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English Version

**Foodstuffs - DNA barcoding of fish and fish products using
defined mitochondrial cytochrome b and cytochrome c
oxidase I gene segments**

Produits alimentaires - Codes-barres d'ADN de
poissons et de produits à base de poissons à l'aide de
segments de gènes mitochondriaux du cytochrome b et
cytochrome c oxydase I

Lebensmittel - DNA-Barcoding von Fisch und
Fischprodukten anhand definierter mitochondrialer
Cytochrom-b- und Cytochrom-c-Oxidase-I-
Genabschnitte

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European foreword

This document (CEN/TS 17303:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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Introduction

Food safety is a key aspect in terms of consumer protection. In the last three decades, globalization has taken place in the trade of food. Fish trade channels are becoming steadily longer and more complicated so that sophisticated traceability tools are needed to ensure food safety. Correct food labelling is a prerequisite to ensure safe fish products and fair trade as well as to minimize illegal, unreported and unregulated (IUU) fishing. In particular, the fact that fish is increasingly being processed in export countries makes the identification of species by morphological characteristics impossible.

The development of harmonized and standardized protocols for the authentication of fish products is necessary to establish reliable methods for the detection of potential food fraud.

1 Scope

This document describes a procedure for the identification of single fish and fish fillets to the level of genus or species.

The identification of fish species is carried out by PCR amplification of either a segment of the mitochondrial cytochrome b gene (*cytb*) [1] or the cytochrome c oxidase I gene (*cox1*, *syn COI*) [2], [3] or both, followed by sequencing of the PCR products and subsequent sequence comparison with entries in databases [4], [5]. The methodology allows the identification of a large number of commercially important fish species.

The decision whether the *cytb* or *cox1* gene segment or both are used for fish identification depends on the declared fish species, the applicability of the PCR method for the fish species and the availability of comparative sequences in the public databases.

This method has been successfully validated on raw fish fillets, however, laboratory experience is available that it can also be applied to processed, e.g. cold smoked, hot smoked, salted, frozen, cooked, fried, deep-fried samples.

This document is usually unsuitable for the analysis of highly processed foods, e.g. tins of fish, with highly degraded DNA where the fragment lengths are not sufficient for amplification of the targets. Furthermore, it is not applicable for complex fish products containing mixtures of two or more fish species.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions (ISO 24276)*

ISO 16577, *Molecular biomarker analysis — Terms and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1 alignment

process or result of matching up the nucleotide residues of two or more biological sequences to achieve maximal levels of identity

[SOURCE: BLAST Glossary]