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STUDIES ON ELECTROPHORETIC IDENTIFICATION OF FISH SPECIES USED IN SURIMI (PRODUCTS) AND THEIR QUALITY EVALUATION

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Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Fisheries Science

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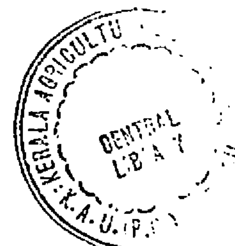

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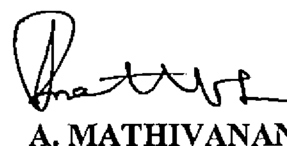

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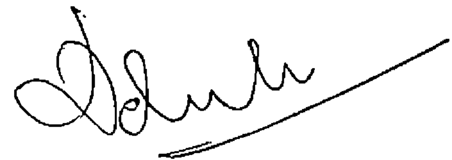

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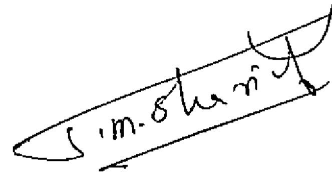

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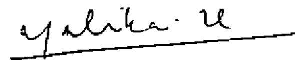

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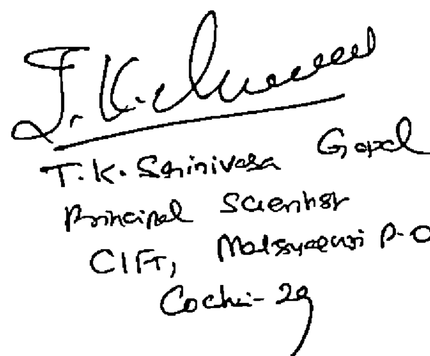

ACKNOWLEDGEMENT

I am deeply indebted to **Dr. D.D. Nambudiri, Dean,** Professor, Department of Processing Technology, College of Fisheries, Panangad for his constant help and guidance throughout the course of my study. His careful attention, suggestions and corrections helped me to prepare the manuscript of the thesis. I am also ever thankful to him for providing all the facilities necessary for the thesis.

I sincerely thank **Dr. P.M. Sherief**, Professor and head, Department of Processing Technology for valuable suggestion and support for the timely completion of my research work.

I am greatly indebted to **Dr. Sajan George**, Professor, Department of Processing Technology for his guidance and support during the course of study. I remain grateful to him for the helpful advice and constructive suggestions given during the course of the experiment and preparation of the thesis.

My sincere thanks to **Sri. S. Krishnakumar**, Assistant Professor, Department of Processing Technology for his cordial and timely help for the preparation of the thesis.

I am also thankful to **Smt. Malika,** Assistant Professor, Department of Management Studies for her encouragement and support.

I wish to express my gratitude to all the staff of Department of Processing Technology for the help extended by them without which completion of my work would not have been successful.

I owe a great deal to **Dr. Sanker**, Principal scientist, CIFT for granting permission and for providing necessary facilities for the successful conduct of research work. My special thanks are also due to **Miss. Dhanya,** Ph.D research scholar, CIFT for her valuable help in analyzing the samples.

The assistance rendered by the library staff of College of fisheries, Panangad.and Central Institute of Fisheries Technology, Cochin are gratefully acknowledged.

I wish to place on record my sincere thanks to **Indus Photo Shop,** Kalamachery for their nice piece of work in printing the thesis.

My special thanks are also due to my friend **Sabin kumar** for his valuable and timely help to collecting the materials for the thesis.

I would like to thank my friends **Laxmi sir, Ajay anand, Sanil, Jithin, Jayaraj, Yuavaraj; Gomathi, Maya, Anju, Indira, Mrs. Sindhu, Navya** for their cooperation and help during my research work.

I wish to extent my sincere thanks to my guardian ***Thiru. K.C.M. Saravanan annan*** for his valuable financial help, guidance and constant encouragement during my studies.

Finally I would like to express my gratitude to my parents and sisters whose encouragement and continuous support helped me to complete my work.

A. Mathivanan.

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INTRODUCTION

1. INTRODUCTION

Seafood and their products are recognized as important sources of nutrients. This has resulted in increased consumption. With the increasing price of commercial seafood products, the willful or unintentional adulteration by substituting lower quality and/or lower priced seafood products for higher priced products has been on the rise (An *et al.,* 1988). This is easy in the case of raw and cooked fish products such as surimi, kamaboko, fish sausage and canned flesh.

Surimi is the stabilized myofibrillar proteins obtained from deboned fish flesh that is washed with water, mixed with cryoprotectants, and then frozen (Moosavi-nasab *et al.*, 2005). A blended surimi is one in which the surimi make-up is more than one species. The use of such blended surimi is likely to increase as industry tries to make production more economical. Surimi is used as a functional protein ingredient along with natural shellfish meat in the manufacture of a variety of fabricated seafood products such as crab legs, scallops, lobster and shrimp analogs (An *et al.,* 1989) which has the potential for adulteration and substitution.

In United States the finished products must be labeled properly to meet the Food and Drug Administration (FDA) guidelines which reflect the nature of the products. Martin (1986) has stressed the need to establish proper nomenclature and labeling for newly developed seafood analogs. These must meet FDA requirements which have an impact on the two major ingredients in the fabricated seafood products: the fish species as the main ingredient and the other species, such as snow crabmeat for crab meat analog, as the additional ingredient.

Problems also exist regarding the labeling of the content of the specific seafood components. Products with claims of 35% crabmeat are widely sold when the use of over 10% crabmeat is known to show detrimental effect to the products (An *et al.*, 1989).

Therefore, there is a need for a reliable, objective method of identification which could be used to establish the species when substitution or adulteration is suspected and which could generally be used as a check on the labeling of fish and fishery products.

There are at least three broad testing methods available for species identification namely, chemical, electrophoretic, and immunological methods (Kurth and Shaw, 1983).

Electrophoretic patterns of the muscle myogens of fish muscle were found to be characteristic of the species. These species specific patterns remained unaltered during processing and preservation. These patterns are found suitable for the species identification in cases of doubtful authenticity. The U.S. Food and Drug Administration decided to recognize electrophoretic pattern of the muscle myogens of fish as an authentic method for species identification and to differentiate seafood species or seafood products (Devadasan, 2002).

Several official electrophoretic methods, such as starch gel-zone electrophoresis, acrylamide disc electrophoresis, thin layer polyacrylamide gel isoelectric focusing and cellulose acetate strip electrophoresis have been accepted as official methods by the Association of Official Analytical Chemists (AOAC, 1990) to differentiate seafood or seafood products (An *et al.*, 1988).

When adulteration or substitution takes place, the electrophoretic system detects the substituted species by species-specific zones. It is also useful to detect the mislabeling of the content of the specific seafood components (minced meat, surimi and canned foods). This is extremely useful for Export Inspection Agency (EIA) for regulatory purposes and also for protection of consumer's interests (An *et al.*, 1989).

Therefore, the objectives of this study were to identify the species used in raw fillets, surimi and sausage using SDS-PAGE; to determine the adulterated or substituted species in surimi samples and their quality evaluation.

1.1. PARAMETERS TESTED

The following parameters were tested.

1. Electrophoretic pattern of water soluble proteins
2. Electrophoretic pattern of salt soluble proteins
3. Quality evaluation of surimi
   3.1. Moisture content

3.2. pH

3.3. Impurities

4. Quality evaluation of sausage

4.1. Folding test

4.2. Sensory evaluation

4.3. Gel strength.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Willful or unintentional adulteration by substituting lower quality and/or lower priced seafood products for higher priced products has led to an increasing demand for identifying species of fish or other seafood in the marketplace (An *et al.,* 1988).

A blended surimi is one in which the surimi make-up is more than one species. The use of such blended surimi is likely to increase as industry tries to make production more economical. Surimi is used as a functional protein ingredient in the manufacture of a variety of fabricated seafood products such as crab legs, scallops, lobster and shrimp analogs (An *et al.,* 1989) which has the potential for adulteration and substitution.

Therefore, there is a need for a reliable, objective method of identification which could be used to establish the species when substitution or adulteration is suspected and which could generally be used as a check on the labeling of fish and fishery products.

There are at least three broad testing methods available for species identification namely, chemical, electrophoretic, and immunological methods (Kurth and Shaw, 1983).

2.1. ELECTROPHORETIC TECHNIQUES

Electrophoresis as an analytical procedure was first introduced by the Swedish scientist, Tiselius, in 1930 (Kleparnik and Bocek, 1991). When first developed, electrophoresis was carried out in free solution in a moving boundary system (Andrews, 1986; Kleparnic and Bocek, 1991).

2.1.1. Significance of Electrophoretic Techniques for Species Identification in Fish and Fishery Products

Electrophoretic patterns of the muscle myogens of fish muscle were found to be characteristic of the species. These species specific patterns remained unaltered during processing and preservation. These patterns are found suitable for the species identification in cases of doubtful authenticity.

The U.S. Food and Drug Administration to recognizes electrophoretic pattern of the muscle myogens of fish as an authentic method for species identification and to differentiate seafood species or seafood products (Devadasan, 2002).

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Other feasible methods have been subsequently applied in identifying fish or shellfish species; these include: sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), urea-isoelectric focusing (urea-IEF), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), immunoassay, enzyme-linked immunosorbent assay (ELISA) and DNA techniques (Chen and Hwang, 2002).

When adulteration or substitution takes place, the electrophoretic system detects the substituted species by species-specific zones. It is also useful to detect the mislabeling of the content of the specific seafood components (minced meat, surimi and canned foods). This is extremely useful for Export Inspection Agency (EIA) for regulatory purposes and also for protection of consumer's interests (An *et al.*, 1989).

2.2. PROTEINS STUDIED FOR SPECIES IDENTIFICATION IN FISH AND FISHERY PRODUCTS

2.2.1. Sarcoplasmic Proteins (Water Soluble Proteins)

The soluble proteins of the sarcoplasm, located within the sarcolemma are referred to as "sarcoplasmic proteins". Among them, some albumins and so called myogens, to which belong most of the enzymes of the glycolytic pathway, are the real water soluble proteins (Wahyuni *et al.*, 1998). The other fractions of sarcoplasmic proteins are soluble in low salt concentrations.

The sarcoplasmic or water soluble proteins make up 20-35% of the total protein content of muscle, depending on the species (Mackie, 1993). They

include about 100 different proteins which are globular or rod shaped in conformation with low viscosity (Asghar *et al.,* 1985) and are low molecular weight (40-60 kD) compounds (Mackie, 1997).

The genetic differences between species are more pronounced in sarcoplasmic proteins compared with other group of proteins, because they are responsible for widely divergent enzymatic transformations in the muscle cell. Hence the separation patterns of profiles obtained on electrophoresis or isoelectric focusing (IEF) can be used for the unequal identification of the species (Mackie, 1997).

2.2.2. Myofibrillar Proteins (Salt Soluble Proteins)

Salt soluble proteins present in the myofibrils within the muscle cell. They contribute about 65-75% of the total proteins (Mackie, 1997). Based on the physiological and structural roles in living tissues, myofibrillar proteins can be further divided into three sub groups.

a) the major contractile proteins, including myosin and actin, which are directly responsible for muscle contraction and are the backbone of the myofibril.

b) regulatory proteins, including tropomyosin, the tropinin complex and several other minor proteins, which are involved in the initiation and control of contraction, and

c) cytosketal or scaffold proteins, including titin or connectin, nebulin, desmin and a number of other minor components, which provide structural support and many functions in keeping the myofibril in alignment or register (Xiong, 1997).

Among the different myofibrillar proteins myosin and tropomyosin find application in species identification in fish and fishery products by electrophoresis.

2.2.2.1. *Myosin*

Myosin contributes 55% of the muscle proteins by weight and forms the thick filaments in myofibrils (Murray *et al.,* 1993). It is the largest myofibrillar protein molecule with a molecular weight of 250 kD and is highly asymmetrical consisting of two globular heads and a long tail (Rayment *et al.,* 1993).

Fish myosin, similar to myosin of other vertebrates, is a hexameric protein consisting of two heavy chains (MW=210 kD each) and four light chains (MW=15-25 kD each) with a total molecular weight of 520 kD (Seki *et al.*, 1998).

Electrophoretic pattern of the light chains is different for different species whereas the heavy chains are same (Martinez *et al.*, 1990 a). Hence an electrophoregram myosin light chain isolated from fish muscle is used for species identification.

2.2.2.2. *Tropomyosin*

Tropomyosin is the most abundant regulatory protein which is a dimeric molecule consisting of two dissimilar subunits designated ∝- and β-tropomyosin with molecular weight of about 34,000 and 36,000 daltons respectively (Xiong, 1997).

Seki (1977) compared SDS-electrophoretic patterns of tropomyosin from over 20 different species of fishes. He observed a single band in mackerel, yellow fin tuna, big eye tuna, and skipjack tuna whose electrophoretic mobility is different for different species.

Tropomyosin is a heat stable protein which can be extracted from heat treated fish products, also making it possible to identify the content of the fish products by SDS electrophoretic pattern of tropomyosin (Suzuki, 1981).

2.3. ELECTROPHORETIC WORKS ON SPECIES IDENTIFICATION

Since each individual species is chemically composed of different proteins at varying levels, techniques that separate proteins may help to identify different species. Electrophoretic methods such as starch gel-zone electrophoresis, acrylamide disc electrophoresis, thin layer polyacrylamide gel isoelectric focusing and cellulose acetate strip electrophoresis have been accepted as official methods by the Association of Official Analytical Chemists (AOAC, 1990) to differentiate seafood or seafood products (An *et al.,* 1988). .

Several workers used electrophoresis as an aid in the species identification of fish and fishery products and is reviewed by LeBlanc (1989).

2.3.1. Raw Fish Flesh

As long as the sarcoplasmic proteins of flesh remain in the native state, they are readily extractable with water or dilute salt solutions and are then in a suitable form for separation by IEF or electrophoresis (Mackie, 1997).

The species of raw fish is normally readily established by comparing the IEF profile of an aqueous extract with those of aqueous extract of authentic species. Although the separation profiles of individual fish, even of the same species, show some degree of variation, they none the less have a sufficient number of major zones in common to enable a profile to be recognized unequivocally (Mackie, 1997).

Cowie (1968) has employed the thin slab polyacrylamide gel electrophoresis technique for the separation of muscle myogen in raw and mixture of fish species with relation to fish taxonomy.

Lundstrom (1980) adopted successfully thin layer polyacrylamide gel isoelectric focusing for species identification. Learson (1970) has described a rapid electrophoretic cellulose acetate method for identifying fresh, frozen, freeze dried fish fillets and breaded raw and precooked sticks.

Yowell and Flurkey (1986) reported that when fish fillets were subjected to freezing and microwave heating, a significant loss in water soluble

proteins with appearance of two high molecular weight proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Chen and Hwang (2002) used SDS-PAGE technique for identification of muscle proteins in different puffer species. They reported that lower molecular weight protein bands (≤30.0 kD) and double staining were more useful for identifying the different puffer species.

IEF profiles of raw fish of the same species have been shown to be remarkably constant, although minor differences attributable to polymorphism are found. Extensive studies on the effects of geographical location of fishing ground have shown that there is little variation in the profiles of a species. These results give confidence in the use of the IEF method for fish species identification. Cod obtained off Baltic Sea, has the typical cod profile which is different from that of Pacific cod (Mackie and Ritchie, 1981).

Sotelo *et al.,* (1992) used IEF technique for identification and differentiation of closely related species. They differentiated scampi (*Nephrops norvegus)* from *Metanephrops andamanicus* from the Pacific.

When adulteration /substitution takes place, the electrophoretic system can also be applied to the problem, provided that the profiles of the species of interest have species-specific zones which do not coincide with those of the other species in the mixture (Sotelo *et al.,* 1992).

Isoelectric focusing is the preferred procedure for the identification of species of raw fish. With recent advances in the use of densitometry and image analysis, data on isoelectric point (pI) values and peak height and areas held in computer data bases. These developments will make the system will identify mixed species products and give an estimate of the quantitative composition of the respective species (Hall, 1997).

IEF has been used by several workers to identify seafood species such as shrimp (An *et al.,* 1988; Wei *et al.,*1990) crab (Gangar, 1992), snapper (Huang *et al.,* 1995).

IEF profiles of the white fish species, cod, haddock and whiting, have species-characteristic zones well separated from those of scampi, thereby enabling the presence of adulterants to be established (Sotelo *et al.,* 1992).

When crustacean species are present in a mixture the absence of species-specific zones, well separated from those of other species, makes

identification of the components' species difficult and in many cases impossible. For this particular problem, the alternative SDS procedure, which separates on the basis of molecular weight, has been found to be more discriminating (Hall, 1997).

