ABSTRACT

In the wake of increasing demand of the fish and fish products, the market for fish products is currently confronting a wide range of difficulties. This increase in the fish demand has paved the ways for the fish adulteration and mislabeling at the various levels of the food chain. The health of consumers is directly impacted by food adulteration. In terms of sustainability and food safety, fish substitution in fish fillet is concerning. There is need to identify adulterated fish species in the restaurant sector for regulatory food controls, consumer confidence, and proper application of traceability. DNA based identifications have been proven useful. In this study a Sensitive, robust and reliable method was developed for verifying the fish adulteration in the processed fish products. The authentication protocol for Rohu, Tilapia, Thalia was developed by Multiplex PCR. The primers were designed using the cytb Mitochondrial gene with the fragment size in the range of (78bp-506bp). Three species specific and one Degenerate Common forward primer was designed. The three species specific primers for *Labeo rohita, Laeo catla* and *Oreochromis niloticus* showed the fragment size of 235bp,186bp,506bp on the Agarose gel respectively. The primers for *Labeo rohita* and *Labeo catla* were sensitive to 0.1ng of DNA template, However the primer for *Oreochromis niloticus* was sensitive to 1ng of DNA template. After the optimization,validation of specificity and Sensitivity of primers the fish samples (3 reference and 20 commercial samples) were screened .The study reported 80% mislabeling in the processed fish samples. This technique developed here can be used for the food inspection and for enforcing the regulatory food control.

Keywords: *Labeo rohita*, *Labeo catla, Oreochromis niloticus*, Multiplex PCR, Cytb, Adulteration