

forms. The DNA was amplified by a set of designed primer exclusively targeting D-loop variable region of mitochondrial genome. The target DNA on PCR amplification yield a 515 bp product which was further sequenced to check the conformity with the available databases. A restriction enzyme, *Tsp509I* was selected for further restriction digestion and the digested DNA fragments were visualized by silver staining. The results showed that the selected single enzyme was able to differentiate all the five closely related chilled snapper species. RFLP digestion of the amplified region gave 3-5 major bands in snapper species. Distinct bands at 240, 165 and 70 bp were noticed in *L. fulvus*, while *L. gibbus* have 3 bands at 270, 165 and 95 bp. *L. argentimaculatus* shared five bands at 165, 130, 105, 70 and 65 bp, while the closely related *L. lemniscatus* lack one band at 70 bp. *L. rivulatus* possessed three distinct bands at 250, 165 and 130 bp. Similar patterns could also be achieved with frozen and cooked snapper species. Hence, the developed PCR-RFLP method targeting D-loop region can be reliably used to authenticate the five major prevalent snapper species more precisely without any discrepancies.

#### SF OR 08

### Sodium benzoate in fish and ice samples from different markets of Kerala

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**S**odium benzoate is a permitted additive used in processed fish and fishery products to inhibit growth of mold, yeast and

many bacteria. The ADI fixed by JEFCA for sodium benzoate is 0-5 mg/kg body weight and the maximum allowable limit is 0.1% as per EU regulations. Due to effective antimicrobial properties, treatment of this preservative is proven to extend shelf life of processed fishery products by many researches. But in fresh and processed fish, use of sodium benzoate is not allowed as per Indian and international regulations. However, clandestine use of sodium benzoate in freshly harvested fish reported from various quarters. Hence a study was attempted to evaluate the presence of sodium benzoate in fresh fish and ice using a rapid and sensitive HPLC method. Ammonium acetate: Acetonitrile (4:1) mixture was used as buffer at a flow rate of 1 ml/min and the detection wavelength was 225 nm. Fifty-five samples including 18 ice sample and 37 fin fish and shellfish samples collected from different markets of Kerala, were analysed. Among the samples, 2% of fish samples contained sodium benzoate below quantification level, 10% fish samples and 50% ice samples contained sodium benzoate below the detection level. The study data indicate minimal risk of health issue from consumption of these fishes because the detected concentration was very low and a long period exposure of this level can only make people vulnerable to adverse health effects. However, frequent monitoring of the fish samples with zero tolerance and awareness about the preservatives and their side effects will help to ensure food safety.