2.3.2. Cooked but Not Autoclaved Fish

During cooking the proteins get heat denaturation, aggregation of proteins leading to precipitation which are inextractable in the normal protein solvents such as water or strong salt solutions as the native properties of the proteins have been destroyed (Hall, 1997).

Mackie (1993, 1994a) reported that the bonds that are formed are largely non-covalent, hydrophobic and hydrogen bonds, possibly with some degree of electrostatic interaction between the charged groups of the denatured proteins. Some -S-S- bonds may also be formed.

Heat-denatured proteins can be dissolved in a concentrated solution of SDS, a solvent which splits hydrogen bonds. A reducing agent such as dithiothreitol is often added to break any -S-S- bonds which may be present. The extracted protein residues can then be separated by SDS electrophoresis and, as for raw species, by comparing the protein profile of the unknown with those of authentic samples, which can been treated in the same way, identification of the species can be made (Hall, 1997).

The SDS profile with silver staining of an unknown (reformed extruded scampi) was compared with those of authentic species of gadoid which could have been used to extend or adulterate the product (Blum *et al.*, 1989; Craig *et al.*, 1995).

Craig *et al.*, (1995) reported that the SDS procedure is preferred method for scampi product identification compared with IEF. Adulterated scampi can be detected from other crustacean such as warm water prawns, Indian white prawn by clear mobility of one or two species-specific zones of SDS electrophoresis, which can made it possible to establish the presence of non-scampi proteins.

The SDS technique is often preferable for differentiating cooked flesh and salted smoked fish compared to IEF (Hall, 1997).

Krzynowek and Wiggin (1981) used thin layer polyacrylamide gel isoelectric focusing for generic identification of cooked and frozen crabmeat with 97.5% accuracy.

Mackie (1969) has described a modified polyacrylamide disc electrophoresis for identifying raw, cooked fish and smoked fish.

An *et al.,* (1988) found that SDS-PAGE was useful in distinguishing shrimp of different genus. This technique was tested and proven in a blind study to be useful for species identification and detection of fabricated products.

SDS electrophoresis has been used successfully to analyse surimi-based gel products which are manufactured into shellfish substitutes. Sufficient differences in the SDS profiles of what are essentially sarcoplasmic proteins-free muscle proteins have been obtained to enable the identity of the species of fish used in the production of surimi to be established (Torry Research Station, 1986; Rehbein, 1992).

Seki *et al.,* (1980) have described a preparative SDS-PAGE method of myosin light chains from both raw and cooked fish products such as kamaboko, fish sausage and canned flesh.

An *et al.,* (1989) used both SDS-PAGE and urea-gel IEF to identity species-specific protein bands of raw, cooked fish and surimi samples from Alaska Pollock and Red hake. They found that this method appears to have potential application for identifying adulterated/substituted fish and surimi mixed samples.

However, the success of the procedure in identifying the species is dependent on the extent of the relationship between the species. Distinct profiles can be obtained from cod, blue whiting and sardine but not between cod and Alaska Pollack, one of the main species used for the manufacture of surimi. The solution to the problem is to separate myosin heavy chains by SDS electrophoresis and, after limited proteolytic digestion, to separate the peptides obtained by SDS electrophoresis to give species-specific profiles.

However, SDS electrophoresis is likely to remain the preferred procedure for species identification of cooked fish products, surimi and raw fish products such as scampi where there is potential adulteration by other crustacea.

Further developments in optimizing the procedure will enable corresponding data on molecular weight and area of protein zone to be determined by image analysis and stored on computer data base. These developments will make that the system will identify mixed species products and give an estimate of the quantitative composition of the respective species (Hall, 1997).

2.3.3. Heat - Sterilized and Autoclaved Products

When fish flesh is subjected to the high temperatures and pressure of the canning process (Horner, 1992), the constituent proteins undergo further denaturation, possibly in the form of increased covalent bond formation.

Mackie *et al.*, (1992) reported that continued denaturation of proteins in the canning process is seen in the loss of protein zones on SDS electrophoresis and background staining. Due to these features, SDS electrophoresis has limited application to canned fish products; the few protein zones that are observed are often common to all the species examined and the strong background staining adds to the problems of differentiation.

Mackie and Taylor (1972) extended the polyacrylamide gel electrophoresis for the identification of heat sterilized canned fish species.

Mackie and Taylor (1972) used cyanogens bromide in formic acid to give IEF species-specific profiles of the protein hydrolytic fragments.

Cyanogen bromide split the methionine residue, which liberates a sufficient number of large polypeptides which can be successfully identified by electrophoresis (Connell, 1973; Anon, 1988 b; Hofmann, 1988).

Mackie *et al.*, (1999) used SDS electrophoresis for identification of canned fish species.

Pineiro *et al.*, (1999) developed SDS-PAGE as a reference method for species identification in raw and heat-processed samples. They yielded well reproducible and discriminant species-specific protein patterns.

However, when the species are closely related, such as members of the tuna or salmon families, the differences in profiles obtained are insufficient to enable differentiation to be made.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. PREPARATION OF SURIMI

3.1.1. Selection of Raw Material

Two species of fish were used for the present study.

The species used were, Threadfin bream (*Nemipterus japonicus*), and Bulls-eyes (*Priacanthus hamrur*).

The above species were selected on the basis of their commercial importance in surimi production and their availability. They were obtained from the Cochin fishing harbour.

The material was brought to the laboratory in iced condition. Care was taken to keep the temperature of the material below 5°C by icing during transportation and the samples were kept in a deep freezer immediately on procurement.

3.1.2. Individual Surimi

Surimi was prepared individually or separately from two selected species as follows,

Surimi sample I : Threadfin bream

Surimi sample II : Indian bulls eye

3.1.3. Mixed Surimi

Mixed surimi samples were prepared by mixing the meat of fishes as follows,

Surimi sample III: Threadfin Bream: Indian Bulls eye = 1:1 ratios.

Surimi sample IV: Threadfin Bream: Indian Bulls eye = 3:1 ratios.

Surimi sample V : Threadfin Bream: Indian Bulls eye = 1:3 ratios.

Surimi sample VI: Threadfin Bream: Indian Bulls eye = 4:1 ratios.

3.1.4. Method of Surimi Preparation

Surimi was prepared following a procedure described by Suzuki (1981). Raw material was washed properly with water and weighed. The material was dressed to remove head, scales, viscera, peritoneal membrane,

skin, and large bones and washed once again. Meat was picked up manually and the yield was determined. Meat was minced by meat mincer. Minced meat was strained to remove pin bones, membranes, connective tissue, etc., by using a strainer. Water leaching was done by adding 7-8 times volume of water to the meat and agitating by using stirrer for about 5-10 minutes. Ice was added to the meat-water mixture during stirring for keeping the temperature below 5°C. Meat was allowed to settle down in the vessel and then supernatant was removed. Water leaching was repeated for about 3-5 times until the meat became white in colour. NaCl was added to the water used for final wash at the rate of 0.05-0.1%. Meat was dehydrated using hand press to achieve the final moisture content to 80%. Sodium tripolyphosphate at 0.3%, sucrose at 4.0% and sorbitol at 4.0% of meat weight were mixed with meat with the help of silent cutter at 10-15 minutes at temperature of <15°C. The yield was determined. Surimi was packed in plastic film lined trays at the rate of 0.5kg/tray with 3cm thickness with no air packet. Surimi was frozen at -35°C to-40°C by using contact plate freezer and it was stored at -30°C.

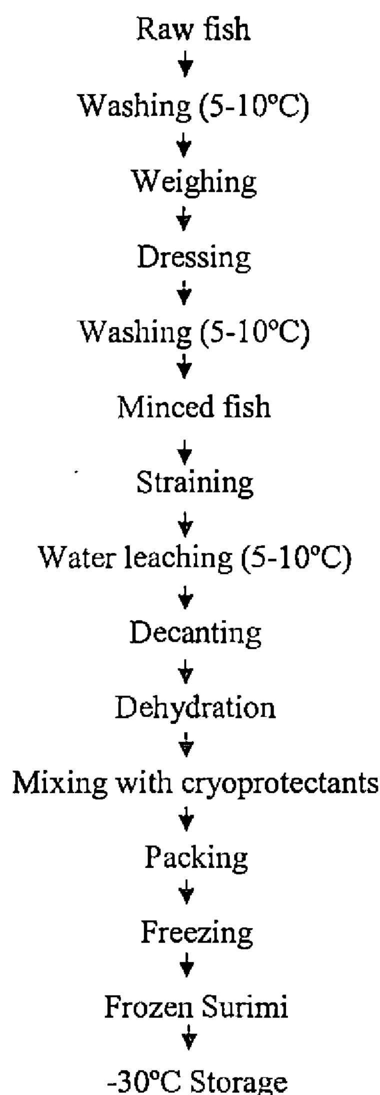

Fig.1. Process Flow of Surimi Production

3.2. PREPARATION OF SAUSAGE

Sausage was prepared following a procedure described by Suzuki (1981). Sausages I, II, III, IV, V, VI were prepared by drawing the samples from corresponding surimi.

Each sample of thawed surimi was divided into four lots of approximately 150g each and was ground using mortar and pestle for 2 minutes each. Three percent salt was added and ground again for 8 minutes. Corn starch was added at the rate of 0%, 3%, 5%, and 10% to the lots respectively. Grinding was continued for another 5 minutes. During grinding the temperature of surimi was kept as low as possible (below 10°C). Cellulose casings were sealed at one end using sealer. Paste meat was stuffed into casings without air pockets using sausage stuffer. The other ends of the casings

were sealed using sealer. It was boiled for 30-40 minutes in a water bath at 88-90°C and then cooled in cold water. The sausage was over wrapped to prevent drying and kept in chill store at 10°C over night.

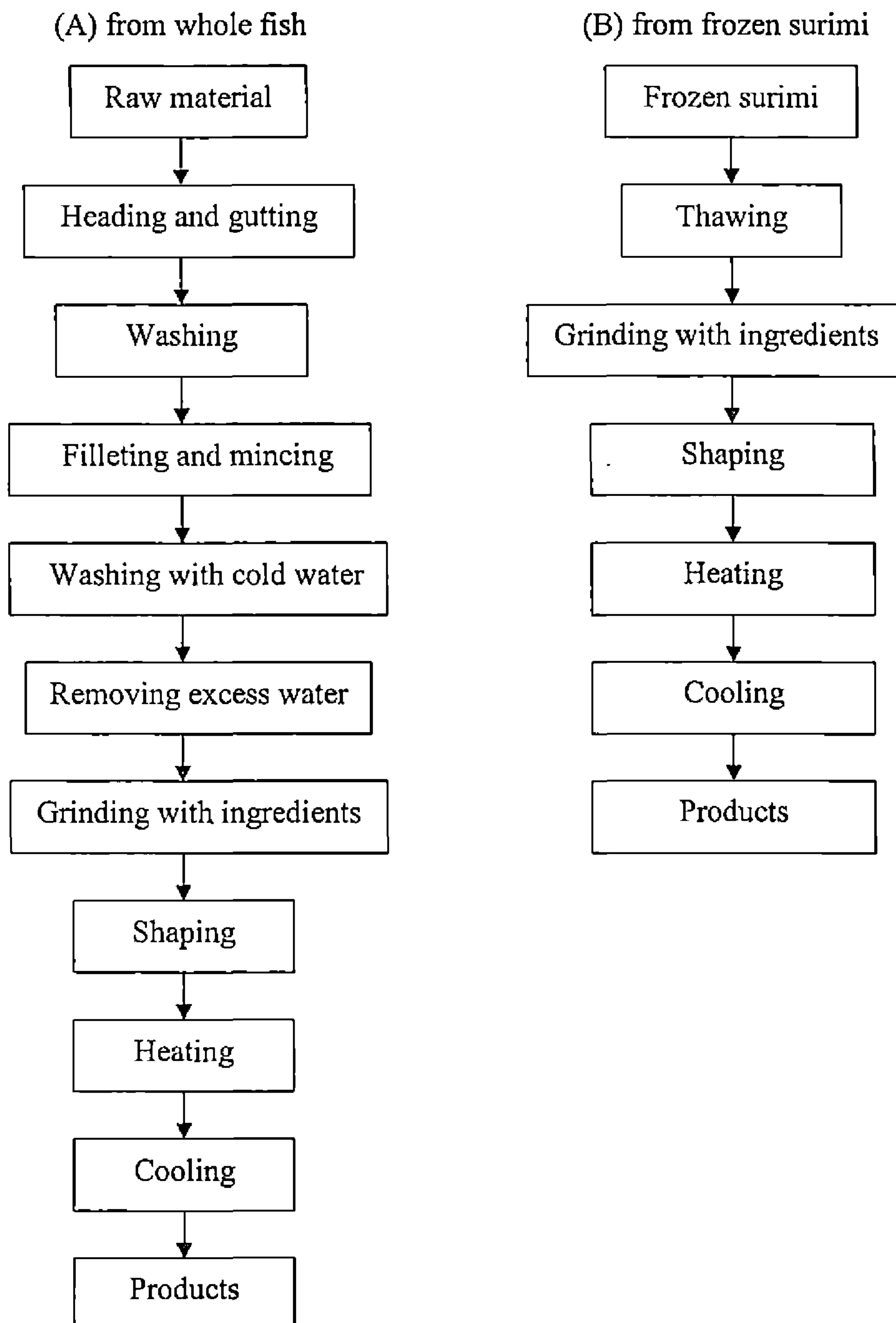

Fig.2. Process Flow of Sausage Production

3.3. QUALITY EVALUATION OF PREPARED SURIMI

Various methods to test the quality of frozen surimi are being employed by companies or associations working in this field. Quality evaluation of prepared surimi was carried out by the method suggested by FAO-Codex Alimentarius Commission (2005).

3.3.1. Preparation of Test Sample

A 50g block of frozen surimi was cut out using a saw or cutter. This test block was placed in a polyethylene bag and left it at room temperature until it reached a temperature of about 0°C and was soft enough to be cut with knife.

3.3.2. Moisture

Moisture content of surimi was determined by the method of AOAC (1975). A sample of about 10g was weighed accurately in a clean dry preweighed 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 surimi upon drying. The determination of the test was done on a three sample basis.

Moisture content was calculated according to the following formula to the first decimal place.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1 - W} \times 100$$

Where,

W - Weight of empty dish

W_1 - Weight of dish + sample before drying

W_2 - Weight of dish + sample after drying

3.3.3. pH

pH of surimi was determined by the method described by Suzuki (1981). A 5g sample of thawed surimi was blended with 45ml of distilled water. The mixture was homogenized and pH of the suspension was measured

using a glass electrode pH meter to the second decimal place. The determination of the test was done on a two sample basis.

3.3.4. Impurities

Impurities of surimi were determined by the method described by Suzuki (1981). A 10g sample of thawed surimi was spread uniformly on glass plate to 1mm thickness and impurities such as skin, small bones, and any objectionable matter other than fish meat was sought out. The impurities were counted as follows; Objects of 2mm or larger was counted as one each, and objects less than 2mm was counted as 1/3 and any unnoticeable matter smaller than 1mm was disregarded. Grades were given according to the scale (Appendix I).

3.4. QUALITY EVALUATION OF PREPARED SAUSAGE

Quality evaluation of prepared sausage was carried out by the method suggested by FAO-Codex Alimentarius Commission (2005).

3.4.1. Folding Test

Folding test of sausage was conducted by the method described by Suzuki (1981). The folding test was conducted by folding a 5mm thick slice of sausage (gel) slowly in half and half again while examining it for signs of structural failure (cracks). Folded state was kept for five seconds, and then the change in the shape was evaluated by five-stage method. The minimum amount of folding was required to produce a crack in the gel was determined the score for this test. Three or more slice pieces of the same inspection sample were tested and the average mark was obtained (Appendix II).

Sausage with AA grade was recorded.

3.4.2. Sensory (Biting) Test

Sensory test of sausage was conducted by the method described by Suzuki (1981). Sensory test was done by a panel of judges by biting 5mm thick slice pieces of the sausage sample and it was evaluated resilience upon touch to teeth and cohesiveness upon bite by 10- point method. Three or more slice pieces of the same inspection sample were tested by a panel and the average score was obtained (Appendix III).

3.4.3. Gel Strength

Gel strength can be defined as the force required to break the surface of the sample. It is expressed as g.cms. The gel strength of sausage was measured by the method suggested by Okada (1974).

Flow rate of water was determined by allowing water to flow into cup at the constant rate for a certain period in seconds. The water was stopped and volume of water was measured. Rate of water flow per second was calculated. Average of few trials was taken and expressed in g/sec.

The kymograph paper was attached on the surface of the drum and the pen was kept in position. The drum was rotated for certain period in seconds. Length of the horizontal line was measured. Distance traveled by surface of drum per second was calculated and expressed in cm/sec.

