

Electrophoretic Identification of Fish Species Used in Surimi (Products) and their Quality Evaluation

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ABSTRACT

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify species-specific water soluble proteins and salt soluble proteins bands 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*). 7.5% SDS was most effective in distinguishing the species-specificity between threadfin bream and bulls-eye of raw fish, surimi, sausage and different combinations of surimi mixtures; sausage mixtures.

Keywords: adulteration/substitution, bulls-eye, electrophoresis, sausage, threadfin bream

Abbreviations: **BF**, bulls-eye fillet; **BS**, bulls-eye surimi; **FDA**, food and drug administration; **MW**, molecular weight; **SDS-PAGE**, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **Std**, standard; **TF**, threadfin bream fillet; **TS**, threadfin bream surimi

INTRODUCTION

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. Due to its light colour, bland odour, and unique gelling properties 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 the 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 to detect the substituted species by species-specific zones when adulteration or substitution takes place. 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).

Electrophoretic system is also useful to detect the mis-labeling of the content of the specific seafood components (minced meat, surimi and canned foods). This is extremely useful 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 threadfin bream and bulls-eye raw fillets, surimi and sausage; identification of adulterated/substituted species in surimi mixtures; sausage mixtures by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and quality evaluation of prepared surimi and sausage.

MATERIALS AND METHODS

All chemicals and reagents used throughout the study were HPLC or analar grade obtained from Sigma-Aldrich, St. Louis, MI, USA.

Threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur*), the two most commonly utilized species in surimi manufacturing in tropical countries including India (Park *et al.* 2005), were used. Fishes were obtained from the Cochin fishing harbour. Surimi, sausage and different combinations (1:1; 3:1; 1:3; and 4:1) of surimi mixtures; sausage mixtures from threadfin bream and bulls-eye were prepared following a procedure described by Suzuki (1981). Surimi samples were stored at -30°C. The sausage samples were over wrapped to prevent drying and kept in chill store at 10°C overnight.

Protein extraction

1. Water-soluble proteins

Minced meat/frozen surimi/sausage (5 g) was mixed and homogenized with cold distilled water (10 ml) for 2 min in a mortar and pestle. The suspension was then centrifuged at $2000 \times g$ for 10 min at 4°C , the supernatant were collected.

2. Salt-soluble proteins

The residue of salt-soluble protein was mixed with Dyer's buffer (40 ml; 5% NaCl in 0.02 M NaHCO_3 , pH 7.0 and stirred using a magnetic stirrer for 1 h at low temperature. The suspension was then centrifuged at $10,000 \times g$ for 20 min, supernatant were collected and made up with the same buffer.

SDS-PAGE

SDS-PAGE was performed according to a modified procedure of Laemmli (1970). Using a Protean II (vertical slab) unit (Bio-Rad), slab gels consisted of a running (7.5%) which was polymerized overnight, and a stacking (4%) gel which was poured 2 h before sample application. The protein samples were run at a constant current of 200 V and protein migrates on the electrical field was indicated by the bromophenol blue added in the sample buffer. Following electrophoresis, the proteins were stained with Coomassie Brilliant Blue (R-250) for 30 min and then destained in 7% acetic acid. The gel was photo documented using a Chemi Doc XRS camera (Bio-Rad., Via Cellini, Italy). 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 Geni., Bangalore, India). Molecular weight of protein was determined by using software, Quantity One.

Quality evaluation

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

Surimi: Moisture content, pH and impurities of surimi were evaluated by using frozen surimi (50 g) on a two-sample basis.

Sausage: Folding test and sensory test of sausage were conducted by the method described by Suzuki (1981). Gel strength of sausage was measured by the method described by Okada (1974), gelled samples were cut into cylindrical slices (25 mm long), and film was removed. Due to weight of water, plunger penetrated into gels, stress-strain curve was drawn on the kymograph paper. Three or more slice pieces of the same inspection sample were evaluated.

RESULTS AND DISCUSSION

Quality evaluation of surimi and sausage

Moisture contents of surimi varied from 75-79.80%; pH varied 6.06-6.90 and No. of impurities various from 11-19. Threadfin bream, bulls-eye and different combinations (1:1; 3:1; 1:3; and 4:1) of mixed sausage had good elasticity and gel strength as shown by sensory score and folding test values. 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 surimi and sausage samples were of good quality.

