all conditions of storage (Frazier and Westhoff, 1978). Fungi can grow at water activity below 0.80 (Katz and Labuza, 1981) in the case of dry fishery product. It can grow in chilled condition and also at low pH, but in frozen condition it cannot grow, because at -20^0C microbial activity is not possible.

Here again number of colonies observed in both the cases were below 30. So, the result can be expressed as less than $3x10^2$ cfu/gm of sample. However, in the cases of both samples numbers of colonies were less, but the test showed comparatively even lower number of colonies. This might be due to antifungal property of acetic acid.

Smruti *et al.* (2004) found that after 21 days of chilled storage, untreated control samples had fungal count of 3.5x 10^3 cfu/ gm as compared with 1x 10^3 cfu/gm for gel incorporated samples of patties.

5.2.8. Sensory evaluation

According to Ryder *et al.* (1993) sensory evaluation is most reliable test for raw material and processed fishery products. Even the biochemical method often employed for determining freshness of flesh foods, cannot be singularly accepted as universal due to the complex nature of flesh foods (Field, *et al.*, 1968; Edwards *et al.*, 1983). Sensory evaluation being a subjective method cannot be singly used. However, coupled with other methods sensory evaluation forms an important quality index (Gill, 1992; Nunes *et al.*, 1992).

Sensory analysis is concerned with measuring physical properties by psychological techniques. Sensory methods are used for measuring the properties that cannot be evaluated directly by physical or chemical tests (Joseph and Iyer, 2002).

The parameters used for organoleptic tests were texture and taste. A score sheet was given to the panelists before the test. The panelists were asked to judge each parameter according to their liking as would be done by consumer for surimi based paste products. Scores for the two sensory parameters showed that products were acceptable throughout storage period. Friedman test showed that there was no significant difference between the two products.

5.2.8.1. Texture

Although score for texture analyzed by Friedman test showed that there was no significant difference between control and test. But Fig. 8 showed some variation in mean score with storage period.

Mean scores for texture always showed more than 5, which is the minimum score for acceptability of product during frozen storage. So we could say that both control and test maintained their texture quality throughout storage period.

5.2.8.2. Taste

However, the Friedman test for taste scores was showing no significant difference between control and test. But graph was showing little variation in mean scores with storage period. The mean score for taste always showing more than 5, which is the minimum score for acceptability of products during frozen storage. So, products maintained their sensory quality during frozen storage. We could say that sausage with protein gel could be an alternative for sausage prepared using starch as binder. Because its protein content is higher than control.

It was found that both control and test samples remained acceptable during the two months of storage period. The test, which contained protein gel provided comparatively good shelflife and showed lower variation in quality parameters. The protein content of test was higher than control and also maintained gel strength during storage. Eventhough starch is a cheap source of binder the product developed had higher protein content. The preparation of protein gel is simple and required only acetic acid. So, it will be feasible to incorporate protein gel as a binder. Sensory evaluation also showed not much variation during storage and the product was acceptable even after two months of storage. Very little work only has been carried out regarding protein gel incorporation. So further studies are required.

Summary

6. SUMMARY

1. The main objectives of the present investigation were preparation of fish protein gel from shark meat, incorporation of gel into fishery product such as sausage and study of the quality changes in the product during frozen storage.

2. Fresh Indian dog shark (*Scoliodon laticaudus*) and Bull's eye (*Pricanthus hamrur*) were transported from the fish market / landing centre to the laboratory in an insulated box.

3. Indian dog shark meat was used for preparation of protein gel using acetic acid induced gelation and Bull's eye meat was used for the preparation of minced meat.

4. Sausage was prepared by forming dough by mixing and grinding minced fish meat, salt, sugar, starch or protein gel, spices and ice water. The dough was stuffed in cellulose casing, ringed, boiled at 85-90^0 C for 50 minutes and then cooled using ice water and again reboiled for one minute in boiling water, fan dried and packed in low density polyethelene bag and stored at –20^{0}C under frozen condition.

5. Product was standardized based on different proportions of starch, protein gel, and ice water.

6. Sausage without protein gel was treated as control and with gel as test.

7. Sausage prepared using starch: protein gel: ice water with proportion 10: 10: 0 was selected as test and that prepared with proportion 10: 0: 10 was used as control.

8. Products both control and test were subjected to freezing and stored at -20^{0}C for a period of two months. Low-density polyethelene bag of thickness 300 gauge was used for packing the product.

9. Quality changes during storage were monitored based on various tests. *viz.*, moisture content, total volatile base nitrogen content, peroxide value, gel strength, pH, total plate count, fungal count and sensory evaluation based on texture and taste.

10. The value of the quality parameters such as TVBN and peroxide value showed increasing trend during storage in the case of both products. Gel incorporated sausage showed comparatively lower rate of change indicating a better quality for the Gel incorporated sausage.

11. Sample from both the products remained acceptable for the entire storage period of two months and no significant difference in the sensory quality was found between the two products. However, products showed some variation in sensory quality with storage period. The Gel incorporated product showed better sensory quality.

12. Physical quality parameters such as pH and gel strength showed distinct variation in case of both the products with storage period.

13. Microbiologically quality of products showed no significant changes during frozen storage.

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INCORPORATION OF FISH PROTEIN GEL FROM SHARK MEAT INTO FISHERY PRODUCTS

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was undertaken for the incorporation of fish protein gel from shark meat into fishery products. Fish sausage was prepared with and without protein gel. Standardization was done based on various combinations of starch, protein gel and ice water and most acceptable combination was selected. Fish meat was mixed with various food additives such as salt, sugar, starch or fish protein gel, monosodium glutamate, colour and water and stuffed into cellulose casings, cooked, cooled, packed using low-density polyethylene of thickness 300 gauge, subjected to freezing and stored at -20^{0}C in frozen condition.

The various starch: protein gel: ice water proportion used for study were 10: 0: 10, 10: 5: 5, 10: 10: 0 and 5: 15: 0. The effect of protein gel was assessed based on gel strength and sensory evaluation of products. Based on these parameters, the following proportion 10: 10: 0 of starch: protein gel: ice water was selected.

Fish sausages were prepared using the standardized method and subjected to storage studies along with a control in frozen condition at temperature of freezing -20^{0}C. Quality changes during storage were monitored at appropriate time interval based on various tests, *viz.*, moisture content, peroxide value, total volatile base nitrogen content, pH, gel strength, sensory evaluation, total plate count and fungal count.

Both control and test were acceptable with no significant reduction in sensory quality during frozen storage. Products were acceptable throughout storage period. Both products showed some changes in moisture content, pH, gel strength and TVBN content with storage period. Products were remained acceptable up to 60 days of storage. The Gel incorporated product appeared to be slightly the better of the two based on sensory responses eventhough statistically there was no significant difference.

Appendix II

Scores sheet for sensory evaluation for storage studies.

Samples of fish sausage are given. Kindly evaluate the texture and taste based on the scale provided. Put a '✓' mark against the appropriate score for each parameter. Minimum score for acceptability was 5.

Sample No.	Score									
	1very poor	2	3	4	5	6	7	8	9	10 excellent
A										
B										

Date: Name

Remarks if any. Signature

Appendix I

Score sheet for standardization of the product

Samples of fish sausage are given. Kindly evaluate the texture and taste based on the scale provided. Put a '✓' mark against the appropriate score for each parameter.

Sample no.		Score									
		1Very poor	2	3	4	5	6	7	8	9	10 Excellent
A	Texture										
	Taste										
B	Texture										
	Taste										
C	Texture										
	Taste										
D	Texture										
	Taste										

Date: Name

Remarks if any.
Signature