From the two values the factor 'F' calculated as follows,

$$F = \frac{\text{Weight of water in g/sec}}{\text{Distance travelled by drum surface in cm/sec.}}$$

'F' was expressed as g/cm.

Then sample was cut into round shaped slices of length 25mm with flat surface at both ends, and then the film was removed. The sample was placed vertically on stand below plunger so that the center of the slice surface was directly below the plunger. Drum was rotated to get a horizontal line. Water was allowed to flow into the cup at the same rate as determined earlier. Plunger moved down due to the weight of water, simultaneously the pen traced stress-strain curve on the kymograph paper. Gel strength of the sample was calculated as, (shown in the figure.3)

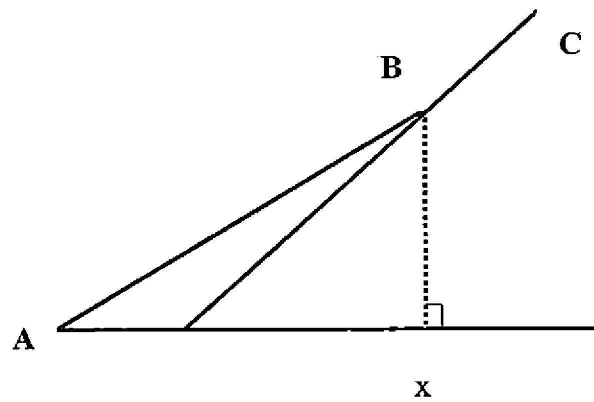

Fig.3. Okada Gelometer's kymogram

Gel strength (g.cm) = ½ (Ax X Bx) X F

Where,

Ax - Load (g)

Bx - Degree of strain (cm)

3.5. EXTRACTION OF PROTEIN FRACTIONS FOR ELECTROPHORESIS

3.5.1. Extraction of Sample for Electrophoresis of Water Soluble Poteins

About 5g of the minced meat was mixed with 10ml cold distilled water and homogenized using a mortar and pestle for 2 minutes under ice cold conditions. The suspension was then centrifuged at 2000 rpm for 10 min at 4°C. The supernatant was used for the electrophoresis of sarcoplasmic proteins.

3.5.2. Extraction of Sample for Electrophoresis of Salt Soluble Proteins

The residue of the previous extraction was used for the extraction of salt soluble protein using Dyer's buffer (5% NaCl in 0.02*M* $NaHCO_3$, pH 7.0). The residue was mixed with about 40 ml Dyer's buffer and stirred at low speed using a magnetic stirrer for 1 h. at low temperature. The suspension was then centrifuged at 10,000 rpm for 20 min. and the supernatant was transferred to a 50 ml standard flask. The volume was made up with the same buffer and used for the elctrophoresis of salt soluble proteins.

3.6. ELECTROPHORESIS OF PROTEINS

Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970).

3.6.1. Principle

In the presence of SDS and 2- mercapto ethanol, proteins dissociate into their subunits and bind large quantities of detergent. The total charge of the protein becomes negative giving a constant charge to mass ratio. So proteins move according to their molecular weight in an electric field.

3.6.2. Reagents

3.6.2.1. ***Tris- Hcl*:** 0.5 *M*, pH 6.8

3.6.2.2. ***Tris- Hcl*****:** 1.5 *M*, pH 8.8

3.6.2.3. ***SDS*****: 10%**

3.6.2.4. **A*crylogel*****:** 30% T, 2.67% C

3.6.2.5. ***Ammonium Persulphate (APS)*****:** 10%

3.6.2.6. ***Coomassie Brilliant Blue (R-250)***

3.6.2.7. ***Acetic Acid (7%)***

3.6.2.8. ***Sample Buffer*****:**

Distilled water	:	3.8 ml
Tris- HCl 0.5 *M*, pH 6.8	:	6.8 ml
Glycerol	:	0.8 ml
10% SDS	:	1.6 ml
2- mercaptoethanol	:	0.4 ml
1% Bromophenol blue	:	0.4 ml

3.6.2.9. ***Electrode Buffer***

Tris base	:	9g
Glycine	:	43.2g
SDS	:	3g

These reagents were dissolved in 600 ml of distilled water.

3.6.2.10. ***Working Solution***

Dilute 100 ml from stock to 500 ml distilled water.

3.6.2.11. *Separating Gel (7.5%)*

Distilled water	:	4.05 ml
Tris- HCl 1.5 *M*	:	2.5 ml
10% SDS	:	100 µl
Acrylamide	:	3.3 ml
APS 10%	:	50µl
TEMED	:	10µl

3.6.2.12. *Stacking Gel (4%)*

Distilled water	:	6.1 ml
Tris- HCl 0.5 *M*	:	2.5 ml
10% SDS	:	100µl
Acrylamide	:	1.33 ml
APS 10%	:	50µl
TEMED	:	10µl

3.6.3. Procedure

0.1 ml of the suitably diluted protein solution was taken in a small centrifuge tube and 0.1 ml of the sample buffer was added, heated in a boiling water bath for 4 min., cooled and stored at -20°C in a cold store until use. Running (7.5%) and stacking (4%) gels were prepared and protein solution in Lamelli buffer is applied on the stacking gel (10µl). Current of 200V was applied and the protein migrates on the electrical field was indicated by the bromophenol blue added in the sample buffer. At the end of the run the gel was removed and was placed in a big petridish containing the stain, Coomassie Brilliant Blue (R-250) for 30 min. and transferred into 7% acetic acid for destaining till the gel gets completely destained. The gel was photo documented.

3.6.4. Molecular Weight Determination

Molecular weights of the protein bands were determined according to the methods of Weber and Osborn (1969) and Davies and Stark (1970) using a broad range molecular weight (MV) protein kit, SDS (Bangalore Genei., Bangalore, India). Molecular weight of protein was determined by using software, Quantity One.

RESULTS

4. RESULTS

The results of the experiment can be considered systematically under the following heads.

1. Quality evaluation of surimi
2. Quality evaluation of sausage
3. Electrophoretic pattern of water soluble proteins
4. Electrophoretic pattern of salt soluble proteins

4.1. QUALITY EVALUATION OF SURIMI

Table 1, shows the quality evaluation of surimi used for the experiment. Moisture contents of surimi varied from 75-79.80%; pH varied 6.06- 6.90 and No. of impurities varied from 11-19.

Table 1 : Quality evaluation of surimi

Surimi samples	I TF	II BF	III TF:BF (1:1)	IV TF:BF (3:1)	V TF:BF (1:3)	VI TF:BF (4:1)
Moisture content (%)	77.70	79.80	77.60	75.40	78.50	75
pH	6.77	6.06	6.90	6.43	6.69	6.60
Impurities (no.)	15	15	19	14	11	14

TF - threadfin bream fillets; BF – bulls-eye fillets

4.2. QUALITY EVALUATION OF SAUSAGE

Table 2, shows the effect of various quality factors on the different starch concentration of threadfin bream, bulls-eye and various mixed sausage samples used for the experiment.

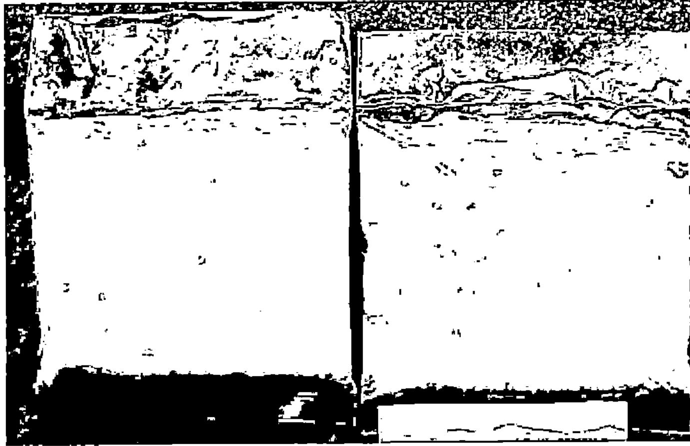

Plate 1.Surimi samples

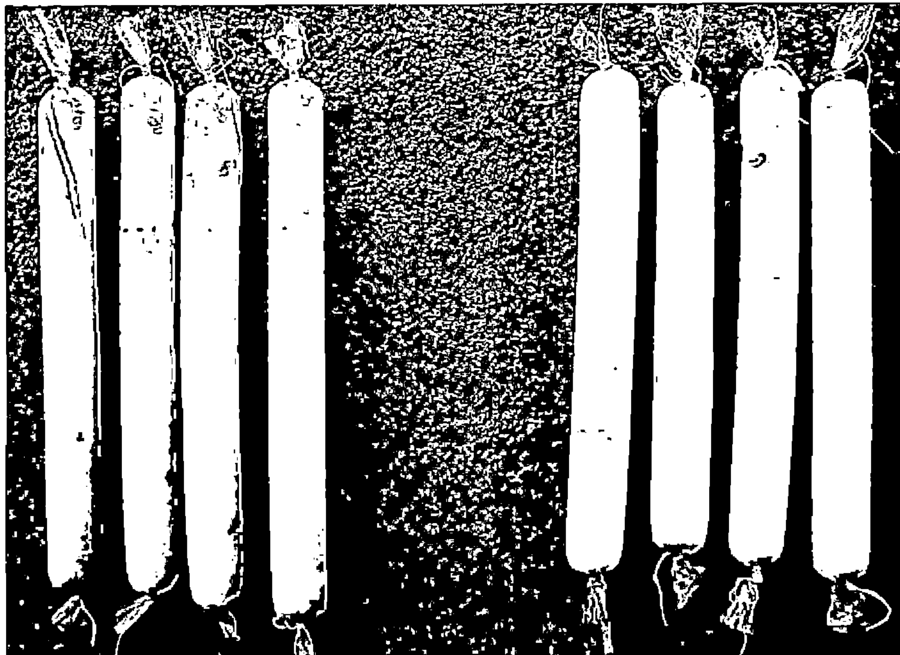

Plate 2. Sausage samples

4.3. ELECTROPHORETIC PATTERN OF WATER SOLUBLE PROTEINS

4.3.1. Raw Fillets

4.3.1.1. *Threadfin bream*

Plate 3 and table 3 shows the electrophoretic pattern of water soluble proteins of threadfin bream raw fillets (TF). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

4.3.1.2. *Bulls-eye*

Plate 3 and table 3 shows the electrophoretic pattern of water soluble proteins of bulls-eye raw fillets (BF). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

4.3.2. Surimi Samples

Plate 3 and table 4 shows the electrophoretic pattern of water soluble proteins of surimi samples. Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

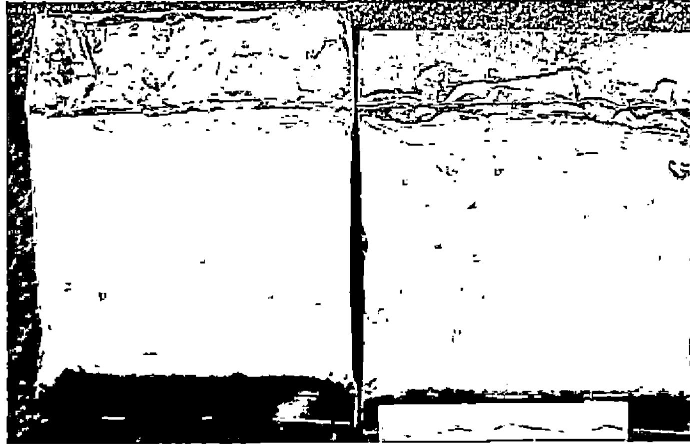

Plate 1.Surimi samples

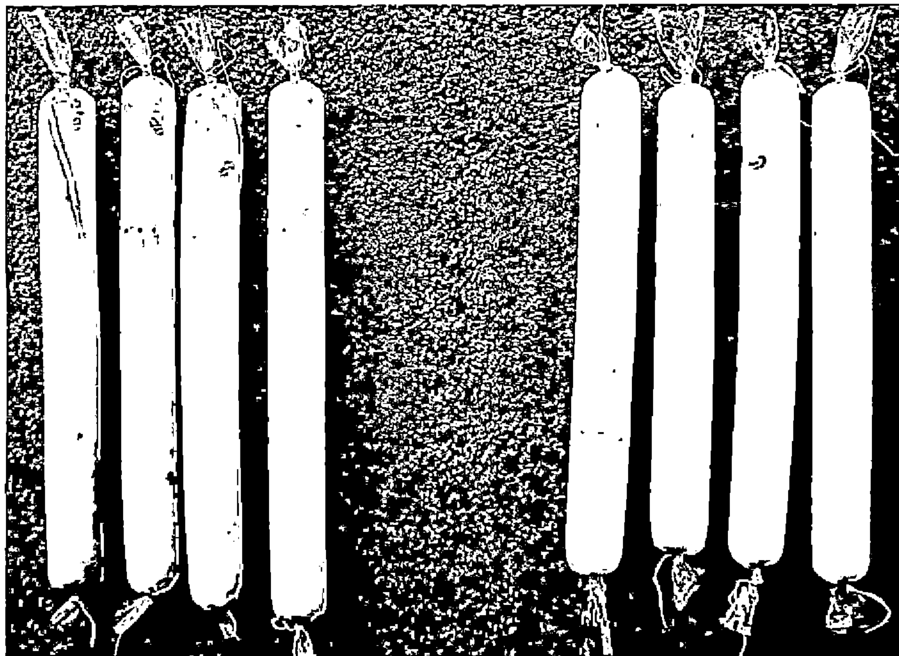

Plate 2. Sausage samples

4.3. ELECTROPHORETIC PATTERN OF WATER SOLUBLE PROTEINS

4.3.1. Raw Fillets

4.3.1.1. *Threadfin bream*

Plate 3 and table 3 shows the electrophoretic pattern of water soluble proteins of threadfin bream raw fillets (TF). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

4.3.1.2. *Bulls-eye*

Plate 3 and table 3 shows the electrophoretic pattern of water soluble proteins of bulls-eye raw fillets (BF). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

4.3.2. Surimi Samples

Plate 3 and table 4 shows the electrophoretic pattern of water soluble proteins of surimi samples. Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

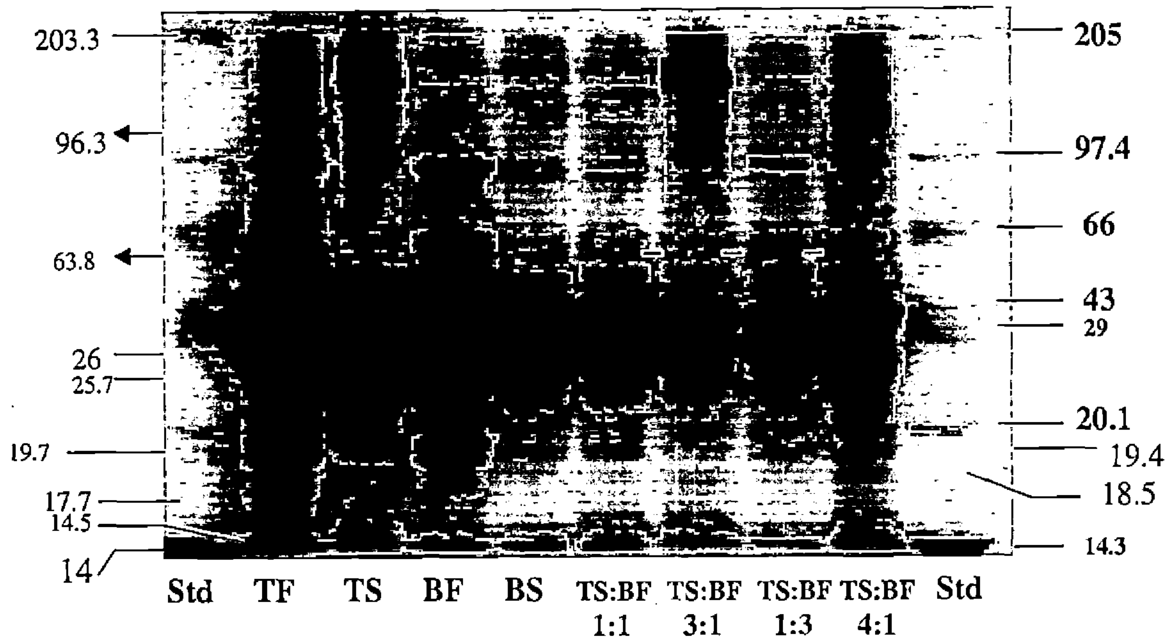

Plate 3.SDS-PAGE pattern of water soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; TS: threadfin bream surimi; BF:bulls-eye fillet; BS:bulls-eye surimi.