Electrophoretic pattern of water-soluble proteins

1. Intact fish and surimi

SDS-PAGE was shown to be effective in demonstrating species differences in water soluble proteins patterns of raw fish and surimi samples (Fig. 1). Using SDS-PAGE, TF showed characteristic bands with MW's of 203.3, 26, 19.4, 18.5 and 14.5 kD while the BF showed specific bands with MW's of 25.7, 19.7, 18.4, 17.7 and 14 kD. All these species-specific bands were found in the fish and their surimi samples (Fig. 1). New bands with molecular weights of 93.6 and 22.3 kD; 63.8 kD were found in TS and 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 (An *et al.* 1989) and enhancement of protein extraction by SDS by solubilizing membrane proteins (Copper 1997): residual proteolytic enzyme activity may together contribute to the appearance of the new bands. Appearances of new bands in surimi due to proteolytic activity were reported in pink and rock shrimp (An *et al.* 1988), Alaska pollock and red hake (An *et al.* 1989), cod and blue whiting (Rehbein 1992), Atlantic croaker (Perezmateos *et al.* 2004) and Alaska pollock (Moosavi-nasab 2005).

Bands with MW's of 93.6, 63.8, 22.3, 19.7, 17.7 and 14.5 kD were found to be common to all the combinations of TS + BS mixture. Among these 93.6, 22.3 and 14.5; 63.8, 19.7 and 17.7 kD were found in threadfin bream and bulls-eye surimi respectively. These bands can be used with reservation to identify the TS + BS mixture when they are mixed with different combinations of 1:1; 3:1; 1:3; 4:1 ratios.

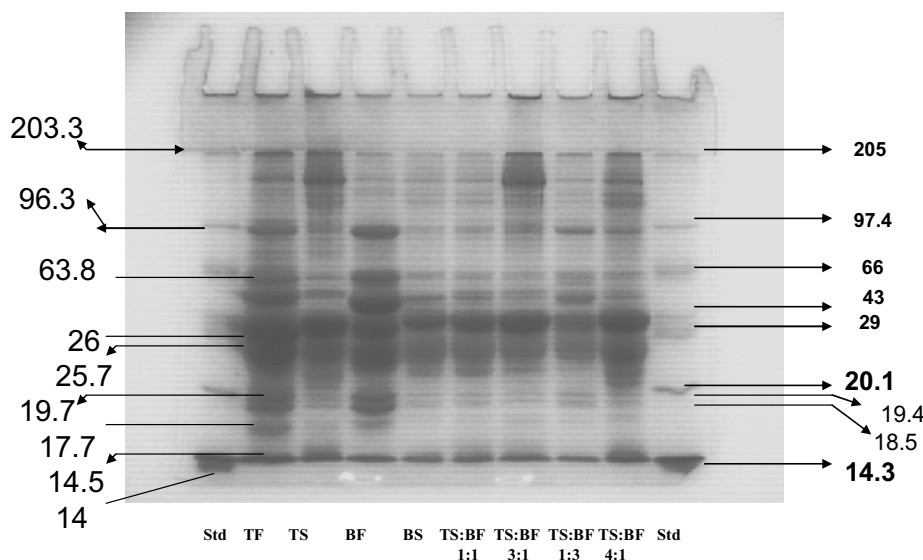


Fig. 1 SDS-PAGE pattern of water soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; TS: threadfin bream surimi; BF: bulls-eye fillet; BS: bulls-eye surimi.

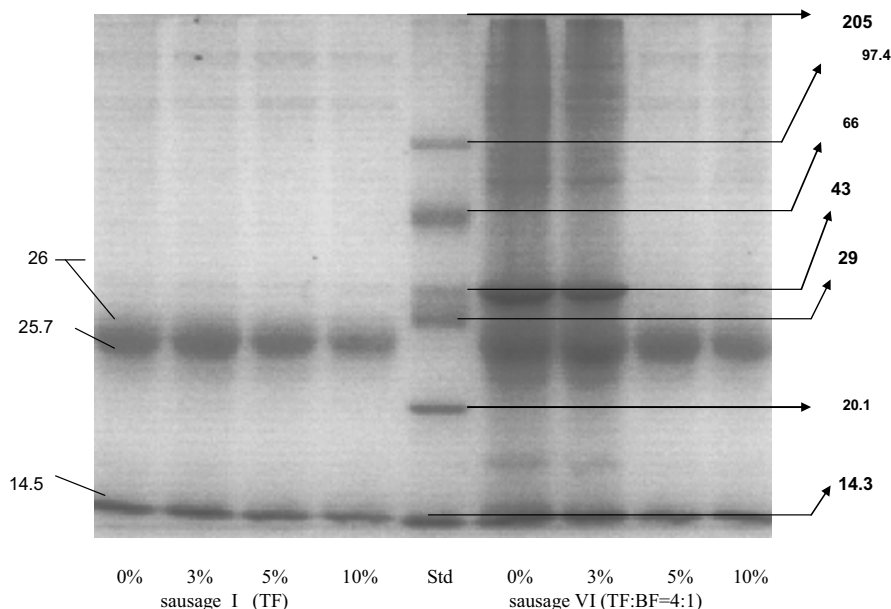


Fig. 2 SDS-PAGE pattern of water soluble proteins of sausage I (TF) and VI (TF:BF = 4:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.

