Rapid and low-cost authentication of common sole (*Solea solea*): everyone, everywhere, every time!

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Fish consumption is responsible for 17 % of the global animal protein intake by humans, putting the fishing and aquaculture industry as a pivotal food provider. However, due to an ever-growing global population and increasing pressure on the industry, seafood is prone to fraudulent activities, such as the substitution of species. When a fish is stripped of its morphological traits, it becomes impossible to identify it by eye. Additionally, the complex supply chains make it easier for fraud to go unnoticed. To identify processed seafood, genetic approaches using DNA extraction and the Polymerase Chain Reaction (PCR) can be used. DNA barcoding is the most commonly used genetic method, but like most current molecular methodologies it is expensive, time-consuming, and requires both expensive lab equipment and expert knowledge. However, in the seafood industry, most products are consumed as fresh as possible, so limiting the time needed for authentication is key if the intent is to authenticate the food before consumption. In this study, we developed a fast, cheap, and easy to apply method for the identification of common sole (*Solea solea*), an expensive flatfish species that is known to be commonly substituted along the Belgian supply chain. First, three crude DNA extraction methods were compared with a commercial extraction method. Second, the PCR was replaced with Loop-mediated Isothermal Amplification (LAMP), which is both faster and technically less demanding because only a heating element is required and colorimetric detection of amplification is used. Third, the specificity of the LAMP assay was evaluated. A comparison was made between the commercial DNA extraction kit (Nucleospin® Food kit) with a dipstick-based, paramagnetic bead-based, and an alkaline-based DNA extraction method. The alkaline-based DNA extraction method turned out to be the most reliable and cheapest (1 Eurocent), capable of extracting DNA in less than 20 min, without requiring a centrifuge, as opposed to the Nucleospin® Food kit, which takes 1 h and costs over 5 Euro per sample. A *S. solea* specific LAMP assay for the mitochondrial *cytochrome b* gene was developed and validated within a working range between 1 and 0.1 ng of DNA. The alkaline-based DNA extraction method was combined with the LAMP assay and tested on ten common sole fillets under different preservation conditions (fresh, frozen, and ethanol stored) and ten previously identified sole meal products. The combination of the alkaline-based DNA extraction and LAMP correctly identified all sole samples as sole and no false positives were detected. The results demonstrate how the LAMP assay can be combined with alkaline DNA-based extraction for the rapid identification of common sole products. The entire authentication takes less than 1 h, costs less than 0.5 Euro, and can be performed on-site since it only requires a few consumables, a few hand tools, and a simple heating element that can reach 85 and 65 °C. We anticipate that our assay is a sound initial scan for sample prioritisation in large-scale audits and identifications or other cases where advanced equipment is not available. Additionally, our method represents a proof-of-concept for the development of similar methodologies for the identification of other important seafood species.

Keywords: CYTB; Authentication; DNA isolation; Isothermal amplification; Sseafood fraud; Substitution