Comparative Effects of Processing Treatments and DNA Extraction Methods on PCR-Based Identification of Two Fish Species

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Seafood authentication has become increasingly important due to widespread species mislabeling and substitution in processed fish products. DNA-based techniques such as polymerase chain reac- tion (PCR) enable accurate species identification; however, their success depends on DNA quality, influenced by processing methods. Therefore, this study evaluated the effects of DNA extraction methods and drying treatments on PCR-based identification of processed fish species. The objectives were to compare extraction efficiency, determine the impact of drying methods on DNA quality, and assess PCR amplification success using different primers. Two fish species, *Sardinella* spp. and *Rastrelliger* spp., were analyzed using fresh and processed (fish muscle) samples subjected to sun drying, salt drying, and oven drying. DNA was extracted using a commercial kit (Wizard® Genomic DNA Purification Kit), phenol–chloroform, and cetyltrimethylammonium bromide (CTAB) methods (n = 3). DNA concentration and purity were measured using a Nanodrop spectrophotometer, followed by PCR amplification using cytochrome c oxidase subunit 1 (COI, 745 bp) and internal transcribed spacer 2 (ITS2, 312 bp) markers. Statistical analysis revealed that DNA concentration was significantly affected by species ($p = 0.008$) and extraction method ($p = 0.006$), while DNA purity was significantly influenced by these factors ($p < 0.001$). Among tested methods, CTAB produced the highest DNA concentration (988.38 ng/µL) and higher DNA purity (2.109) ($p < 0.05$), (A260/A280 1.8–2.0.) Drying treatments reduced DNA quality, with oven drying (270.58 ng/µL) causing greatest degradation, whereas salt-dried samples retained higher DNA concentration (692.72 ng/µL) and purity (1.95). PCR results indicated that ITS2 markers produced clear amplification in processed samples, whereas COI showed limited amplification due to degradation of longer DNA fragments. Overall, findings suggest the CTAB method combined with ITS2 markers provides more reliable PCR- based identification of processed fish species and supports seafood authentication. This study provides insights for improving molecular authentication protocols in processed seafood and enhancing reliability in food traceability systems.

Keywords: *DNA extraction, Processed seafood, PCR identification, Rastrelliger spp., Sardinella spp.*