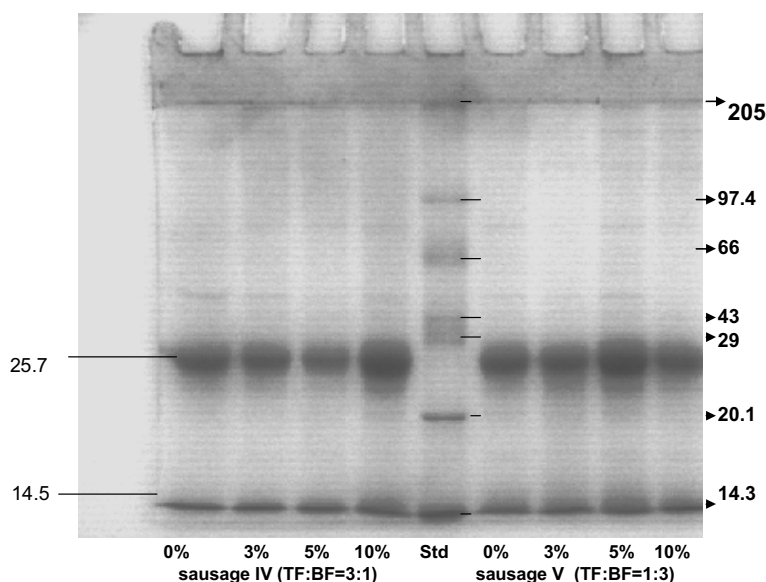


Fig. 3 SDS-PAGE pattern of water soluble proteins of sausage II (BF) and III (TF:BF = 1:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.

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; Park *et al.* 2005a) which may lead to disappearance of these bands.

2. Sausage

Using SDS-PAGE, TF sausage showed characteristic strong bands with MW's of 26 and 14.5 kD while the BF sausage showed specific bands with MW's of 25.7 and 14 kD. All these species-specific bands were common to the different starch concentration (0, 3, 5 and 10%) of sausage and also found in the fish and their surimi samples (Figs. 2, 3). Bands with MW's of 203.3, 19.4 and 18.5 kD of TS; and the 19.7, 18.4 and 17.7 kD of BS, 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 (An *et al.* 1988, 1989), and also due to release of renatured internal proteases (Erikson *et al.* 1983; Venugopal *et al.* 1983). Yowell

and Flurkey (1986) reported that 76% of water-soluble proteins are decreased from heated samples when compared to fresh one (Mackie 1968; Roberts and Lawrie 1974; Nishioaka and Shimizu 1979; Davis and Anderson 1984). Bands with MW's of 25.7 and 14.5 kD were found common to all different combinations (1:1; 3:1; 1:3; 4:1) of TF + BF sausage mixture (Figs. 2-4) and also found in the BS and TS, respectively. These bands can be used with reservation to identify the TF and BF sausage mixture when they are mixed with different combinations of 1:1; 3:1; 1:3; 4:1 ratios. 8% gel of SDS-PAGE has been successfully used for the identification of Atlantic croaker in their water soluble protein extracted from sausage heated at 30°C, 40°C and 90°C were reported by Perez-Mateos *et al.* (2004). SDS-PAGE with 15% gel was used to identify raw, frozen and batter-coated squid rings with clear bands in their water soluble protein extract heated at the temperature of 180°C/3 min (Llorca *et al.* 2007). Water soluble protein extract from Alaska Pollock and Pacific whiting surimi crab stick and their mixture was identified and detected using SDS-PAGE (Reed and Park 2008).

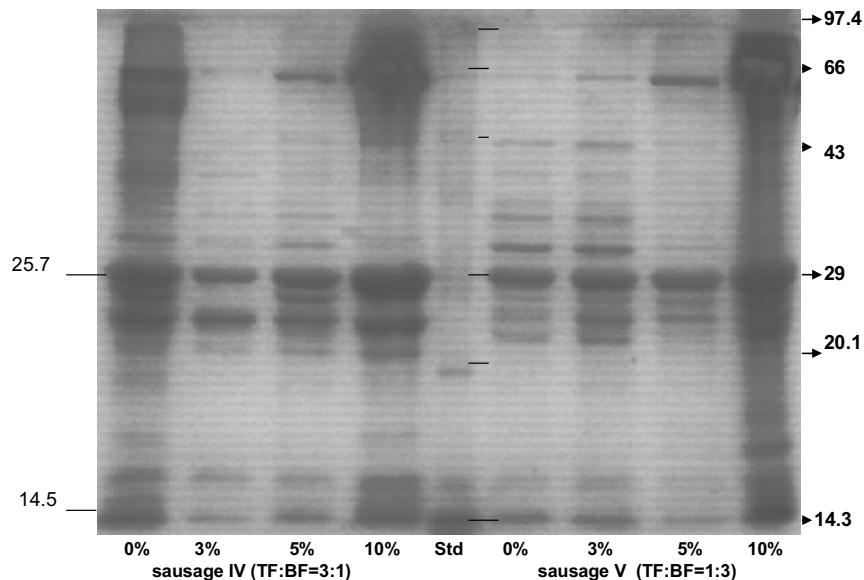


Fig. 4 SDS-PAGE pattern of water soluble proteins of sausage IV (TF:BF = 3:1) and V (TF:BF = 1:3) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.

