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Electrophoretic identification of threadfin bream, bulls-eye and their Surimi

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ABSTRACT
Objectives: A good method for the identification of the species origin of surimi has not been reported in the literature (An *et al*., (1988). Identification of adulterated/substituted species in surimi mixtures threadfin bream and bulls-eye raw fish by Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE).
Methodology and results: SDS-PAGE was performed using threadfin bream and bulls-eye raw fillets (obtained from Cochin fishing harbour, kerala, India); their surimi and different combinations (1:1; 3:1; 1:3; and 4:1) of their surimi mixtures. 7.5% SDS was more effective in distinguishing the species in both raw fish fillets and surimi.
Conclusions and application of findings: SDS-PAGE was shown in this study to be effective in distinguishing the species between threadfin bream and bulls-eye of raw fish, surimi and different combinations (1:1; 3:1; 1:3; and 4:1) of surimi mixtures. The SDS-PAGE protein patterns have potential application in detecting/differentiating the adulteration/substitution in the fish mixtures and surimi when they are prepared from these two species in the tested combinations.

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 led to an increasing demand for methods to identify the species of fish or other seafood in the market place (An *et al.,* 1988). 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 is made-up of 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.

The finished products must be labeled properly to meet the Food and Drug Administration (FDA) guidelines which reflect the nature of the products. 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, i.e. chemical, electrophoretic and immunological methods (Kurth & Shaw, 1983).

Electrophoretic patterns of the muscle myogens of fish have been found to be characteristic of the species. These species specific patterns remain unaltered during processing and preservation, and are thus 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)

Adulteration by substituting lower quality and/or lower priced seafood products for higher priced products is easy in the case of raw and cooked fish products such as surimi, kamaboko, fish sausage and canned flesh (An *et al.,* 1988). Therefore identification of species used for the preparation of surimi is highly necessary. Threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur*) are the two most commonly utilized species in surimi processing in tropical countries (Park et al, 2005a). Therefore, the objectives of this study were to identification of threadfin bream and bulls-eye raw fillets and surimi, and detection/identification of adulterated/substituted species in surimi mixtures by SDS PAGE.

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Threadfin bream (*Nemipterus japonicus*)

Bulls-eye (*Priacanthus hamrur)*,

MATERIALS AND METHODS

Threadfin bream (*Nemipterus japonicus*), and bulls-eye (*Priacanthus hamrur)*, were used. The fishs were obtained from the Cochin fishing harbour, kerala, India . Surimi and different combinations (1:1; 3:1; 1:3; and 4:1) of surimi mixtures from threadfin bream and bulls-eye were prepared following the procedure described by Suzuki (1981). Surimi samples were stored at -30ºC. for the period of 7 days.

Protein extraction:

Water soluble proteins: Minced meat/frozen surimi (5g) was mixed and homogenized with cold distilled water (10ml) for 2 min using mortar and pestle. The suspension was then centrifuged at 2000x g for 10 min at 4ºC, and the supernatant was collected.

Salt soluble proteins: The residues of water soluble protein were mixed with Dyer's buffer (40ml; 5% NaCl in 0.02*M* $NaHCO_3$, pH 7.0) and stirred using a magnetic stirrer for 1 h. at low temperature of 4ºC . The suspension was then centrifuged at 10,000 x g for 20 min, supernatant was collected and made up to 50ml with the same buffer.

Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was performed according to the modified procedure of Laemmli (1970) using a Protean II (vertical slab) unit (Bio-Rad). Slab gels consisted of a running gel (7.5%) which was polymerized overnight, and a stacking gel (4%) which was poured 2h before sample application.

The protein samples were run at a constant current of 200V and protein migration 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. 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 the software, Quantity One, Bio-Rad Laboratories, Hercules, California, U.S.A.

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RESULTS AND DISCUSSION

Electrophoretic pattern of water soluble proteins

Intact fish and Surimi: SDS-PAGE was shown to be effective in differentiating between species using water soluble protein patterns of raw fish and surimi samples (Fig. 1). Threadfin bream (TF) showed characteristic bands with MW's of 203.3, 26, 19.4, 18.5 and 14.5 kD while the bulls-eye (BF) showed specific bands with MW's of 25.7, 19.7, 18.4, 17.7 and 14 kD. The difference in the distance between the bands is not so narrow except 18.5 and 18.4 kD of threadfin bream and bulls-eye water soluble proteins. The distance between the bands is significant which are calculated based on Quantity One software (Bio-Rad Laboratories, Hercules, California, U.S.A.)

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 threadfin bream surimi (TS) and bulls-eye surimi (BS), respectively and also in all the combinations of TS + BS mixture. These new bands can be used, 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 scampi (Torry Research Station, 1986); cod (Yowell and Flurkey, 1986); 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 (Perez-mateos *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 kD were found in threadfin bream surimi while 63.8, 19.7 and 17.7 kD were found in bulls-eye surimi. These bands can be used to identify the TS and BS mixture when they are mixed at different combinations of 1:1; 3:1; 1:3; 4:1 ratios.

