Conventional Triplex Polymerase Chain Reaction: A Reliable Tool for Detection of Species Adulteration in Canned Tuna Products

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Abstract

Authentication of canned tuna fish product is mandatory due to the growing occurrence of fraud in the processed fish industry. Different values and quality of tuna species can lead to the replacement or mixing of expensive fish by less expensive ones. Detection of species adulteration in canned tuna is much more difficult because of the DNA degradation and fragmentation results by high temperature and high pressure throughout the canning process. In this study Yellow-fin Tuna, Big eye Tuna and Skipjack Tuna species were selected for the adulteration detection and thirteen canned tuna samples were taken and two sardine samples and one mackerel sample were taken as negative samples from local and foreign markets which were dipped in different solutions. Isolation of DNA was performed by 200mg small fragment protocol of DNeasy Mericon food kit, QIAGEN. Extracted DNA was confirmed using spectrophotometer and fluorometer. Confirmed DNA were subjected to the conventional one-way triplex polymerase Chain Reaction (PCR) targeting 284 bp Yellow fin Tuna, 140 bp Big eye Tuna and 251 bp Skipjack Tuna regions from cytochrome b gene. Six samples out of thirteen were identified as adulterated against the label indicated in the cans in three replicated triplex PCR trials. LT1, LT2, LT6 labeled as Skipjack Tuna, but PCR products correspond to big eye for LT1, Yellow fin and Skipjack for LT2 and Yellow fin and big eye for LT3. LT7, LT8, MT13 labeled as yellow fin, but all resulted positive for big eye tuna. 2% agarose gel which has a higher resolution allowed the precise band separation. In this study conventional triplex PCR methodology proved to be an inexpensive, reliable and sensitive tool for detecting canned tuna DNA fragments (longer than 100 bp) present in canned tuna products.

Keywords - Canned tuna, DNA, Triplex polymerase chain reaction, Adulteration