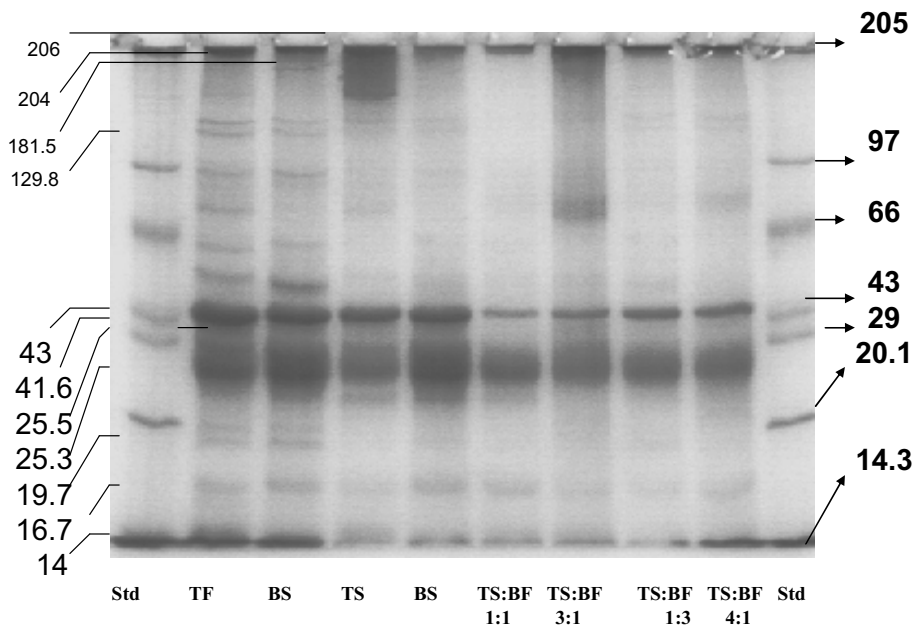


Fig. 5 SDS-PAGE pattern of salt soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; TS: threadfin bream surimi; BF: bulls-eye fillet; BS: bulls-eye surimi.

Electrophoretic pattern of salt soluble proteins

1. Intact fish and surimi

Using SDS-PAGE, TF showed characteristic myofibrillar protein bands with MW's of 204, 181.5, 43 and 14.3 kD while the BF showed characteristic bands with MW's of 206, 129.8, 25.5, 19.7, 16.7 and 14 kD. All these species-specific bands were found in the fish and their surimi samples (Fig. 5). Some of the species-specific bands, such as the 26 kD of TF and 42.3 kD of BF were missing in the respective surimi (Fig. 5). This may be due to the loss of myofibrillar proteins during the successive water leaching process (Lin and Park 2005; Moosavi-nasab 2005; Park *et al.* 2005a). New bands of 25.3 and 41.6 kD were found in threadfin bream and bulls-eye surimi respectively, also in all TS + BS mixtures (Fig. 5) and these bands can be used with reservation to differentiate surimi mixtures. Chen (1979); Perez-Mateos *et al.* (2004) reported that significant myosin degradation of surimi with NaCl, which leads to increase in protein bands. All the combination of TS + BS mixture had common bands of 204, 41.6, 25.3 and 14.3 kD

(Fig. 5). Among these bands 204, 25.3, and 14.3, 41.6 kD were also found in TS and BS, respectively, and 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. Various combinations of mixtures myofibrillar proteins of pink and rock shrimp using 10.4% SDS-PAGE (An *et al.* 1988); Alaska pollock and red hake mixtures using 10.4% SDS-PAGE and isoelectric focusing techniques for traceability study (An *et al.* 1989; Dreyfuss *et al.* 2006); for surimi-based products (Pepe *et al.* 2007) were successfully reported. Liu *et al.* (2009) successfully used 7.5% SDS-PAGE for the identification of protein extract from raw and cooked meat (40, 50 and 60°C) of triangle shell pearl mussel.