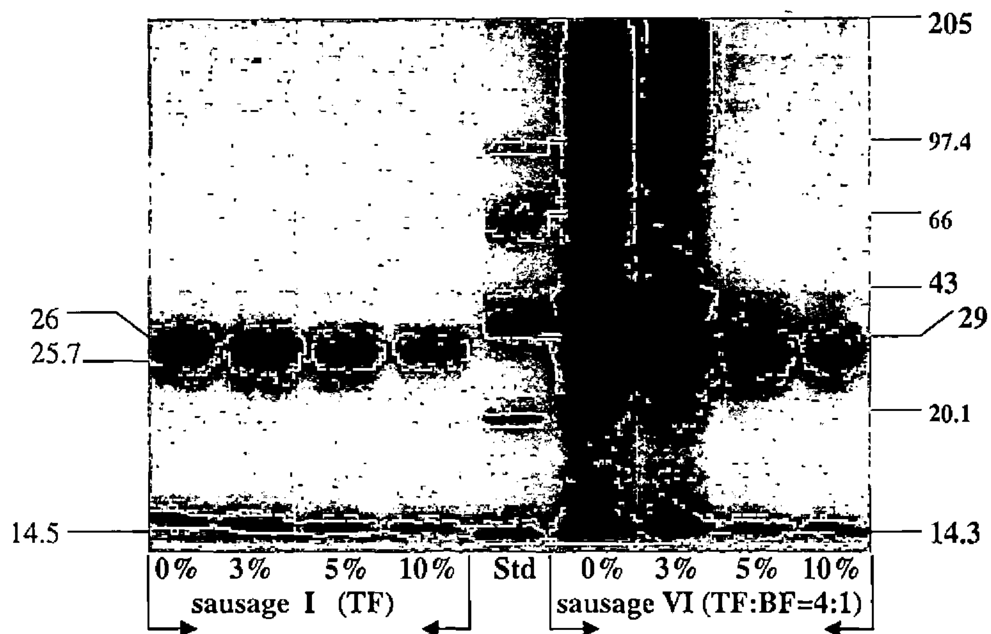

Plate 4.SDS-PAGE pattern of water soluble proteins of sausage I (TF) and VI (TF:BF=4:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

Table 2 : Effect of various quality factors on the different starch concentration of threadfin bream, bulls-eye and various mixed sausage

Sausage	Starch concentration (%)	Sensory score	Folding test Grade	Gel strength (g.cm)
I) TF	0	6.4	AA	630.63
	3	6.5	AA	722.09
	5	8.6	AA	819.00
	10	6.0	AA	962.33
II) BF	0	8.1	B	568.75
	3	6.4	A	705.25
	5	6.7	AA	815.36
	10	7.0	AA	912.73
(III) TF:BF (1:1)	0	7.7	AA	673.40
	3	6.4	AA	737.78
	5	5.0	AA	834.01
	10	7.2	AA	1004.64
IV) TF:BF (3:1))	0	7.7	AA	412.45
	3	5.9	AA	638.82
	5	6.4	AA	911.14
	10	7.6	AA	1108.38
V) TF:BF (1:3)	0	8.4	AA	543.04
	3	7.3	AA	649.74
	5	7.0	AA	765.77
	10	7.4	AA	942.31
VI) TF:BF (4:1))	0	7.6	B	649.74
	3	6.3	A	780.55
	5	6.6	AA	919.55
	10	6.5	AA	1091.32

TF - threadfin bream fillets; BF – bulls-eye fillets

Table 3 : Molecular weights of the protein bands of fillets extracted with distilled water

Band No.	Mol.wt. of protein bands (kD) of fillets	
	Threadfin bream	Bulls-eye
1	203.3*	202.1
2	157.4	158
3	92.4*	137.5
4	60.9*	91*
5	51.3*	61.4*
6	26.0*	47.8*
7	19.4*	31.1*
8	18.5*	25.7*
9	16.6	19.7*
10	14.5*	18.4*
11		17.7
12		14*

*strong bands; remaining narrow and less intensive bands.

Table 4 : Molecular weights of the protein bands of surimi samples extracted with distilled water

Band No.	Mol.wt. of proteins bands (kD) of surimi samples					
	I) TF	II) BF	III) TF:BF (1:1)	IV) TF:BF (3:1)	V) TF:BF (1:3)	VI) TF:BF (4:1)
1	203.3*	196.1	194.5	203.3*	201.8*	203.3*
2	154.4*	155	155.3	152.3*	157.6	156.2*
3	131.6	123.8	136.5	132.4	135.6	132.9
4	120.8	94.3	124	124.2	124.4	123.2
5	99.7	63.8	103.3	100.2	93.6*	93.6
6	93.6	51.4	93.6	93.6	81.4	63.8
7	86.9	38.4*	88.8	63.8	63.8	52.5
8	78.9	25.7*	79.8	53.2	59.7	36.3*
9	63.1	22.8	63.8	35.8*	51.1*	25.7*
10	54.5	19.7	52.3	26.0*	37.5*	22.3*
11	33.2*	18.4	37*	22.3	22.3*	21.2
12	26*	17.7	25.7*	19.7	19.7	19.7
13	22.3	14*	22.3	19.3	19.1	17.7
14	19.4		19.7	17.7	17.7	14.5*
15	18.5		19	14.5*	14.5*	
16	14.5*		17.7			
17			14.5*			

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands;   remaining narrow and less intensive bands

4.3.3. Sausage Samples

4.3.3.1. *Sausage (I) TF and (VI) TF:BF (4:1)*

Plate 4 and table 5 shows the electrophoretic pattern of water soluble proteins of sausage I (TF) and (VI) TF:BF (4:1). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

Table 5 : Molecular weights of the protein bands of sausage samples extracted with distilled water

Band No.	Mol.wt. of protein bands (kD) of sausage samples with different starch concentration							
	(I) TF				(VI) TF:BF (4:1)			
	0%	3%	5%	10%	0%	3%	5%	10%
1	197	205	205	207	191.2 *	193.1*	209.1	209.1
2	44.2	161.5	159.9	163.1	80.1	153.7	163.1	197
3	26.0*	123.5	122.3	122.3	40.4*	132.4	123.5	161.5
4	14.5*	44.6	104.4	114.1	25.7*	119.9	81.6	121.1
5	123.5	26.0*	83.8	105.4	16.9	80.1	41.7	104.4
6	166.4	14.5*	45	43.8	14.5*	41.7*	25.7*	82.3
7			26.0*	26.0*		25.7*	14.5*	54.5
8			16.6	14.5*		16.9		44.2
9			14.5*			14.5*		25.7*
10								14.5*

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands;   remaining narrow and less intensive bands

4.3.3.2. *Sausage (II) BF and (III) TF:BF (1:1)*

Plate 5 and table 6 shows the electrophoretic pattern of water soluble proteins of sausage (II) BF and (III) TF:BF (1:1). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

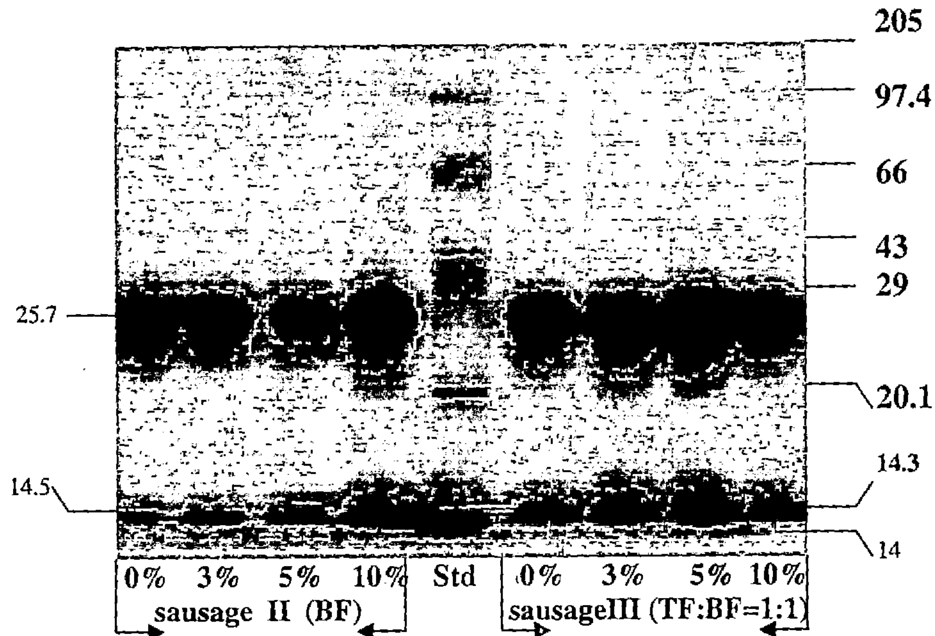

Plate 5.SDS-PAGE pattern of water soluble proteins of sausage II (BF) and III (TF:BF=1:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

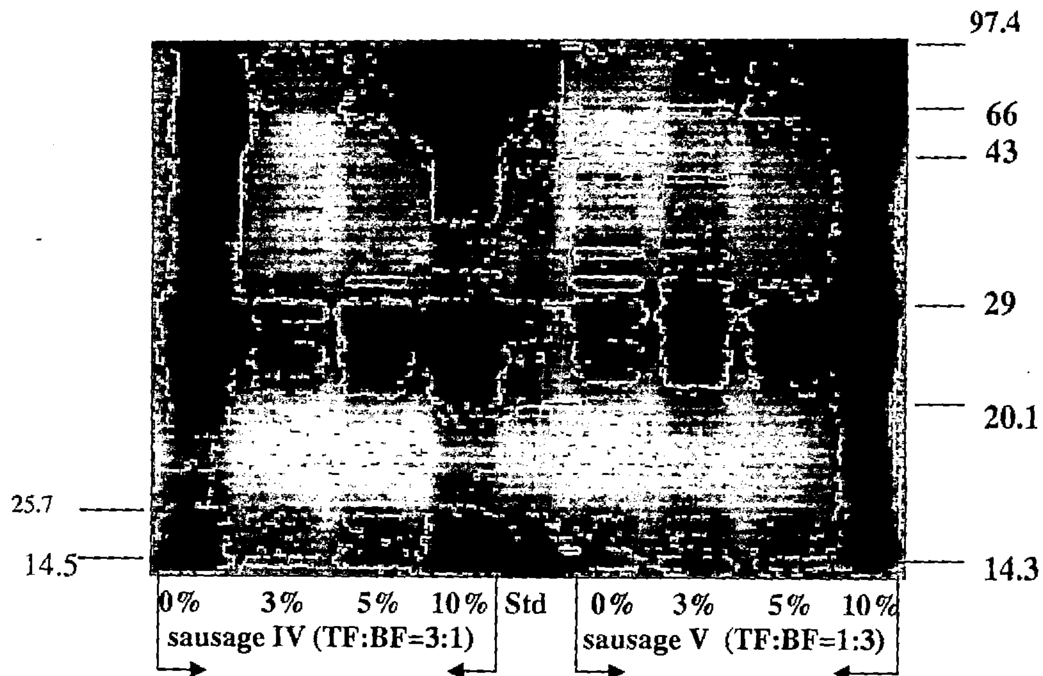

Plate 6.SDS-PAGE pattern of water soluble proteins of sausage IV (TF:BF=3:1) and V (TF:BF=1:3) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

4.3.3.3. *Sausage (IV) TF:BF (3:1) and (V) TF:BF (1:3)*

Plate 6 and table 7 shows the electrophoretic pattern of water soluble proteins of sausage (IV) TF:BF (3:1) and (V) TF:BF (1:3). Bands were obtained in the electropherogram of water soluble proteins in a 7.5% gel.

Table 6 : Molecular weights of the protein bands of sausage samples extracted with distilled water

Band No.	Mol.wt. of protein bands (kD) of sausage samples with different starch concentration							
	(II) BF				(III) TF:BF (1:1)			
	0%	3%	5%	10%	0%	3%	5%	10%
1	205	201	203	81	206.9	209	205	205
2	164.1	165.7	167.3	70.1	165.7	170.6	164.1	164.1
3	151.9	137.9	136.6	64.3	128.9	127.6	126.4	126.4
4	79.1	126.4	121.6	49.3	81.9	117	117	106.2
5	50.1	119.3	104.2	41.7	50.1	47.2	80.5	81.9
6	25.7*	82.6	81	25.7*	44.8	25.7*	50.1	39.2
7	20.9	50.1	73.2	20.4	25.7*	20.9	25.7*	25.7*
8	14*	25.7*	44.4	14*	20.6	19.8	14.5*	17.4
9	13.8	14*	25.7*		17.2	14.5*		14.5*
10		13.8	14*		14.5*			

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

4.4. ELECTROPHORETIC PATTERN OF SALT SOLUBLE PROTEINS

4.4.1. Raw fillets

4.4.1.1. *Threadfin bream*

Plate 7 and table 8 shows the electrophoretic pattern of salt soluble proteins of threadfin bream raw fillets (TF). Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

4.4.1.2. *Bulls-eye*

Plate 7 and table 8 shows the electrophoretic pattern of salt soluble proteins of bulls-eye raw fillets (BF). Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

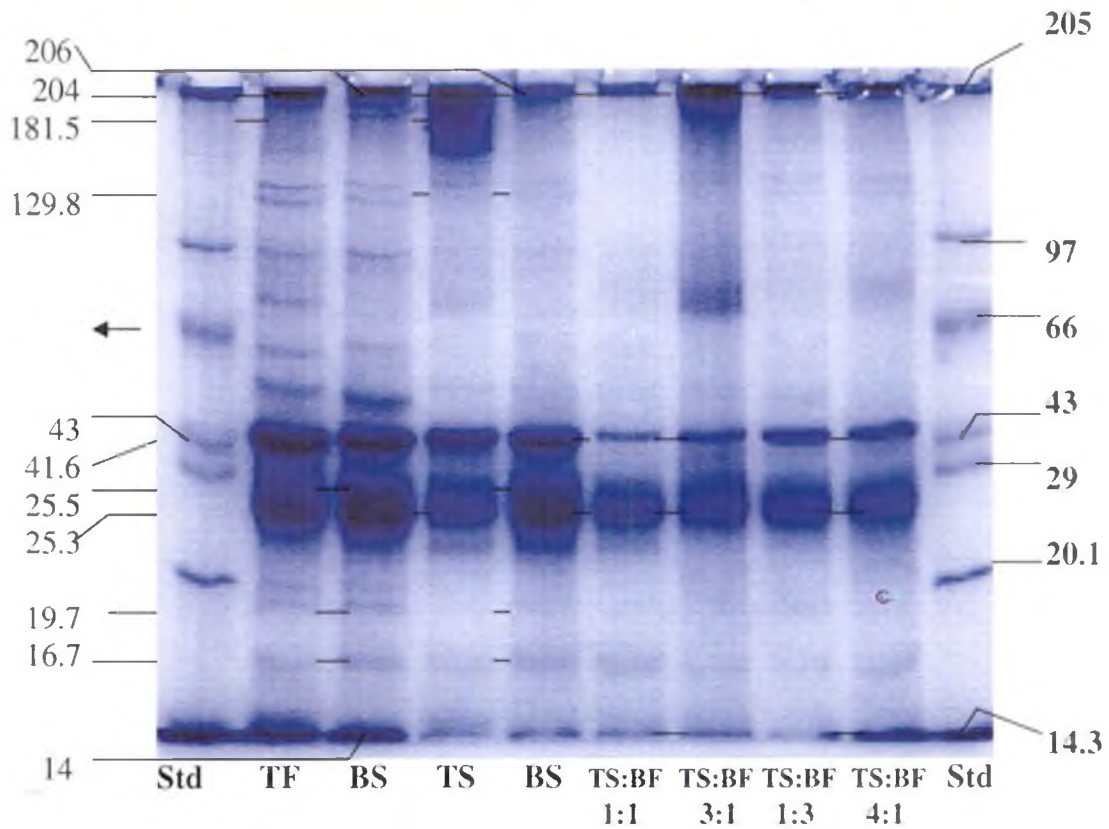

Plate 7.SDS-PAGE pattern of salt soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; TS: threadfin bream surimi; BF:bulls-eye fillet; BS:bulls-eye surimi.