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, Lee 1986a and Park *et al*., 2005a) which may lead to disappearance of these bands. Sarcoplasmic proteins are so important

for identifying the different types of fish in the raw fillets but water-soluble compounds includes sarcoplasmic proteins, digestive enzymes, inorganic salts, and low molecular substances should be removed from the minced meat by water leaching to increases the concentration of myofibrillar proteins, which is primarily responsible for gel formation of surimi (Lee, 1984).

Electrophoretic pattern of salt soluble proteins

Intact fish and Surimi: Using SDS-PAGE, threadfin bream (TF) showed characteristic myofibrillar protein bands with MW's of 204,181.5, 43 and 14.3 kD while the bulls-eye (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. 2). Some of the species-specific bands, such as the 26 kD of threadfin bream and 42.3 kD of bulls-eye were missing in the respective surimi (Fig. 2). This may be due to the loss of myofibrillar proteins during the successive water leaching process (Park *et al*., 2005a; and Moosavi-nasab, 2005)

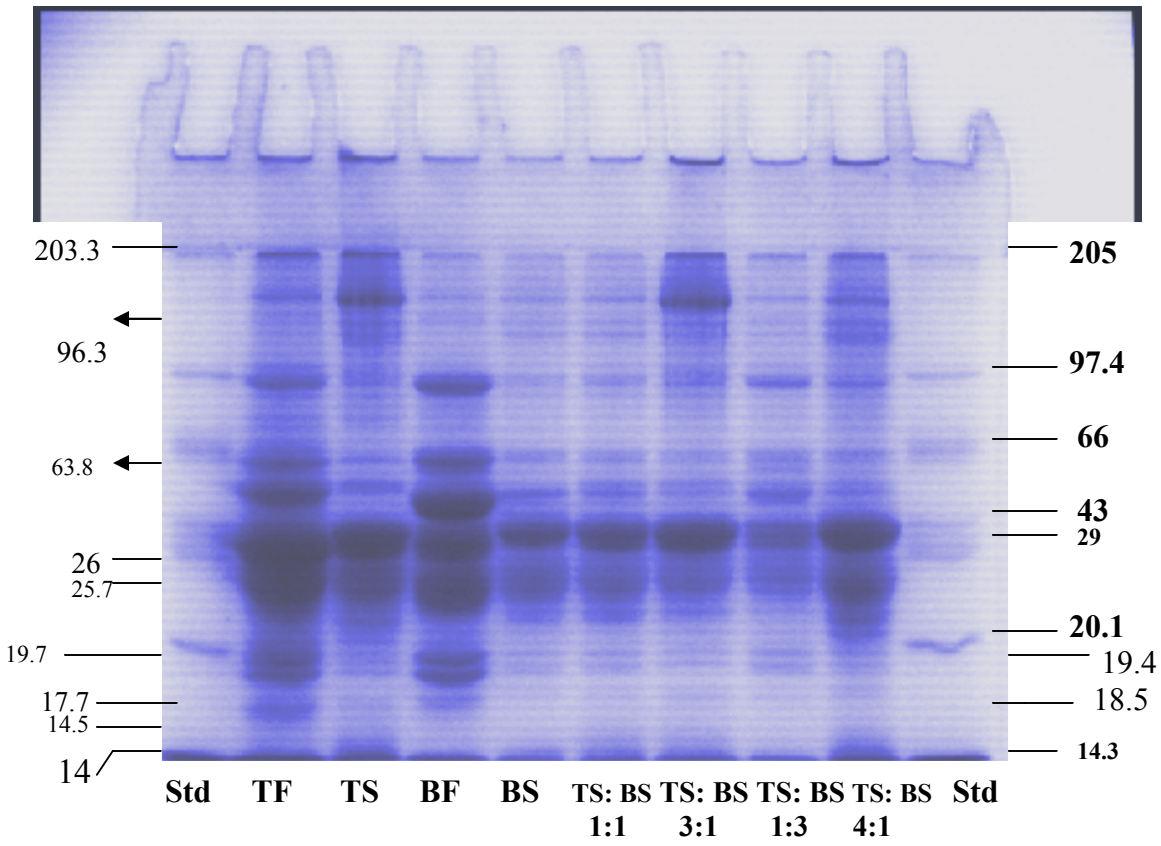

Figure1: SDS-PAGE pattern of water soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF: threadfin bream fillet; TS: threadfin bream surimi; BF:bulls-eye fillet; BS:bulls-eye surimi.