2. Sausage

Species-specific myofibrillar protein bands with MW's of TF sausage; BF sausages were 25.3 kD; 25.5 and 19.7 kD were found in their SDS-PAGE, respectively (Fig. 6). All these species-specific bands were found in their surimi samples and also in different starch concentration (0, 3, 5 and

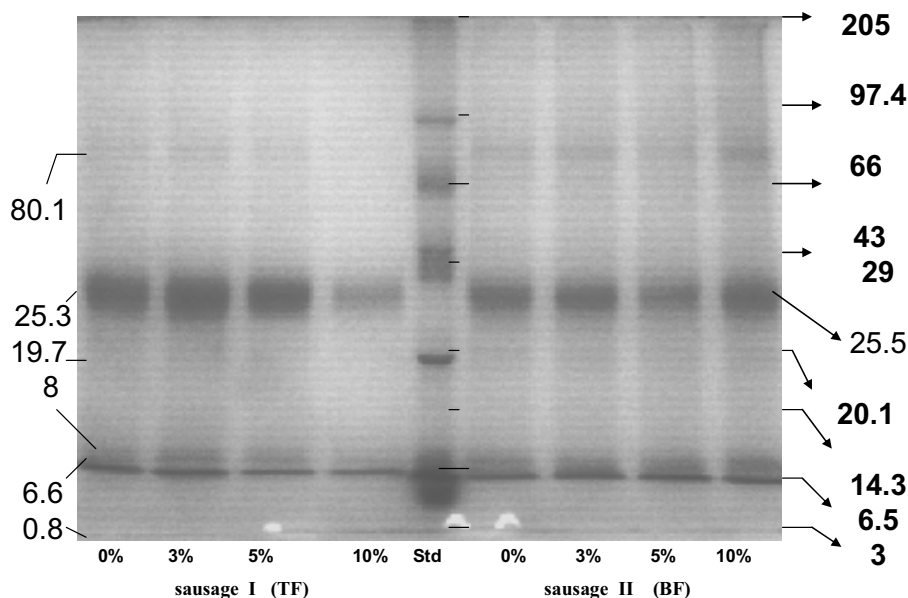


Fig. 6 SDS-PAGE pattern of salt soluble proteins of sausage I (TF) and II (BF) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.

10%) of sausage. New bands such as, 8 and 6.6 kD in TF sausage and 80.1 and 0.8 kD in BF sausage (**Fig. 6**) were found. This may be due to some of the relative percent of the minor proteins had been increased after washing due to the loss of major proteins in surimi gel samples from Alaska pollock and red hake (An *et al.* 1989); in chicken surimi prepared from spent hen breast muscle with 3 washing cycle (Kang *et al.* 2009) and enhancement of protein extraction from the cooked samples by SDS by solubilizing membrane proteins (Copper 1997). Residual proteolytic enzyme activity may also together contribute to the appearance of the new bands. Appearances of new bands in surimi gel due to proteolytic activity were reported in cod (Yowell and Flurkey 1986), Alaska pollock (Moosavinasab 2005) and protein extract of surimi gel from pork and beef (Park *et al.* 2006). Hamm and Hofmann (1965) and Itoh *et al.* (1980, 1980a) reported that at high temperature (<70°C) myosin molecules dissociated from actin, protein aggregation takes place which reduces the solubility of myofibrillar protein which in turn leads to loss of protein bands. Bands with MW's of 204, 181.5, 43 and 14.3 kD of threadfin bream surimi and the 206, 129.8, 16.7 and 14 kD of bulls-eye

surimi, were missing in the corresponding sausage samples (**Fig. 6**). Loss of myofibrillar protein bands also attributed to the extracellular protease activity (Venugopal *et al.* 1983; Yowell and Flurkey 1986; Llorca *et al.* 2007); the addition of starch also caused the disappearance of protein bands in bulls-eye, croaker and threadfin bream sausage samples using SDS-PAGE (Benjakul *et al.* 2002; Elton and Ewart 2006); in sausage samples of soy proteins and meat were reported (Ramezani *et al.* 2006) and sausage samples of Atlantic salmon and rainbow trout using capillary electrophoresis were reported by Acuria *et al.* (2008). Addition of starch at different concentration (12%, 11%, 10% and 9%) in Alaska pollock and Pacific whiting surimi crabstick and sausage caused disappearance of SDS-PAGE protein bands (Reed and Park 2008).