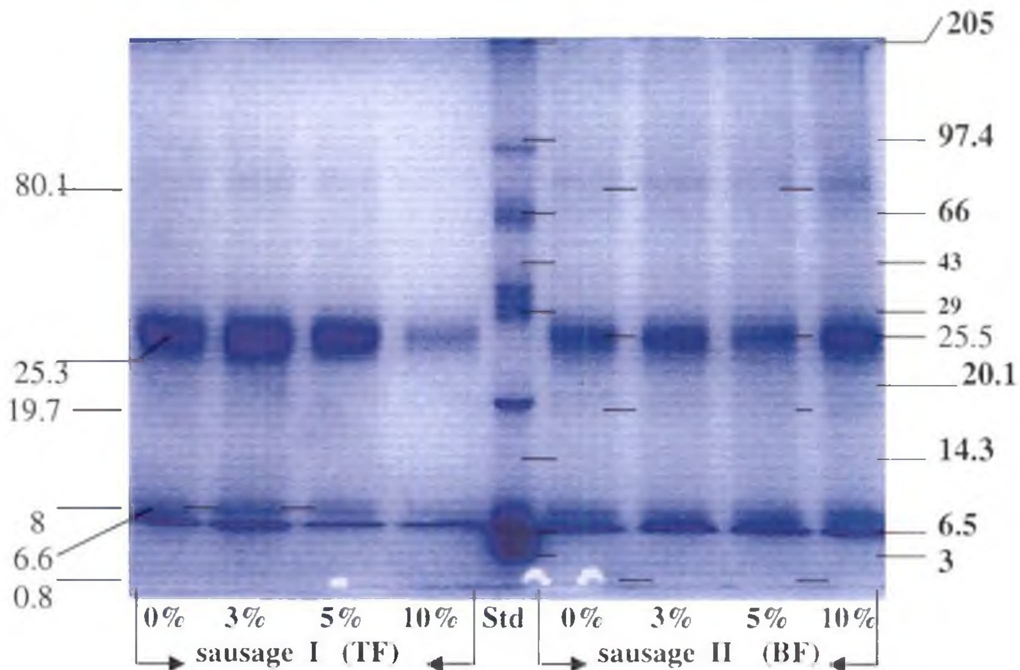

Plate 8.SDS-PAGE pattern of salt soluble proteins of sausage I (TF) and II (BF) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

Table 7 : Molecular weights of the protein bands of sausage samples extracted with distilled water

Band No.	Mol.wt. of the protein bands (kD) of sausage samples with different starch concentration							
	(IV) TF:BF (3:1)				(V) TF:BF (1:3)			
	0%	3%	5%	10%	0%	3%	5%	10%
1	205*	267.9	96.3	147.7 *	193.1	224.1	193.1	156.8*
2	96.3*	100.3	66	96.3*	97.4	96.3	93.3*	89.5*
3	81.4*	59.1	59.1	71.7	64.6	64.3	65	79.7
4	66.6	52.2	52.2	60.3	58.8	58.8	47	70.2
5	59.1*	48.2	48.2	48.2	54	53.5	40*	59.7
6	55.9	43.4*	43.4*	42*	51.9	51.7	39.5	43*
7	53.2	36.3*	40.1*	34.3*	47*	47*	36.6	35.8*
8	48.2	32.4	36.3*	31.5	43*	43*	34.5	25.1
9	43.4*	25.7	31.7	23.3	37.5	36.3	25.7	22.7
10	40.1*	14.5*	28.6	25.7	33.7	33.4	14.5*	25.7
11	36.3*		25.7	14.5*	25.7	28.2		14.5*
12	29		14.5*		14.5*	25.7		
13	27		6.0		7.5	14.5*		
14	25.7							
15	14.5*							
16	4.8							

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

4.4.2. Surimi Samples

Plate 7 and table 9 shows the electrophoretic pattern of salt soluble proteins of surimi samples. Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

4.4.3. Sausage Samples

4.4.3.1. Sausage I (TF) and II (BF)

Plate 8 and table 10 shows the electrophoretic pattern of salt soluble proteins of sausage I (TF) and II (BF). Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

Table 8 : Molecular weights of the protein bands of fillets extracted with Dyer's buffer

Band No.	Mol.wt of the protein bands (kD) of fillets	
	Threadfin bream	Bulls-eye
1	204*	206*
2	181.5*	129.8
3	161.1	120
4	129.0	92.4
5	120.5	76.1
6	93.6	70.9
7	75.7	62.2
8	61.1	49.9
9	52.4	42.3*
10	43*	25.5*
11	26*	19.7
12	19.8	18.9
13	19.1	16.7
14	16.6	14*
15	14.3*	

*strong bands; remaining narrow and less intensive bands

Table 9 : Molecular weights of the protein bands of surimi samples extracted with Dyer's buffer

Band No.	Mol.Wt. (kD) of the protein bands of surimi samples					
	(I) TF	(II) BF	(III) TF:BF (1:1)	(IV) TF:BF (3:1)	(V) TF:BF (1:3)	(VI) TF:BF (4:1)
1	204*	206*	204*	204*	204*	204*
2	181.5*	129.8	41.6	132.2	175.8	130.1
3	140.8	47.4	25.3*	75.1	131.8	121.3
4	122.7	41.6*	16.5	41.6	122.9	78.7
5	76.4	25.5*	14.3	25.3*	41.6*	41.6*
6	73.5	20.3		16.4	25.3*	25.3*
7	43*	19.7		14.3	19.8	21.4
8	25.3*	16.7			14.3	14.3*
9	22.7	14				
10	14.3					

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

Table 10 : Molecular weights of the protein bands of sausage samples extracted with Dyer's buffer

Band No.	Mol.wt. of the protein bands (kD) of sausage samples with different starch concentration							
	(I) TF				(II) BF			
	0%	3%	5%	10%	0%	3%	5%	10%
1	212.5	90.6	129.9	77.7	163.1	212.5	212.5	167.1
2	108.5	80.1	95.4	49.1	139.6	103.4	161.2	104.6
3	98.5	25.3*	79.3	30.2	98.5	88.8	99.7	88.8
4	77.7	20.9	66	25.3*	80.1	80.1	80.1	80.1
5	25.3*	16	34.5	21.1	70.1	68.7	66	68.7
6	21.6	8	25.3*	12.6	62.5	59.2	57.7	58.5
7	19.5	6.6*	20.4	10.9	36	25.5*	25.5*	33.8
8	17.6	1.7	17.1	8	25.5	19.7	19.7	25.5*
9	8	0.7	14.3	6.6*	19.7	13.3	9.3	19.7
10	6.6*		10.9	1.3	12.8	8.5	7.2	16.3
11	1.6		8	0.7	7.2	6.1*	6.1*	13
12			6.6*		6.1	2.3	1.6	7.2
13			1.8		2.1	0.8	0.8	6.1*
14			0.7		0.8			1.9
15								0.8

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

4.4.3.2. *Sausage (III) TF:BF (1:1) and (IV) TF:BF (3:1)*

Plate 9 and table 11 shows the electrophoretic pattern of salt soluble proteins of sausage (III) TF:BF (1:1) and (IV) TF:BF (3:1). Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

4.4.3.3. *Sausage (V) TF:BF (1:3) and (VI) TF:BF (4:1)*

Plate 10, 11 and table 12 shows the electrophoretic pattern of salt soluble proteins of sausage (V) TF:BF (1:3) and (VI) TF:BF (4:1). Bands were obtained in the electropherogram of salt soluble proteins in a 7.5% gel.

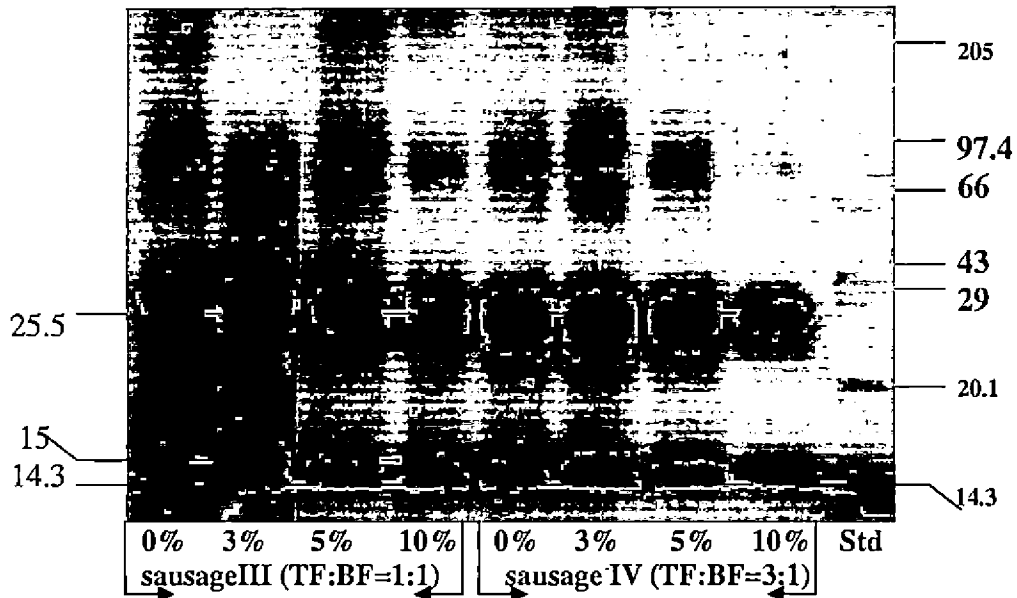

Plate 9.SDS-PAGE pattern of salt soluble proteins of sausage III (TF:BF=1:1) and IV (TF:BF=3:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

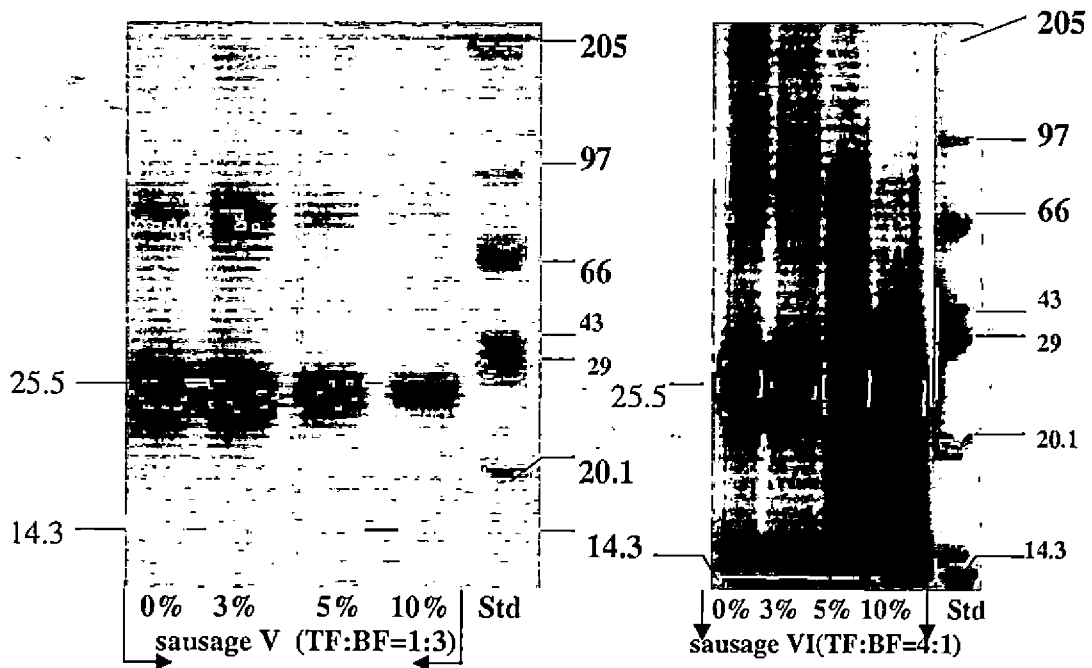

Plate 10 and 11.SDS-PAGE pattern of salt soluble proteins of sausage V (TF:BF=1:3) and VI (TF:BF=4:1) with different starch concentration respectively. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; BF:bulls-eye fillet.

Table 11 : Molecular weights of the protein bands of sausage samples extracted with Dyer's buffer

Band No.	Mol.Wt. of the protein bands (kD) of sausage samples with different starch concentration							
	(III) TF:BF (1:1)				(IV) TF:BF (3:1)			
	0%	3%	5%	10%	0%	3%	5%	10%
1	201.7	198.4	265.5	116.3	222.2	215.1	208.3	252.9
2	82.6	168.8	180.1	82.6	139	198.4	139	211.7
3	72.3	81	122.1	68.7	116.3	139	120.1	139
4	47.3	46	82.6	27.4	90.6	110.8	80.1	92.5
5	29*	28.1*	70.1	25.5*	79.3	88.8	59.8	81
6	25.5	25.5	58.7	15.0	69.4	77.7	25.5*	68.7
7	20.2	17.2	28.1*	14.3*	61	67.3	16.8	61
8	18.4	15.0	25.5		25.5*	59.8	14.3*	47.3
9	15.0	14.3*	15.0		17.8	41.8	4	25.5*
10	14.3*		14.3*		14.3*	25.5*		24
11						17.5		14.3*
12						14.3*		4

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

Table 12 : Molecular weights of the protein bands of sausage samples extracted with Dyer's buffer

Band No.	Mol.Wt. of the protein bands (kD) of sausage samples with different starch concentration							
	(V) TF:BF (1:3)				(VI) TF:BF (4:1)			
	0%	3%	5%	10%	0%	3%	5%	10%
1	80.3	78.3	197	189.4	189.2	189.2	189.2	107.5
2	62.5	25.5*	168.3	95.4	193.1	84.15	97.1	47.3
3	25.5*	20.5	79.6	82.3	107.5	72.2	76.1	25.5*
4	14.3*	14.3	69.3	25.5*	97	43	72.2	14.3*
5			60.3	14.3	84.1	25.5*	25.5*	
6			25.5*		72.2	20.4	21.4	
7			20.5		25.5*	14.3*	14.3*	
8			14.3		22.7	7.6		
9					14.3*			
10					7.2			

TF - threadfin bream fillets; BF – bulls-eye fillets

*strong bands; remaining narrow and less intensive bands

DISCUSSION

5. DISCUSSION

5.1. QUALITY EVALUATION OF SURIMI

As per Table 1, moisture contents of surimi samples were estimated in the range of 75-79.80%. The highest moisture content (79.80%) was found in Bulls-eye surimi and the lowest moisture content (75%) was found in threadfin bream: bulls-eye (4:1) mixed surimi. The present results showed that surimi used for the experiment had good moisture content. This results are in agreement with findings of Suzuki, (1981); Sonu, (1986); Alvarez *et al.*, (1995); Park *et al.*, (2005a) and Hultin *et al.*, (2005).

pH of surimi samples were estimated in the range of 6.06-6.90. The highest pH (6.90) was found in threadfin bream: bulls-eye (1:1) mixed surimi and the lowest (6.06) was found in bulls-eye surimi. The present results showed that surimi used for the experiment had required level of pH. These findings are in agreement with earlier studies of Suzuki, (1981); Theodore *et al.*, (2003) and Park *et al.*, (2005a).

Numbers of impurities in surimi samples were estimated in the range of 11-19. The highest no. of impurities (19) were found in threadfin bream: bulls-eye (1:1) mixed surimi. This may be due to the manual method of surimi preparation. The lowest no. of impurities (11) were found in threadfin bream: bulls-eye (1:3) mixed surimi. The present results showed that surimi used for the experiment had acceptable level of impurities. This result is in agreement with findings of Suzuki, (1981); Hultin *et al.*, (2005) and Park *et al.*, (2005a).

The results show that surimi samples used for the experiment were of good quality.

5.2. QUALITY EVALUATION OF SAUSAGE

Threadfin bream and bulls-eye sausage had good elasticity (Table 2) except 0% starch concentration of Bulls-eye sausage which had fair elasticity compared to others as shown by sensory score and folding test values. This result is in agreement with findings of Suzuki, (1981); Hultin *et al.*, (2005) and Park *et al.*, (2005a).

Threadfin bream: bulls-eye (1:3) mixed sausage had good elasticity compared to other combination of 1:1; 3:1 and 4:1, because it had higher proportion of meat from bulls-eye and others had acceptable/fair elasticity as shown by sensory score and folding test values. This result is in agreement with findings of Park *et al.*, (2005a).

Threadfin bream, bulls-eye sausage and mixed sausage had good gel strength. This result is in agreement with findings of Hultin *et al.*, (2005) and Park *et al.*, (2005a). Gel strength of the sausage was increasing with respect to their starch concentration. This increasing gel strength with starch was also reported by Suzuki, (1981); Alvarez *et al.*, (1995); Park *et al.*, (2005a).

The results show that sausage samples used for the experiment were of good quality.

