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DNA-based methods for species identification of fishery products

Ute Schröder, Kristina Kappel

Max Rubner-Institut, Department of Safety and Quality of Milk and Fish Products, Kiel/Hamburg

The controlling of the labelling of fishery products is a prerequisite for an effective consumer protection. Regardless of the reasons consumers should know what they are buying or eating. For this reason effective traceability tools as DNA-based methods for species identification have been established for several years in many European control laboratories, particularly the "Gold Standard" PCR Sanger sequencing. In its Food and Feed Law (LFGB) Germany has anchored different validated methods for species identification in fish and crustacean species. Beside the protein based method isoelectric focusing (IEF), established at the beginning of the 21st century and followed by the DNA-based restriction-fragment-length-polymorphism method (RFLP), the PCR sequencing technology is the method of choice. As seafood has become increasingly globalized and traditional fish species have been replaced by new species from Asia, Africa and South America, the identification method should be independent of reference material. PCR sequencing meets this requirement in species assignment by comparing the generated DNA sequence with reference sequences in public databases as GenBank (NCBI) and BOLD (Barcode of Live Database). In Germany, the PCR sequencing methods for fish and crustacean species were validated with the mitochondrial gene marker *cytb* (cytochrome b) and 16S rDNA, respectively, and successfully established since 2012. Due to their short DNA fragment length of approx. 464 bp and 312 bp processed fishery products such as smoked, salted and heated can also be identified by this technology.

The latest development in standardization of DNA based methods for the identification of fish species was achieved within the framework of the European project Labelfish. A detailed Standard-Operating-Procedure (SOP) for a PCR sequencing approach on the mitochondrial marker cytochrome-c-oxidase I (*cox1*, COI) was written and an international ring trial was successfully performed. The collaborative study showed satisfactory results so that a standardized sequencing method with both markers *cytb* and COI was developed and finalized on CEN level (European Committee for Standardization) as a technical specification which will be adopted in the next few months.

However, there are further challenges on species identification that cannot be solved with the presented PCR sequencing method. Regarding some closely related fish species like tuna or redfish other gene markers have to be used. In addition, DNA sequences are not found in public databases for all species, especially tropical fish species, or are sometimes ambiguous, making valid identification impossible. In this case FINS (Forensically-Informative-Nucleotide-Sequencing) is one option that is used in research institutes: The construction of a phylogenetic tree of DNA sequences can help to correctly classify the affected fish species within the genus or family. Further problems are highly processed products such as tuna cans and mixtures of different species. Normal Sanger sequencing does not allow more species to be identified than one at the same time. New

technologies like NGS (Next-Generating Sequencing) with the possibility for massive parallel sequencing can be the answer.

Time is money, for this reason scientists are looking for new rapid methods which can be implemented in the control labs. Depending on the equipment of laboratories, PCR sequencing takes three to five days. The incoming goods shall be controlled at the boarder or in the plant just in time. Moreover, high throughput technologies are required to cope with a high volume of samples. The MRI is working on these issues and tries to find solutions which will be presented by means of examples such as a real-time PCR (qPCR) method and a DNA-Chip. The national and international network based on projects with other fish research institutes, universities and the fishing industry is an important prerequisite for this successful research work.