All TF + BF sausage mixtures had common bands with MW's of 25.5 and 14.3 kD, among these 25.5 and 14.3 kD were also found in the BS and TS, respectively (**Figs. 7, 8**). These unaltered species-specific bands can be used to identify the TF + BF sausage mixture when they are mixed with different combinations of 1:1; 3:1; 1:3; and 4:1. SDS-PAGE has been used successfully to detect the adulterated/substi-

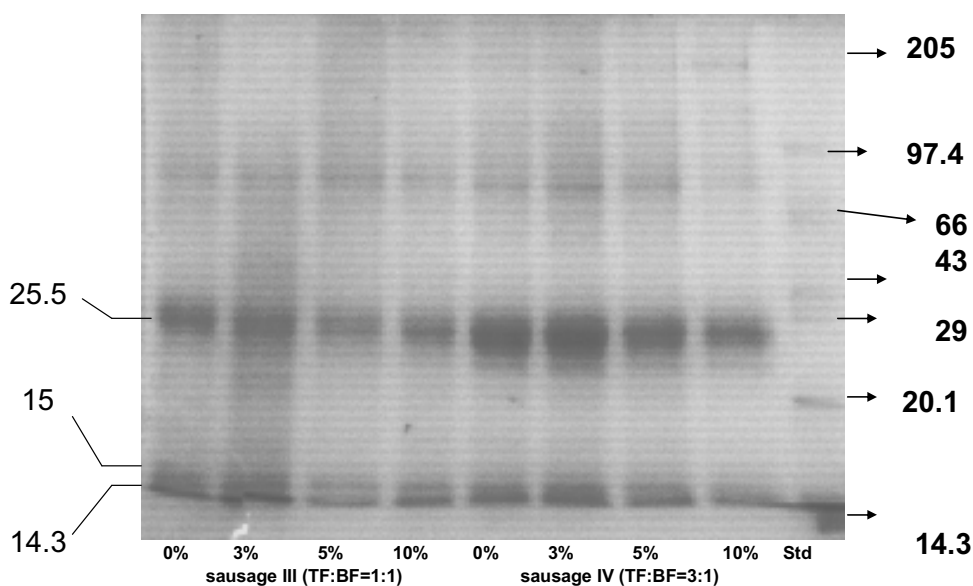
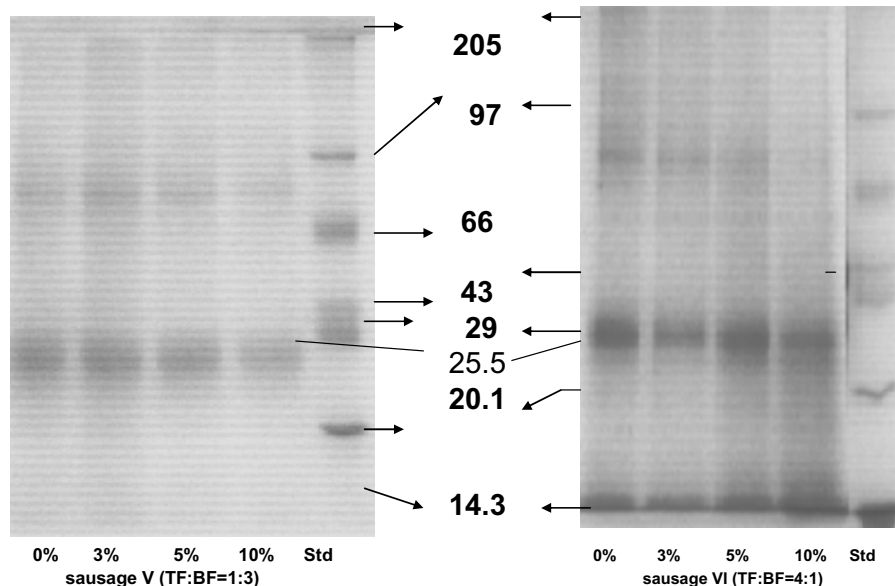


Fig. 7 SDS-PAGE pattern of salt soluble proteins of sausage III (TF:BF = 1:1) and IV (TF:BF = 3:1) with different starch concentration. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.



Figs. 8 and 9 SDS-PAGE pattern of salt soluble proteins of sausage V (TF:BF = 1:3) and VI (TF:BF = 4:1) with different starch concentrations, respectively. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; BF: bulls-eye fillet.

tuted species in sausage and their various mixtures of myofibrillar protein extract from cod, blue whiting, sardine and Alaska pollock by Torry Research Station (1986) and Rehbein (1992). 10.4% SDS-PAGE were found to be more useful for species identification and detection of adulteration in surimi products from pink and rock shrimp (An *et al.* 1988). An *et al.* (1989) successfully used both SDS-PAGE and isoelectric focusing for traceability studies of different combinations of raw fish, surimi and surimi-based products from Alaska pollock and red hake. SDS-PAGE with 15% gel was used to identify raw, frozen and different forms of battered squid rings with clear bands in their salt soluble protein extract heated at the temperature of 180°C/3 min (Llorca *et al.* 2007). SDS-PAGE was used successfully not only for the species identification and also for detection with the accuracy level of 98% of species substitution/adulterant used in the surimi crabstick and sausage prepared from the Alaska pollock and Pacific whiting with different combination were reported by Reed and Park (2008).