5.3. ELECTROPHORETIC PATTERN OF WATER SOLUBLE PROTEINS

5.3.1. Electrophoretic Pattern of Water Soluble Proteins of Intact fish and Surimi

Table 3 and 4 show that, using SDS-PAGE, threadfin bream (TF) showed characteristic bands with molecular weights of 203.3, 26, 19.4, 18.5 and 14.5 (all in kilo Daltons) while the bulls-eye (BF) showed characteristic bands with molecular weights of 25.7, 19.7,18.4, 17.7 and 14 (all in kilo Daltons). All these species-specific bands were found in the fish and their surimi samples. This result was similar to those reported by An *et al.*, (1988); An *et al.*, (1989); Chen and Hwang, (2002); Cowie (1968); Mackie, (1993); Perez-mateos *et al.*, (2004); and Moosavi-nasab,(2005); Mackie and Ritchie, (1981); Pineiro *et al.*, (1999); Rehbein, (1992); Sotelo *et al.*, (1992); Torry Research Station, (1986); Yowell and Flurkey (1986). A portion of the sarcoplasmic proteins (up to 21%) of minced meat was washed away from the fish proteins during surimi manufacturing (Lee, 1986a and Park *et al.*, 2005a). This may cause complications in the species identification process of surimi samples, especially when the specific proteins in samples were only present in minor quantities in the fish. Generally, the surimi samples still showed the basic patterns as those of the fish fillet from which they were made. So these bands can be used for the identification of threadfin bream and bulls-eye surimi

when adulteration/substitution takes place. Similar observations were made by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Rehbein, (1992); Torry Research Station, (1986).

New bands with molecular weights of 93.6 and 22.3 (all in kilo Daltons); 63.8 (all in kilo Daltons) were found in threadfin bream surimi (TS) and bulls-eye surimi (BS) respectively and also in all the combinations of TS + BS mixture and they can be used with reservation to differentiate surimi mixtures. Some of the relative percent of the minor proteins had been increased after washing due to the loss of major proteins and the presence of residual proteolytic enzyme activity may together contribute to the appearance of the new bands. Similar findings were reported by An *et al.*, (1988); An *et al.*, (1989); Torry Research Station, (1986); Rehbein, (1992); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Yowell and Flurkey (1986).

Bands with molecular weights of 93.6, 63.8, 22.3, 19.7, 17.7 and 14.5 (all in kilo Daltons) were found to be common to all the combinations of TS + BS mixture. Among these 93.6, 22.3 and 14.5 (all in kilo Daltons); 63.8, 19.7 and 17.7 (all in kilo Daltons) were found in threadfin bream and bulls-eye surimi respectively. This shows that these species-specific bands remained unaltered in different combination of TS + BS mixture (1:1; 3:1; 1:3; 4:1). Similar observations were reported by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Devadasan, 2002; Hall, 1997; Torry Research Station, (1986); Rehbein, (1990 and 1992); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Pineiro *et al.*(1999); Mackie, (1993). So these bands can be used with reservation to identify the TS and BS mixture when they are mixed with different combinations of 1:1; 3:1; 1:3; 4:1 ratios. Similar finding with various combinations of fish, minced meat and surimi mixtures were reported by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Moosavi-nasab, (2005) and Rehbein, (1992).

Some of the characteristic protein bands, such as the 26, 19.4, 18.5 kD of TS and the 18.4 kD of BS were missing in the different combinations of TS + BS mixture. The major portions of sarcoplasmic proteins were removed during the water leaching (Lee, 1984, 1986a and Park *et al.*, 2005a) which may leads to disappearance of these bands in their mixtures. This is in agreement with previous studies of An *et al.*, (1988); An *et al.*, (1989); Dowdie and Biede,

(1983); Davis and Anderson, (1984); Moosavi-nasab, (2005); Park *et al.*, (1997); Yowell and Flurkey (1986).

As per Table 4, several new minor bands but not species-specific bands were developed in the surimi and surimi mixtures. This may be due to the membrane proteins solubilized by SDS. These responsible for the higher recovery and the presence of new bands with high and low molecular weight. Similar finding reported by Copper. (1997); An *et al.*, (1988) and An *et al.*, (1989).

5.3.2. Electrophoretic Pattern of Water Soluble Proteins of Sausage Samples

According to Table 5, using SDS-PAGE, threadfin bream (TF) sausage showed characteristic strong bands with molecular weights of 26 and 14.5 (all in kilo Daltons) while table 6 shows that, the bulls-eye (BF) sausage characteristic strong bands with molecular weights of 25.7 and 14 (all in kilo Daltons). All these species-specific bands were common to the 0%, 3%, 5% and 10% starch concentration of sausage and also found in the fish and their surimi samples. So these bands can be used for the identification of threadfin bream and bulls-eye sausage when adulteration/substitution takes place. Similar finding were reported by An *et al.*, (1988); An *et al.*, (1989); Blum *et al.*, (1989); Craig *et al.*, (1995); Hall, (1997); Perez-mateos *et al.*, (2004); Rehbein, (1990); Pineiro *et al.*(1999); Sotelo *et al.*, (1992) and Yowell and Flurkey (1986).

Some of the characteristic bands, such as the 203.3, 19.4 and 18.5 (all in kilo Daltons) of threadfin bream surimi and the 19.7, 18.4 and 17.7 (all in kilo Daltons) of bulls-eye surimi, were missing in the corresponding sausage samples. This may be due to, at high temperature (88-90°C) of cooking caused both protein denaturation and dissociation of subunits. These results are in agreement with other reports of An *et al.*, (1988) and An *et al.*, (1989). Erikson *et al.*, (1983) reported that decrease in sarcoplasmic proteins could be attributed to the release of renatured internal proteases and also by Venugopal *et al.*, (1983). Yowell and Flurkey (1986) reported that, a decrease of 76% of water soluble proteins extracted from heated samples when compared to soluble proteins extracted from fresh fish samples which leads to loss of specific

proteins bands. These results are in agreement with other reports of Davis and Anderson, (1984); Mackie, (1968); Nishioka and Shimizu (1979) and Roberts and Lawrie (1974). Disappearance of band was observed by Dowdie and Biede, (1983) in heated blue crab meat; reduction of protein bands was also observed by Lee *et al.*, (1974) as the processing temperature of bovine muscle was increased from 65°C to 90°C; Band loss also reported by Caldironi and Bazan, (1980); Dowdie and Biede, (1983) and Koohmaraie *et al.*, (1984).

During cooking the addition of the starch affected/disappeared the above characteristic minor bands. Starch decreased/disappeared the protein solubility in the mixture of SDS in the bulls-eye, croaker and threadfin bream sausage samples were reported previously by Benjakul *et al.*, (2002) and also Elton and Ewart, (2006) and Ramezani *et al.*, (2006)

According to Tables 5,6 and 7, bands with molecular weights of 25.7 and 14.5 (all in kilo Daltons) were found common to all different starch concentration of sausage (0%, 3%, 5% and 10%) of the different combinations (1:1; 3:1; 1:3; 4:1) of TF + BF sausage mixture. These species-specific bands of 25.7 and 14.5 (all in kilo Daltons) were also found in the bulls-eye surimi (BS) and threadfin bream surimi (TS) respectively. This shows that these species-specific bands remained unaltered in the sausage preparation process, especially cooking. Similar findings were reported by An *et al.*, (1988); An *et al.*, (1989); Blum *et al.*, (1989); Craig *et al.*, (1995); Davis and Anderson, (1984); Dowdie and Biede, (1983); Devadasan, 2002; Hall, (1997) and Mackie, (1968); Perez-mateos *et al.*, (2004); Pineiro *et al.*(1999) and Yowell and Flurkey (1986). Based on the previous studies on the cooked samples of An *et al.*, (1988); An *et al.*, (1989); Blum *et al.*, (1989); Craig *et al.*, (1995); Hall, (1997); Perez-mateos *et al.*, (2004); Yowell and Flurkey (1986) these two bands can be used to identify the TF + BF sausage mixture at different combinations of 1:1; 3:1; 1:3; 4:1 ratios.

Table 5, 6 and 7 show that, several new minor bands but not species-specific bands were developed in the sausage due to the enhancement of protein extraction from the cooked sample by SDS. These results are in agreement with other reports of Tanford, (1968); Copper, (1977); Thakker and Grady, (1984); An *et al.*, (1988); An *et al.*, (1989) and Yowell and Flurkey (1986).

5.4. ELECTROPHORETIC PATTERN OF SALT SOLUBLE PROTEINS

5.4.1. Electrophoretic Pattern of Salt Soluble Proteins of Intact fish and Surimi

Salt soluble proteins of intact fish and surimi were extracted by using Dyer's buffer. According to Tables 8 and 9, using SDS-PAGE, threadfin bream (TF) showed characteristic myofibrillar protein bands with molecular weights of 204,181.5, 43 and 14.3 (all in kilo Daltons) while the bulls-eye (BF) showed characteristic bands with molecular weights of 206, 129.8, 25.5, 19.7, 16.7 and 14 (all in kilo Daltons). All these species-specific bands were found in the fish and their surimi samples. These species-specific bands of fish and surimi were observed in Alaska pollock and red hake by An *et al.*, (1989); in pink and rock shrimp by An *et al.*, (1988) and also in various species by Chen and Hwang, (2002); Cowie (1968); Mackie, (1993); Perez-mateos *et al.*, (2004); and Moosavi-nasab,(2005); Mackie and Ritchie, (1981); Pineiro *et al.*(1999); Rehbein, (1992); Sotelo *et al.*, (1992); Torry Research Station, (1986); Yowell and Flurkey (1986). These species-specific bands of fish and surimi can be used for detection of adulterated/substituted species in their respective samples. Similar observations were reported by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Rehbein, (1992); Torry Research Station, (1986).

Some of the species-specific myofibrillar protein bands, such as the 26 kD of TF and 42.3 kD of BF were missing in the respective surimi. This may be due to the loss of myofibrillar proteins during the successive water leaching process of surimi preparation. This result was in agreement with other reports of Lin and Park, (2005); Park *et al.*, (2005a) and Moosavi-nasab, (2005).

From table 9, the new bands of 25.3 kD in TS and 41.6 kD in BS were found. These new bands were also found in the all different combinations (1:1; 3:1; 1:3; 4:1) of TS + BS mixture. This may be due to the significant myosin degradation of surimi with NaCl, which leads to increase in protein bands. The similar results were reported by Chen, (1979) and Perez-mateos *et al.*, (2004) in Atlantic croaker surimi and Choi and Park, (2002); Olsen *et al.*,

(2002) in various species. These bands can be used with reservation to differentiate surimi mixtures. This is in agreement with previous studies of An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Devadasan, 2002; Hall, 1997; Torry Research Station, (1986); Rehbein, (1990) and 1992); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Pineiro *et al.*(1999); Mackie, (1993). Due to the membrane proteins solubilized by SDS several new minor bands were developed in the surimi and surimi mixtures which are all not found in their fish fillets. These findings are in agreement with previous studies of Copper, (1997); An *et al.*, (1988) and An *et al.*, (1989).

Different combination of TS + BS mixture (1:1; 3:1; 1:3; 4:1) had common bands of 204, 41.6, 25.3 and 14.3 (all in kilo Daltons). Among these bands 204, 25.3, and 14.3; 41.6 kD were also found in threadfin bream and bulls-eye surimi respectively and it shows that these species-specific bands remained unaltered in different combinations of TS + BS mixture (1:1; 3:1; 1:3; 4:1). Similar observations were reported by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Devadasan, 2002; Hall, 1997; Torry Research Station, (1986); Rehbein, (1990) and 1992); Perez-mateos *et al.*, (2004); and Moosavi-nasab, (2005); Pineiro *et al.*(1999); Mackie, (1993). So these species-specific bands can be used to identify/detect the substitute in the TS and BS mixture when they are mixed with different combinations of 1:1; 3:1; 1:3; 4:1 ratios. Similar findings with various combinations of fish and shellfish substitutes in the minced meat and surimi mixtures of myofibrillar proteins was successfully reported by Rehbein, (1992); Sotelo *et al.*, (1992) and Torry Research Station, (1986) and also in various species by An *et al.*, (1988); An *et al.*, (1989); Cowie (1968); Moosavi-nasab, (2005).

5.4.2. Electrophoretic Pattern of Salt Soluble Proteins of Sausage Samples

As per table 10, Bands with molecular weights of 25.3 kD; 25.5 and 19.7 kD were found that characteristic bands of threadfin bream (TF) sausage and bulls-eye (BF) sausage respectively. These species-specific bands were found in their surimi samples and also these bands common to the 0%, 3%, 5% and 10% starch concentration of sausage. So these bands can be used for the identification of threadfin bream and bulls-eye sausage. Similar findings were reported in Alaska pollock and red hake by An *et al.*, (1989); in

pink and rock shrimp by An *et al.*, (1988) and also in various species were reported by Blum *et al.*, (1989); Craig *et al.*, (1995); Hall, (1997); Perez-mateos *et al.*, (2004); Rehbein, (1990); Pineiro *et al.*, (1999); Sotelo *et al.*, (1992) and Yowell and Flurkey (1986).

Bands with molecular weights of 8 and 6.6 kD in threadfin bream (TF) sausage and 80.1 and 0.8 kD of bulls-eye (BF) sausage were found to be newly developed. Development of new bands may be enhancement of protein extraction from the cooked sample by SDS. Similar findings in cooked codfish fillets and their mixtures were reported by Yowell and Flurkey (1986); in cooked surimi gel reported by An *et al.*, (1988); An *et al.*, (1989) and Perez-mateos *et al.*, (2004); Tanford, (1968); Copper, (1977); Thakker and Grady, (1984).

Some of the characteristic bands, such as the 204,181.5, 43 and 14.3 (all in kilo Daltons) of threadfin bream surimi and the 206, 129.8, 16.7 and 14 (all in kilo Daltons) of bulls-eye surimi, were missing in the corresponding sausage samples. This may be due to, at high temperature (<70°C) myosin molecules dissociated from actin, which leads to formation -S-S- linkage and hydrophobic interactions, finally protein aggregation takes place which reduces the solubility of myofibrillar protein which in turn leads to loss of protein bands. This result was in agreement with reports of Hamm and Hofmann, (1965); Itoh *et al.*, (1980, 1980a); Devadasan (1985); Mackie (1968); Park *et al.*, (2005a); Loss of myofibrillar protein bands also attributed to the extracellular protease activity. This result was in agreement with previous studies of Yowell and Flurkey (1986); Venugopal *et al.*, (1983). Addition of starch also causes disappearance of protein bands in the mixture of SDS. Similar findings in bulls-eye, croaker and threadfin bream sausage samples were reported by Benjakul *et al.*, (2002) and also Elton and Ewart, (2006) and Ramezani *et al.*, (2006).

Different combinations (1:1; 3:1; 1:3; 4:1) of TF + BF sausage mixture had common bands with molecular weights of 25.5 and 14.3 (all in kilo Daltons) were found (Table 11 and 12) common to all different starch concentration of sausage (0%, 3%, 5% and 10%). These species-specific bands of 25.5 and 14.3 (all in kilo Daltons) were also found in the bulls-eye surimi (BS) and threadfin bream surimi (TS) respectively. These unaltered species-

specific bands can be used to identify the TF + BF sausage mixture at different combinations of 1:1; 3:1; 1:3; 4:1 when adulteration/ substitution suspected. This result is in agreement with previous studies of Torry Research Station, (1986); Rehbein, (1990) in the cod, blue whiting, sardine and Allaska Pollock; An *et al.*, (1988) in Alaska pollock and red hake; An *et al.*, (1988) in pink and rock shrimp and also in various species by Blum *et al.*, (1989); Craig *et al.*, (1995); Pineiro *et al.*, (1999) and Sotelo *et al.*, (1992).

According to tables 11 and 12, new band with molecular weights of 15 kD of TF + BF sausage mixture (1:1) were found common to all different starch concentration of sausage (0%, 3%, 5% and 10%). Also several new minor bands but not common to all different starch concentrations of sausage were found in the different combinations of TF + BF sausage mixture. This may be due to the enhancement of protein extraction from the cooked sample by SDS. These results are in agreement with other reports of An *et al.*, (1988); An *et al.*, (1989); Pineiro *et al.* (1999) and Yowell and Flurkey (1986). Perez-mateos *et al.*, (2004) were observed that development of the new bands at the temperature of 40°C and 90°C in Atlantic croaker surimi gels (sausage).

This study showed that threadfin bream and bulls-eye could be effectively identified by using their SDS-PAGE pattern of water soluble proteins and salt soluble proteins separately. Threadfin bream and bulls-eye surimi and their sausage samples were also effectively identified by using their species-specific SDS-PAGE pattern of water soluble proteins and salt soluble proteins separately. The SDS-PAGE pattern of water soluble proteins and salt soluble proteins were shown in this study to be effective in distinguishing the species specificity between threadfin bream and bulls-eye of raw fish fillets, surimi and sausage samples. The different mixture combinations (1:1; 3:1; 1:3 and 4:1) of threadfin bream and bulls-eye surimi and their sausage gave effective species-specific SDS-PAGE pattern in their extract of water soluble proteins and salt soluble proteins with loss of few bands in the sausage but with the presence of major species-specific bands. So, these different mixture species-specific SDS-PAGE protein patterns can be used with reservation to detect/differentiate the adulteration/substitution in the fish mixtures, surimi and sausage when these two species mixed with the combination of 1:1; 3:1; 1:3 and 4:1 ratios.

