Molecular Identification of Fresh and Cooked Tuna Samples Using Triplex-Polymerase Chain Reaction Assay

S.M. Efrem[1,2], W.W.P. Rodrigo[1] and S.M.P. Lukshman[1,2]

[1]Biotechnology Unit, Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka
[2]Business Management School, 591, Galle road, Colombo 06, Sri Lanka

During trade and processing of tuna products, it is very important to detect commercial frauds regarding substitution of species. As a consequence, a guarantee can be given to the consumers about the safety and origin of the tuna product. Identification of the correct tuna species is important to detect and prevent food adulterations. The main objective of this project is to differentiate most available tuna species of yellowfin, bigeye and skipjack tuna from other fish species in fresh and cooked samples using triplex PCR method. DNA was extracted from fresh samples by salt method and from cooked samples by DNeasy mericon food kit (Qiagen). Fish specific PCR was carried out to confirm that the samples are actually belonging to a fish species and to check the quality of the DNA for the amplification purpose due to the presence of PCR inhibiters especially in the cooked samples. Band size of 251 bp was obtained for all fish samples as expected and band sizes of 284 bp, 140 bp and 242 bp were obtained for yellowfin, bigeye and skipjack tuna respectively in triplex PCR. Out of 10 fresh tuna samples five were identified as adulterated samples and no PCR product was obtained for non tuna samples as expected. Cooked tuna samples labeled as yellowfin and skipjack produced expected bands, and the product labeled as "tuna ambulthiyal" detected as skipjack. Product labeled as "fish ambulthiyal" and the rest of the cooked fish samples were not obtained any band which demonstrated the tested samples not belongs to the yellowfin, bigeye or skipjack. Therefore, this assay can be used successfully for the identification of adulterated fresh and cooked tuna samples and did not get any band for other fish samples which confirm that this assay was specific for above mentioned tuna species.

Keywords: Triplex PCR, Fish specific PCR, Fresh and cooked tuna samples, Molecular identification