CONCLUSION

A good method for the identification of the species origin of surimi and surimi products (sausage) has not been reported in the literature (An *et al.* 1988). There is no appropriate/standard study to detect/trace the adulterated/substituted species in adulterated surimi.

The SDS-PAGE was shown in this study to be effective in distinguishing the species-specificity between threadfin bream and bulls-eye of raw fish, surimi, sausage and different combinations (1:1; 3:1; 1:3; and 4:1) of surimi mixtures; sausage mixtures. These different mixtures species-specific SDS-PAGE protein patterns appears to have potential application to detect/differentiate the adulteration/substitution in the fish mixtures, surimi and sausage when they are prepared from these two species with the combinations of 1:1; 3:1; 1:3; and 4:1 ratios. Findings of this research show that this study can be applied to detect/trace the adulterated/substituted species in adulterated surimi from threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur*) at different combinations (1:1; 3:1; 1:3; and 4:1). Findings of this research show that traceability/detection of adulteration is possible even in very low and higher levels of adulterant used in the surimi.

REFERENCES

Acuria G, Ortiz-Riario E, Vinagre J, Garcia L, Kettlun AM, Puente J, Collados L, Valenzuela MA (2008) Application of capillary electrophoresis

- for the identification of Atlantic salmon and rainbow trout under raw and heat treatment. *Journal of Capillary Electrophoresis and Microchip Technology* **10**, 93-99
- Alvarez C, Couso I, Tejada M (1995) Sardine surimi gel as affected by salt concentration, blending, heat treatment and moisture. *Journal of Food Science* **60**, 622-626
- An H, Marshall MR, Otwell WS, Wei CI (1988) Electrophoretic identification of raw and cooked shrimp using various protein extraction systems. *Journal of Food Science* **53**, 313-318
- An H, Marshall MR, Otwell WS, Wei CI (1989) Species identification of raw and cooked shrimp by urea gel isoelectric focusing techniques. *Journal of Food Science* **54**, 233-236
- An H, Wei CI, Zhao J, Marshall MR, Lee CM (1989) Electrophoretic identification of fish species used in surimi products. *Journal of Food Science* **54**, 253-257
- Benjakul S, Visessanguan W, Riebroy S, Ishizaki S, Tanaka M (2002) Gel-forming properties of surimi produced from bigeye snapper (*Priacanthus tayenus*) and (*P. macracanthus*) stored in ice. *Society of Chemical Industry* **60**, 412-416
- Cheng CS, Hamann DD, Webb NB (1979) Effect of thermal processing on minced fish gel texture. *Journal of Food Science* **44**, 1080-1086
- Copper TG (1997) *The Tools of Biochemistry*, John Wiley and Sons, New York, 381 pp
- Cowie WP (1968) Identification of fish species used by thin slab polyacrylamide gel electrophoresis. *Journal of the Science of Food and Agriculture* **19**, 226-229
- Davis CE, Anderson JG (1984) Size exclusion/HPLC of heated water soluble bovine and porcine muscle proteins. *Journal of Food Science* **49**, 598-602
- Davies G, Stark G (1970) Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. *Proceedings of the National Academy of Sciences USA* **66**, 651-656
- Devadasan K (2002) Proteins and lipids of marine products and their changes during processing and preservation. In: Gopakumar K (Ed) *Textbook of Fish Processing Technology*, Indian Council of Agricultural Research, New Delhi, pp 122-158
- Dreyfuss S, Cutrafelli E, Mageau P, McNamara M (2006) Agar-gel immunodiffusion test for rapid identification of Pollock surimi in raw meat products. *Journal of Food Science* **62**, 972-975
- Elton H, Ewart JAD (2006) Effect of starch in the protein mobility and their changes in the protein pattern. *Journal of the Science of Food and Agriculture* **13**, 62-72
- Erickson MC, Gordon DT, Anglemier AF (1983) Proteolytic activity in the sarcoplasmic fluids of parasitized Pacific Whiting (*Merluccius productus*) and unparasitized true cod (*Godus macrocephalus*). *Journal of Food Science* **48**, 1315-1320
- FAO (2005) *Codex Committee on Fish and Fishery Products*, FAO, Rome, 57 pp
- Hamm R, Hofmann K (1965) Changes in the sulfhydryl and disulfide groups in beef muscle protein during heating. *Nature* **207**, 1269-1274
- Hultin O, Kristinsson G, Lanier C, Park W (2005) Process for recovery of functional proteins by pH shifts. In: Park W (Ed) *Surimi and Surimi Seafood* (2nd Edn), Taylor and Francis Group, Boca Raton, pp 107-139
- Itoh Y, Yoshinaka R, Ikeda S (1980a) Changes to higher molecular weight of protein molecules during the gel formation of carp actomyosin by heating