The results showed that surimi, sausage and surimi mixtures; sausage mixtures of threadfin bream and bulls-eye used for the experiment were of good quality.

SUMMARY

6. SUMMARY

1. The main objectives of the present investigations were identification of species of raw fillets and the species used in surimi and sausage; identification of adultered/substituted species in the mixtures of surimi and sausage prepared from it by SDS-PAGE species-specific pattern; and quality evaluation of prepared surimi and sausage.

2. Two species- threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur*) were used for the present study.

3. Surimi and their mixtures (1:1; 3:1; 1:3; and 4:1); sausage and their mixtures (1:1; 3:1; 1:3; and 4:1) were prepared from threadfin bream and bulls-eye.

4. SDS-PAGE pattern of water soluble proteins and salt soluble proteins of raw fillets of threadfin bream and bulls-eye were studied and this study gave characteristics bands of five and four for threadfin bream and five and six for bulls-eye respectively.

5. SDS-PAGE pattern of water soluble proteins of surimi and sausage of threadfin bream gave characteristics bands of eight and two respectively, and salt soluble proteins gave five and three respectively; water soluble proteins of surimi and sausage of bulls-eye gave characteristics bands of six and two respectively, and salt soluble proteins gave seven and four respectively.

6. SDS-PAGE pattern of water soluble proteins of mixture of different combinations (1:1; 3:1; 1:3; and 4:1) of surimi and sausage of threadfin bream and bulls-eye gave characteristics bands of six and two respectively, and salt soluble proteins gave characteristics bands of four and two respectively.

7. Quality evaluation of prepared surimi, sausage and different combinations of surimi mixtures; sausage mixtures were carried out.

8. SDS-PAGE gave the species-specific pattern of threadfin bream and bulls-eye in their extract of water and salt soluble proteins separately.

9. Threadfin bream and bulls-eye surimi and sausage were identified by comparing their SDS-PAGE pattern with species-specific SDS-PAGE pattern in their extract of water soluble proteins and salt soluble proteins.

10. Species-specific SDS-PAGE pattern of extract of water and salt soluble proteins of raw fillets were also found in their surimi and sausage.

11. Species-specific SDS-PAGE pattern of surimi and sausage were also found in their different combinations (1:1; 3:1; 1:3; and 4:1) of mixtures of extract of water and salt soluble proteins.

12. Adultered/substituted species in the different mixture combinations (1:1; 3:1; 1:3 and 4:1) of threadfin bream and bulls-eye surimi and sausage were identified by comparing their mixture SDS-PAGE pattern with species-specific SDS-PAGE pattern in their extract of water soluble proteins and salt soluble proteins.

13. Surimi, sausage and different combinations of surimi mixtures; sausage mixtures were in good quality which was showed by quality evaluation study (sensory score, folding grade values and gel strength).

REFERENCES

7. REFERENCES

*Akroyd, P. 1967. Acrylamide gel slab electrophoresis in a simple glass cell for improved resolution and comparison of serum proteins. *Amal. Biochem.* 19 (3): 399-410

Alvarez, C., Couso, I. and Tejada, M. 1995. Sardine surimi gel as affected by salt concentration, blending, heat treatment and moisture. *J. Fd. Sci.* 60 (3): 622-626

*Anon. 1988b. Analysis of foods. Detection of the animal species of native muscle tissue by isoelectric focusing. *Nutr. Fd. Sci.* 111: 5-6

An, H., Marshall, M.R., Otwell, W.S. and Wei, C.I. 1988. Electrophoretic identification of raw and cooked shrimp using various protein extraction systems. *J. Fd. Sci.* 53 (2): 313-318

An, H., Marshall, M.R., Otwell, W.S. and Wei, C.I. 1989. Species identification of raw and cooked shrimp by urea gel isoelectric focusing techniques. *J. Fd. Sci.* 54 (2): 233-236

An, H., Wei, C.I., Zhao, J., Marshall, M.R. and Lee, C.M. 1989. Electrophoretic identification of fish species used in surimi products. *J. Fd. Sci.* 54 (2): 253-257

*Andrews, A.T. 1986. Monographs on physical biochemistry. *Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications* (eds. Peacock, A.R. and Harrington, W.F.). Second edition. Clarendon Press, Oxford.

*AOAC. 1975. *Official Methods of Analysis*. Twelfth edition. Association of Official Analytical Chemists, Washington, 1094 p.

AOAC. 1990. *Official Methods of Analysis*. Fifteenth edition. Association of Official Analytical Chemists, Arlington, VA, 885-886

*Asghar, A., Saejima, K. and Yasni, T. 1985. Functionality of muscle proteins in gelation mechanism of structured meat products. C.R.C.*Crit. Rev.Fd. Sci. Nutr.* 22 (1): 27-105

Ashurst, P.R. and Dennis, M.J. 1996. *Food Authentication.* Blackie Academic and Professional, London, pp.140-170

Benjakul, S., Visessanguan, W., Riebroy, S., Ishizaki, S. and Tanaka, M. 2002. Gel-forming properties of surimi produced from bigeye snapper (*Priacanthus tayenus*) and (*P.macracanthus*) stored in ice. *Soc.of Chem. Ind.* 60: 412-416

Blum, H., Beier, H. and Gross, H.J. 1989. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gel. *Electrophoresis.* 8: 93-99

*Caldironi, H.A. and Bazan, N.G. 1980. Quantitative determination of lowsalt soluble protein patterns of bovine muscle cooked at different temperatures, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Fd. Sci.* 45: 901-905

*Cheng, C.S., Hamann, D.D. and Webb, N.B. 1979. Effect of thermal processing on minced fish gel texture. *J. Fd. Sci.* 44: 1080-1086

Chen, T.Y. and Hwang, D.F. 2002. Electrophoretic identification of muscle proteins in 7 buffer species. *J. Fd. Sci.* 67 (3): 936-942

Choi, Y.I. and Park, J.W. 2002. Acid-aided protein recovery from enzyme-rich Pacific whiting. *J. Fd. Sci.* 67 (8): 2962-2967

*Connell, J.J. 1973. *Proc. Soc. Anal. Chem.* 10 (7): 176-178

*Copper, T.G. 1977. *The Tools of Biochemistry.* John Wiley and Sons, New York, 381 p.

Cowie, W.P. 1968. Identification of fish species used by thin slab polyacrylamide gel electrophoresis. *J. Sci. Fd. Agr.* 19: 226-229

Craig, A., Ritchie, A.H. and Mackie, I.M. 1995. Determining the authenticity of raw reformed breaded scampi (*Nephrops norvegicus*) by electrophoretic techniques. *Fd. Chem.* 52: 451-454

*Davis, C.E.and Anderson, J.G. 1984. Size exclution/HPLC of heated water soluble bovine and porcine muscle proteins. *J. Fd. Sci.* 49: 598-602

*Davies, G., Stark, G. 1970. *Proc. Nat. Acad. Sci. USA*, 66: 651

*Devadasan, K., Viswanathannair, P.G. and Antony, P.D. 1985. *Fish Technol.* 27 (1): 70-74

Devadasan, K. 2002. Proteins and lipids of marine products and their changes during processing and preservation. *Textbook of Fish Processing Technology* (ed. Gopakumar, K.). Indian Council of Agricultural Research, New Delhi, pp.122-158

*Dhulkhed,.M. and Narasimharao, S. 1976. Electrophoretic identification of serum protein of sardine and Makeral. *Fishery Technol.* 13 (1): 16-19

Dowdie, O.G. and Biede, S.L. 1983. Influence of processing temperature on distribution of tissue and water-soluble proteins in blue crabs (*Callinectes sapidus*). *J. Fd. Sci.* 48: 804-809

Dreyfuss, S., Cutrufelli, E., Mageau, P. and Mcnamara. 1997. Agar-gel immunodiffusion test for rapid identification of Pollock surimi in raw meat products. *J. Fd. Sci.* 62 (5): 972-975

*Elton, H. and Ewart, J.A.D. 2006. Effect of starch in the protein mobility and their changes in the protein pattern. *J. Sci. Fd. Agri.* 13 (1): 62-72

Erickson, M.C., Gordon, D.T. and Anglemier, A.F. 1983. Proteolytic activity in the sarcoplasmic fluids of parasitized Pacific Whiting (*Merluccius productus*) and unparasitized true cod (*Godus macrocephalus*). *J. Fd. Sci.* 48: 1315-1320

*Etienne, M., Jerome, M., Fleurence, J., Rehbein, H., Kundiger, R.,Yman, I.M., Ferm, M., Craig, A., Mackie, I.M., Jessen, F., Smelt, A. and Luten, J. 1999. A standardized method of identification of raw and heat-processed fish by urea isoelectric focusing: A collaborative study. *Electrophoresis.* 20: 1923-1933

FAO. 1998. Expert consultation on bycatch utilization in tropical fisheries. Beijing, China, 154 p.

FAO. 2005. Codex Committee on Fish and Fishery Products. FAO, Rome, 57 p.

Gallardo, M., Sotelo, G., Pineiro, C. and Perez-Martin, I. 1995. Use of capillary zone electrophoresis for fish species identification. Differentiation of flatfish species. *J. Agric. Fd. Chem.* 43: 1238-1244

Gangar, V. 1992. Electrophoretic identification of (*Callinecfes sapidus*) meat. M.S. Thesis, University of Florida.

*Hafez, Y.S., Mohamad, A.I., Hewedy, F.M. and Singh, G. 1985. Effects of microwave heating on solubility, digestability and metabolism of soy protein. *J. Fd. Sci.* 50: 415-419

Hall, G.M. 1997. *Fish Processing Technology*. Second edition. Blackie, London. 292 p.

Hamilton, W.D. 1982. Fish species identification by thin layer agarose isoelectric focusing and densitometric scanning. *J. Assoc. Off. Anal. Chem.* 65: 119-122

*Hamm, R. and Hofmann, K., 1965. *Nature.* 207: 1269-1274

*Hasnain, A., Tamura, H. and Arai, K. 1976. *Bull. Jap. Soc. Sci. Fish.* 42: 783-791

Hill, W.S., Learson, R.J. and Lane, J.P. 1996. Identification of fish species by agar gel electrophoresis. *J. Assoc. Off. Anal. Chem.* 49: 1245-1247

*Hofmann, K. 1988. *Nature.* 210: 1569-1574

*Horner, W.F.A. 1992. Canning fish and fish products. *Fish Processing Technology* (ed. Hall, G.M.). Blackie, London, pp. 114-153

*Hsieh, Y.H.P., Woodward, B.B. and Blanco, A.W. 1995. Species substitution of retail snapper fillets. *J. Fd. Quality.* 18: 131-140

*Huang, T., Marshall, M.R. and Wei, C.I. 1995. Identification of red snapper (*Lutjanus campechanus*) using electrophoretic techniques. *J. Fd. Sci.* 60: 279-283

Hultin, O., Kristinsson, G., Lanier, C. and Park, W. 2005. Process for recovery of functional proteins by pH shifts. *Surimi and Surimi Seafood* (ed. Park, W.). Second edition. Taylor & Francis Group, Boca Raton, pp. 107-139

*Itoh, Y., Yoshinaka, R. and Ikeda, S. 1980a. *Bull. Jap. Soc. Sci. Fish.* 46 (5): 617-620

*Itoh, Y., Yoshinaka, R. and Ikeda, S. 1980. *Bull. Jap. Soc. Sci. Fish.* 46 (5): 621-624

Keenan, C.P. and Shaklee, B. 1985. Electrophoretic identification of raw and cooked fish fillets and other marine products. *Fd. Technol.* 37: 117-128

*Kimura, I., Murozuka, T.and Arai, K. 1977. *Bull. Jap. Soc. Sci. Fish.* 43: 315-321

*Kleparnik, K. and Bocek, P. 1991. Theoretical background for clinical and biochemical applications of electromigration techniques. *J. Chromatag.* 569: 3-42

Kokuryo, H., Seki, N. 1978. Identification of fish species of dried fish sticks (fushi) by SDS-polyacrylamide gel electrophoresis. *Bull. Jap. Soc. Sci. Fish.* 44 (1): 67-70

Koohmaraie, M., Kennick, W.H., Anglemier, A.F., Elgasim, E.A. and Jones, T.K. 1984. Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. *J. Fd. Sci.* 49: 290-295

Krzynowek, J. and Wiggin, K. 1979. Identification of species in cooked crabmeat by thin layer polyacrylamide gel isoelectric focusing. *J. Assoc. Off. Anal. Chem.* 62: 630-634

Krzynowek, J. and Wiggin, K. 1981. Generic identification of cooked and frozen crabmeat by thin layer polyacrylamide gel isoelectric focusing: Collaborative study. *J. Assoc. Off. Anal. Chem.* 64 (3): 670-673

*Kurth, L. and Shaw, F.D. 1983. Identification of the species of origin of meat by electrophoretic and immunological methods. *Fd. Technol.* 35: 328-331

Laemmli, U.K. 1970. Cleavage of structural proteins assembly of the head of bacteriophage T4. *Nature.* 227: 680-685

Lane, J.P., Hill, W.S. and Learson, R.J. 1996. Identification of species in raw processed fishery products by means of cellulose polyacetate strip electrophoresis. *Comm. Fish. Rev.* 28: 10-13

*Lanier, T.C. 1986. Functional properties of surimi. *J. Fd. Sci.* 40: 107

Lanier, C., Carvajal and Yongsawatdigul. 2005. Surimi gelation chemistry. *Surimi and Surimi Seafood* (eds. Park, W.). Second edition. Taylor and Francis Group, Boca Raton, pp. 435-489

*Lanier, T.C. and Lee, C.M. 1992. *Surimi Technology*. Marcel Dekker, New York.

Learson, J. 1970. Collaborative study of a rapid electrophoretic method for fish species identification. II. Authentic flesh standards. *J. of the AOAC*. 53 (1): 7-9

*LeBlanc, E.L. and LeBlanc, R.J. 1989. Separation of cod (*Gadus morhua*) fillet proteins by electrophoresis and HPLC after various frozen storage treatments. *J. Fd. Sci*. 54:827-834

*LeBlanc, E.L., Singh, S. and LeBlanc, R.J. 1994. Capillary zone electrophoresis of fish muscle sarcoplasmic proteins. *J. Fd. Sci*. 59 (6): 1267-1270

Lee, Y.B., Rickansrud, D.A., Hagberg, E.C. and Brisky, E.J. 1974. Application of SDS-polyacrylamide gel electrophoresis for determination of the maximum temperature to which bovine muscle have been cooked. *J. Fd. Sci*. 39: 428-431

*Lee, C.M. 1984. Surimi process technology. *Fd. Technol*. 38 (11): 69-80

Lee, C.M. 1986a. Surimi manufacturing and fabrication of surimi-based products. *Fd. Technol*. 40 (3): 115

*Lee, C.M. 1986b. A pilot plant study of surimi making properties of red hake (*Urophcis chuss*). *International Symposium on Engineered Seafood Including Surimi*, 1986 (eds. Martin, R., and Collette, R.). National Fisheries Institute, Washington, pp.225

Lundstorm, R.C. 1980. Fish species identification by thin-layer polyacrylamide gel isoelectric focusing: Collaborative study. *J. Assoc. Off. Anal. Chem.* 63 (1): 69-73

Lundstorm, R.C. 1981. Rapid fish species identification by agarose gel isoelectric focusing of sarcoplasmic proteins. *J. Assoc. Off. Anal. Chem.* 64: 38-43

*Lundstorm, R.C. 1983. Identification of pacific rock fish (sebastes) species by isoelectric focusing of sarcoplasmic proteins. *J. Assoc. Off. Anal. Chem.* 66: 974-980

*Mackie, I.M. 1968. *Analyst.* 93: 458-463

*Mackie, I.M. 1969. Identification of species by a modified polyacrylamide gel electrophoresis technique. *J. Assoc. Publ. Anal.* 7: 83-87

Mackie, I.M. 1980. A review of some recent application of electrophoresis and isoelectric focusing in the identification of species of fish and fish products. *Advances in Fish Science and Technology* (ed. Connell, J.J.). Fishing News Books, Farnham, pp. 444-451

Mackie, I.M. 1990. Identifying species of fish. *Analytical Proceedings,* Royal Society of Chemistry. 27: 89-92

*Mackie, I.M. 1992. Surimi from fish. *The Chemistry of Muscle-based Foods* (eds. Johnston, D.E., Knight, M.K. and Leeward, D.A.). Royal Society of chemistry, Cambridge, pp. 200-207