Loss of myofibrillar proteins can be prevented by reducing excessive water leaching process and also it depends on water temperature, the degree of agitation, and contact time between water and meat particles during surimi preparation (Lee, 1984 and 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. 2) and these bands can be used to differentiate surimi mixtures. Cheng (1979) and Perez-mateos *et al.* (2004) reported that significant myosin degradation of surimi with NaCl leads to increase in protein bands. All the combinations of TS + BS mixture had common bands of 204, 41.6, 25.3 and 14.3 kD (Fig. 2). Among these bands 204, 25.3,14.3 and 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 at different combinations of 1:1; 3:1; 1:3; 4:1 ratios. Various combinations of mixtures myofibrillar proteins of scampi and shrimp (Torry Research Station, 1986); cod and blue whiting (Rehbein, 1992); cod, haddock and whiting (Sotelo *et al.,* 1992); Cowie (1968); pink and rock shrimp (An *et al*., 1988); Alaska Pollock and red hake (An *et al*., 1989 & Dreyfuss *et al*., 2006); for surimi based products (Pepe *et al*., 2007) was successfully reported

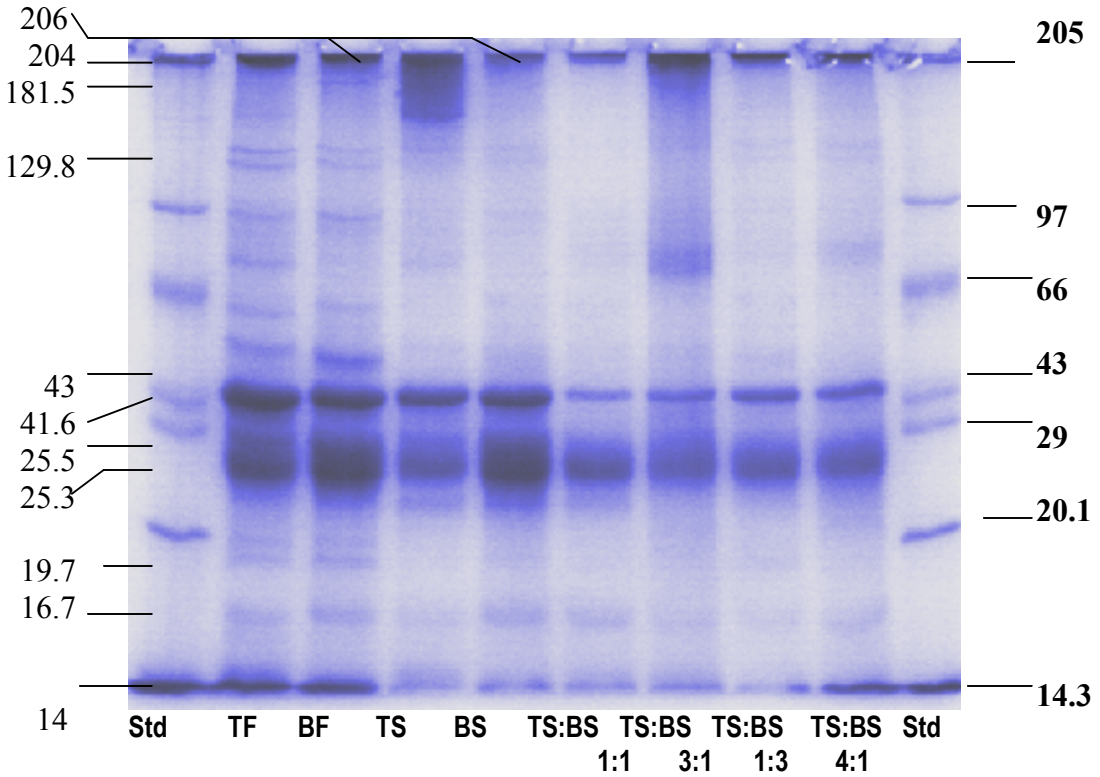

Figure 2: SDS-PAGE pattern of salt soluble proteins of intact fillets, surimi and surimi mixtures of threadfin bream and bulls-eye. The numerical values are molecular weights (kD) of the protein bands. TF:threadfin bream fillet; TS: threadfin bream surimi; BF:bulls-eye fillet; BS:bulls-eye surimi.

CONCLUSION

The objective of this research was to develop a suitable/standard technique to detect/trace the adulteration in the surimi and surimi products even in very low and higher level of adulterant used.

Identification of species by SDS-PAGE is well known, but there is no appropriate study to identification of species used in the fishery products especially surimi and surimi products. So application of this study will be useful to identify species used in the surimi products. There is no appropriate/standard study to detect/trace the adulterated/substituted species in the adulterated surimi. Findings of this research show that application of this study can be used to detect/trace the adulterated/substituted species in the 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 shows that level of traceability/detection of adulteration possible even in very low and higher level of adulterant used in the surimi.

Further research required in this area is finding out a suitable technique for detecting the adulteration level and type of adulterant used in the fishery products especially surimi, sausage and canned products.

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