- and participation of SH groups in these changes. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 617-620
- Itoh Y, Yoshinaka R, Ikeda S** (1980b) Formation of polymeric molecules of protein resulting from intermolecular SS bonds formed during gel formation of carp actomyosin by heating. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 621-624
- Kang GH, Kim SH, Kim JH, Kang HK, Kim DW, Na JC, Yu DJ, Suh OS, Choi YH** (2009) Effects of washing methods on gel properties of chicken surimi prepared from spent hen breast muscle. *Poultry Science* **88**, 1438-1443
- Kurth L, Shaw FD** (1983) Identification of the species of origin of meat by electrophoretic and immunological methods. *Food Technology* **35**, 328-331
- Laemli UK** (1970) Cleavage of structural proteins assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
- Lee CM** (1984) Surimi process technology. *Food Technology* **38**, 69-80
- Lee CM** (1986a) Surimi manufacturing and fabrication of surimi-based products. *Food Technology* **40**, 115
- Liu C, Zhou H, Su Y, Li Y, Li J** (2009) Chemical compositions and functional properties of protein isolated from by-product of Triangle shell pearl mussel *Hyriopsis cumingii*. *Journal of Aquatic Food Product Technology* **18**, 193-208
- Llorca E, Hernando I, Perez-Munuera I, Quiles A, Larrea V, Lluch MA** (2007) Protein breakdown during the preparation of frozen batter-coated squid rings. *European Food Research Technology* **225**, 807-813
- Mackie IM** (1968) Identification of fish species by a modified polyacrylamide disc electrophoresis technique. *Analyst* **93**, 458-463
- Martin RE** (1986) Developing appropriate nomenclature for structured seafood products. *Food Technology* **40**, 127-130
- Moosavi-Nasab M, Alli I, Ismail A, Ngadi O** (2005) Protein structural changes during preparation and storage of surimi. *Journal of Food Science* **70**, 448-453
- Okada M** (1974) *Fish Meat Paste Products*, Koseisha-koseikaku KK, Tokyo, 180 pp
- Park JW, Yongsawatdigul J, Lin M** (2005a) Surimi: Manufacturing and evaluation. In: Park W (Ed) *Surimi and Surimi Seafood* (2nd Edn), Taylor and Francis Group, Boca Raton, pp 33-106
- Park JW** (2005b) Surimi seafood: Products, marketing, and manufacturing. In: Park W (Ed) *Surimi and Surimi Seafood* (2nd Edn), Taylor and Francis Group, Boca Raton, FL, pp 375-434
- Park S, Brewer MS, McKeith FK, Bechtel PJ, Novakofski J** (2006) Salt, cryoprotectants and preheating temperature effects on surimi-like material from beef or pork. *Journal of Food Science* **61**, 790-795
- Pepe T, Trotta M, Marco ID, Anastasio A, Bautista JM, Cortesi ML** (2007) Fish species identification in surimi-based products. *Journal of Agricultural Food Chemistry* **55**, 3681-3685
- Perez-Mateos M, Amato PM, Lanier TC** (2004) Gelling properties of Atlantic croaker surimi processed by acid or alkaline solubilization. *Journal of Food Science* **69**, 328-333
- Ramezani JT** (2006) Effect of chemically modified soy proteins and ficin-tenderized meat on the quality attributes of sausage. *Journal of Food Science* **68**, 85-88
- Reed ZH, Park JW** (2008) Qualification and quantification of fish protein in prepared surimi crabstick. *Journal of Food Science* **73**, 329-334
- Rehbein H** (1990) Electrophoretic techniques for species identification of fishery products. *Zeitschrift für Lebensmittel – Untersuchung und Forschung* **191**, 1-10
- Rehbein H** (1992) Fish species identification by peptide mapping of the myosin heavy chain. *Electrophoresis* **13**, 805-806
- Suzuki T** (1981) *Fish and Krill Protein: Processing Technology*, Applied Science Publisher, London, 260 pp
- Torry Research Station** (1986) *Internal Report, Fish Processing and Handling*, London, 389 pp
- Venugopal V, Alur MD, Lewis NF** (1983) Extracellular protease from *Pseudomonas marinoglutinosa*: Some properties and its action on fish actomyosin. *Journal of Food Science* **48**, 671-676
- Weber K, Osborn M** (1969) The reliability of molecular weight determination by dodecyl sulfate–polyacrylamide gel electrophoresis. *Journal of Biological Chemistry* **244**, 4406-4412
- Yowell K, Flurkey WH** (1986) Effect of freezing and microwave heating on proteins from cod fish fillets: Analysis by SDS polyacrylamide gel electrophoresis. *Journal of Food Science* **51**, 508-509