Mackie, I.M. 1993. The effects of freezing on flesh protein. *Fd. Rev. Int.* 9 (4): 575-610

*Mackie, I.M. 1994a. Fish protein. *New and Developing Sources of Food Proteins*. Chapman & Hall, London, pp. 95-143

*Mackie, I.M. 1994b. Fish speciation. *Fd. Techno. Int. Europe*. 177-180

Mackie, I.M., 1997. Methods of identifying species of raw and processed fish. *Fish Processing Technology* (ed. Hall, G.M.). Second edition. Blackie, London, pp. 160-199

*Mackie, I.M. and Taylor, T. 1972. Identification of species of heat-sterilized canned fish by polyacrylamide disc electrophoresis. *Analyst.* 99: 609-611

Mackie, I.M. and Ritchie, A.H. 1981. Differentiation of Atlantic cod (*Gadus morhua*) and Pacific cod (*Gadus macrocephalus)* by electrophoresis and isoelectric focusing of water-soluble proteins of muscle tissue. *Comp. Biochem. Physiol.* 68B: 173-175

Mackie, I.M., Chalmers, M., Reece, P., Scobbie, A.E. and Ritchie, A.H. 1992. The application of electrophoretic techniques to the identification of species of canned tuna. *Pelagic Fish, the Resource and Its Exploitation* (eds. Burt, J.R., Hardy, R. and Whittle, K.W.). Fishing News Book, London, pp. 200-207

Mackie, I.M., Pryde, S.E., Sotelo, C.G., Medina, I., Martin, R., Quinteiro, J., Mendez, M. and Rehbein, H. 1999. Challenges in the identification of canned fish. *Trends in Fd. Sci. and Technol.* 10 (1): 9-14

*Martin, R.E. 1986. Developing appropriate nomenclature for structured seafood products. *Fd. Technol.* 40 (3): 127-130

*Martinez, I., Olsen, R.L., Ofstad, R., Janmot, C. and d'-Albis, A. 1989. Myosin isoforms in mackerel (*Scomber scombrus*) red and white muscles. *FEBS-LETT.* 252 (1-2): 69-72

*Martinez, I., Ofstad, R. and Olsen, R.L. 1990a. Electrophoretic study of myosin isoforms in white muscle of some teleost fishes. *Comp. Biochem. Physiol.* 96B: 221-227

*Martinez, I., Ofstad, R. and Olsen, R.L. 1990b. Intraspecific myosin light chain polymorphism in the white muscle of herring (*Clupea harengus harengus, L*) *FEBS-LETT.* 265: 23-26

Moosavi-Nasab, M., Alli, I., Isamail, A. and Ngadi, O. 2005. Protein structural changes during preparation and storage of surimi. *J. Fd. Sci.* 70 (7): 448-453

MPEDA. 2002. *An Illustrated Guide on Commercial Fishes and Shellfishes of India*. The Marine Products Export Development Authority, Kochi, 187 p.

Murray, R.K., Granner, D.K., Mayer, P.A. and Rodwell, V.W. 1993. *Harper's Biochemistry*. Prantice-Hall International Inc, New Jercey, pp. 806

*Nakagawa, T., Nagayama, F., Ozaki, H., Watabe, S. and Hashimoto, K. 1989. *Nippon Suisan Gakkaishi*. 55: 19-45

*Nakagawa, T. and Nagayama, F. 1989. *Nippon Suisan Gakkaishi*. 55: 165-171

Nippon Suisan Kaisha Ltd. 1980. *Standard procedure for quality evaluation of frozen surimi*. Tokyo, Japan, pp. 565

*Nishiokafujis and Shimizu, Y. 1979. *Bull. Jap. Soc. Sci. Fish.* 45 (12): 1557-1561

*Okada, M. 1974. *Fish meat paste products* (eds. Okada, M., Yokozeki, M. and Kinumaki, T.). Koseisha-koseikaku, K.K., Tokyo, pp. 162-180

Olsen, S.O., Steinsbo, E.E. and Skara, T. 2002. The influence of potential pre-treatment and processing parameters on general proteolytic activity characteristics in Atlantic salmon (*Salmo salar*), studied in model system. *J. Aquat. Fd. Prod. Technol.* 11 (3/4): 65-85

*Park, J.W., Lin, M. and Yongsawatdigul, J. 1997. New developments in manufacturing of surimi and surimi seafood. *Fd. Rev. Int.* 13: 577-610

Park, J.W. and Morrissey, M.T. 2000. Manufacturing of surimi from light muscle fish. *Surimi and Surimi Seafood* (ed. Park, W.). New York, pp. 23-58

Park, J.W., Yongsawatdigul, J. and Lin, M. 2005a. Surimi: Manufacturing and evaluation. *Surimi and Surimi Seafood* (ed. Park, W.). Second edition. Taylor & Francis Group, Boca Raton, pp. 33-106

Park, J.W. 2005b. Surimi seafood: Products, marketing, and manufacturing. *Surimi and Surimi Seafood* (ed. Park, W.). Second edition. Taylor & Francis Group, Boca Raton, pp. 375-434

*Patterson, R.L.S. 1985. *Biochemical Identification of Meat Species*. Elsevier Applied Science, London, 380 p.

Perez-mateos, M., Amato, P.M. and Lanier, T.C. 2004. Gelling properties of Atlantic croaker surimi processed by acid or alkaline solubilization. *J. Fd. Sci.* 69 (4): 328-333

Pineiro, C., Sotelo, C.G., Medina, I., Gallardo, M. and Martin, R.I. 1997. *Z. Lebensm- Unts- Forsch.* 204 (6): 1125-1128

Pineiro, C., Velazquez, B.J., Perez-Martin, R.I., Martinez, I., Jacobsen, T., Rehbein, H., Kundiger, R., Mendes, R., Etienne, M., Jerome, M., Craig, A., Mackie, I.M. and Jessen, F. 1999. Development of sodium dodecyl sulfate-polyacrylamide gel electrophoresis reference method for the analysis and identification of fish species in raw and heat-processed samples: A collaborative study. *Electrophoresis*. 20: 1425-1432

*Plummer, T. 1979. An Introduction to Practical Biochemistry. Second edition. Tata Mcgraw-Hill Publishing Company Ltd, New Delhi, 362 p.

Ramezani, J.T. 2006. Effect of chemically modified soy proteins and ficintenderized meat on the quality attributes of sausage. *J. Fd. Sci.* 68 (1): 85-88

*Rayment, I., Rypniewski, W.R., Schmidt-Base, K., Smith, R., Tomchick, D.R., Benning, M.M., Winkelmann, D.A., Wesenberg, G. and Holden, H.M. 1993. Three dimensional structure of myosin sub fragment-1. *A molecularmotor Sci.* 261: 50-65

*Regenstein, J. and Lanier, T.C. 1986. Surimi: Boon or Boondoggle? *Seafood Leader.* 6: 152-156

*Rehbein, H. 1990. Electrophoretic techniques for species identification of fishery products. *Z. Lebensum, Unters-Forsch.* 191: 1-10

Rehbein, H. 1992. Fish species identification by peptide mapping of the myosin heavy chain. *Electrophoresis.* 13: 805-806

*Rehbein, H., Etienne, M., Huttula, T., Knudsen, L.B., Jessen, F., Luten, J.B., Bouquet, W., Mackie, I.M., Ritchie, A.H., Martin, R. and Mendes, R. 1995. Influence of variation in methodology on the reliability of the isoelectric focusing method of fish species identification. *Fd. Chem.* 52: 193-197

Rehbein, H., Kundiger, R.,Yman, R.I.M., Ferm, M., Etienne, M., Jerome, M., Craig, A., Mackie, I., Jessen, F., Martienz, I., Mendes, R., Smelt, A., Luten, J., Pineiro, C. and Perez-Martin, R. 1999. Species identification of cooked fish by urea isoelectri focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis: A collaborative study. *Fd. Chem.* 67: 333-339

Rehbein, H. 2005. Electrophoretic techniques for species identification of fishery products. *Z. Lebensm- Unts- Forsch.* 191 (1): 1-10

*Roberts, P.C.B. and Lawrie . 1974. *J. Fd. Technol.* 9: 345-350

*Samejima, K., Hashimoto, Y., Yasin, T. and Fukazawa, T. 1969. *J. Fd. Sci.* 34: 242-247

Sano, T., Noguchi, S.F., Matsumoto, J. and Tsuchiya, T. 1989. Contribution of tropomyosin to fish muscle gei characteristics. *J. Fd. Sci.* 54: 258-265

Scobbie, A.E. and Mackie, I.M. 1988. The use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis in fish species identification- A procedure suitable for cooked and raw fish. *J. Sci. Fd. Agric.* 44: 343-357

Scobbie, A.E. and Mackie, I.M. 1990. The use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis in species identification of fish eggs. *Comp. Biochem. Physiol.* 96B (4): 743-746

Scobbie, A.E. and Mackie, I.M. 1995. The use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis to confirm the presence of salmon (*Salmo salar*) eggs in illegal fish bait. *Electrophoresis*. 16: 306-307

*Seki, N. 1976. Identification of fish species by SDS-polyacrylamide gel electrophoresis of the myofibrillar proteins. *Bull. Jap. Soc. Sci. Fish.* 48 (2): 1163-1176

Seki, N., and Arai, K. 1974. Gel filtration and electrophoresis of the myofibrillar proteins in the presence of sodium dodecyl sulfate. *Bull. Jap. Soc. Sci. Fish.* 40: 1187-1194

*Seki, N. 1977. *Proteins of Fish. Jap. Soc.Sci. Fish* (ed. Koseikaku, K.K.). Tokoyo, pp.7-23

Seki, N., Nakahara, C., Takeda, A., Maruyama, N. and Nozawa, H. 1998. Dimerization site of carp myosin heavy chains by the endogenous transglutaminase. *Fisheries Sci.* 64 (I): 314-319

*Seki, N., Takayasu, M. and Kokuryo, H. 1980. Preparation of myosin light chain as an index for identification of fish species from raw muscle and cooked products. *Bull. Jap. Soc. Sci. Fish.* 40 (8): 1001-1006

Sherief, P.M. 2003. Species Identification by Electrophoresis. *ICAR Sponsored short course on Taxonomy and Biodiversity of Fishes and Crustaceans* (eds. Jayachandran, K.V. and Nair, J.). Kerala Agricultural University, Kochi, pp.75-78

Singhal, R.S., Kulkarini, P.R. and Rege, D.V. 1997. *Handbook of Indices of Food Quality and Authenticity.* Woodhead Publishing, London. 560 p.

Smith, L., Hill, L., Lehman, I. and Lefkowitz, J. 1979. *Principle of Biochemistry : General aspects.* Seventh edition. McGraw-Hill Company, Tokyo, 886 p.

Sonu, C.S. 1986. *Surimi.* National Marine Fisheries Service, California.

Sotelo, C.G., Pineiro, C., Gallardo, J.M., and Perez-Martin, R.I. 1992. Identification of fish species in smoked fish products by electrophoresis and isoelectrofocusing. *Z. Forsch Lebensm. Unters.* 195: 224-227

Sotelo, C.G., Pineiro, C., Gallardo, J.M. and Perez-Martin, R.I. 1993. Fish species identification in seafood products. *Trends Fd. Sci. Technol.* 4: 395-401

*Sumner, J.L. and Mealy, S.W. 1983. A survey of substitution of fish fillets in Melbourne. *Fd. Technol.* 35: 332-334

Suzuki, T. 1981. *Fish and Krill Protein: Processing Technology.* Applied Science Publisher, London, 260 p.

*Tanford, C. 1968. Protein denaturation. *Adv. Prot. Chem.* 23: 121-125

*Takashi, R., Murozuka, T. and Arai, K. 1974. Light chains of myosin from fish dorsal and rabbit skeleted muscles. *Bull. Jap. Soc. Sci. Fish.* 40 (8): 1063-1069

*Thakker, K.D. and Grady, L.T. 1984. Solubility. *Food Analysis* (eds. Gruenwedel, D.W. and Whitaker, J.R.). Marcel Dekker, New York.

Theodore, A., Kristinssion, H.G. and Crynen, S. 2003. The effect of pH and salt on the gelation properties of catfish surimi and catfish protein isolates from acid and alkali-aided processing. IFT Annual Meeting. Chicago. *Abstract*: 76 A-10

Thompson, R.R. 1967. Disc Electrophoresis method for the identification of fish species . *J. Assoc. Off. Anal. Chem.* 50: 282-285

*Tiecco, G. 1980. Electrophoretic patterns of fresh and frozen meat. *Archivio Veterinario Italiano*. 31 (3/4): 108-110

*Torry Research Station. 1985. Internal Report.

Torry Research Station. 1986. Internal Report.

*Venugopal, V., Alur, M.D. and Lewis, N.F. 1983. Extracellular protease from Pseudomonas marinoglutinosa: Some properties and its action on fish actomyosin. *J. Fd. Sci.* 48: 671-676

*Wahyuni, M., Ishizaki, S. and Tanaka, M. 1998. Improvement of thermal stability of fish water soluble proteins with glucose-6-phosphate through the maillard reaction. *Fisheries Sci.* 64 (6): 973-978

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determination by dodecyl sulfate–polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244: 4406-4412

Wei, C.I., An, H., Chen, J. and Marshall, M.R. 1990. Use of a modified urea gel isoelectri focusing method for species identification of raw or broiled white, pink and rock shrimp. *J. Fd. Biochem.* 14: 91-102

*Xiong, L.Y. 1997. Structure function relationships of muscle proteins. *Food Proteins and their Applications* (Damodaran, S. and Parant, A.). Marcel Dekker Inc. New York, pp. 341-386

*Yman, I.M. 1992. Meat and fish species identification. *European Food and Drink Review*, Autumn, 381 p.

Yowell, K. and Flurkey, W.H. 1986. Effect of freezing and microwave heating on proteins from cod fish fillets: Analysis by SDS polyacrylamide gel electrophoresis. *J. Fd. Sci.* 51: 508-509

* Not referred original paper

ABSTRACT

STUDIES ON ELECTROPHORETIC IDENTIFICATION OF FISH SPECIES USED IN SURIMI (PRODUCTS) AND THEIR QUALITY EVALUATION

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Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Fisheries Science

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2008

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8. ABSTRACT

SDS-PAGE pattern of water soluble proteins and salt soluble proteins of raw fillets, surimi, sausage and different combinations (1:1; 3:1; 1:3; and 4:1) of surimi mixtures; sausage mixtures from the two species of fish, threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur*) were studied to see that whether these pattern are useful in identifying the species used for the preparation of surimi and sausage; and their quality evaluation.

SDS-PAGE gave the effective species-specific pattern of raw fillets, surimi, sausage and surimi mixtures; sausage mixtures of extract of water and salt soluble proteins of threadfin bream and bulls-eye separately.

Threadfin bream and bulls-eye were effectively identified by using their SDS-PAGE pattern of extract of water soluble proteins and salt soluble proteins separately.

Threadfin bream and bulls-eye surimi and sausage were also effectively identified by comparing their SDS-PAGE pattern with species-specific SDS-PAGE pattern in their extract of water soluble proteins and salt soluble proteins separately.

The SDS-PAGE pattern of water soluble proteins and salt soluble proteins were shown in this study to be effective in distinguishing the species specificity between threadfin bream and bulls-eye of raw fish fillets, surimi and sausage samples.

Adultered/substituted species in the different mixture were identified by comparing their mixture SDS-PAGE pattern with species-specific SDS-PAGE pattern in their extract of water soluble proteins and salt soluble proteins separately.

Different mixtures species-specific SDS-PAGE protein patterns can be used with reservation to detect/differentiate the adulteration/substitution in the fish mixtures, surimi and sausage when these two species mixed with the combinations of 1:1; 3:1; 1:3 and 4:1 ratios.

The quality evaluation study showed that surimi, sausage and surimi mixtures; sausage mixtures of threadfin bream and bulls-eye used for the experiment were of good quality.

APPENDIX

APPENDIX I

Scale for grade and number impurities of frozen surimi

Grade	Impurities	Grade	Impurities
10	0	5	12-15
9	1-2	4	16-19
8	3-4	3	20-25
7	5-7	2	26-30
6	8-11	1	>30

APPENDIX II

Grade sheet for folding test of sausage

Grade	Condition
AA	No crack showing after folding twice
A	No crack showing after folding in half
B	Cracks gradually when folded in half
C	Cracks immediately when folded
D	Breaks by finger pressure

APPENDIX III

Score sheet for sensory (biting) test of sausage

Points	Structural strength	Points	Structural strength
10	Extremely strong	5	Slightly weak
9	Very strong	4	Weak
8	Strong	3	Very weak
7	Slightly strong	2	Extremely weak
6	Fair	1	Fragile