# Fish, seafood and related products

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## General overview of the product

It is reported that over 171 million tonnes of fish (seafood) are harvested in one year [1], corresponding to the highest ever consumption of 20.3 kg per capita in 2016: production in aquaculture is steadily increasing, but traditional fishery remains the major part of the production process with about 91 million tonnes.

Seafood is currently in a critical situation. On one side, there is an increasing trend towards consumption of fish and seafood because of perceived health benefits, such as their content in omega-3 polyunsaturated fatty acids, and as an alternative source of protein to meat. On the other hand, the sustainability of fishery, coupled with increasing pollution, requires caution. In recent times newspapers reported on mercury pollution, microplastics in seafood and antibiotics in farmed fish. It is clear that these contrasting trends can be conducive to fraud and mislabelling. Different countries in the world have different standards and requirements, complicating the situation. Illegal, unreported and unregulated (IUU) fishing is the other main problem for the market of safe, nutritious and healthy seafood. IUU practices concern many aspects of fishery: species, age of fish, geographic area, amounts of catch, timing, and equipment [2].

Mislabelling is a common problem for fish, and seafood in general (cf. the recent paper on "snapper" identity [3]). This has been evidenced in many studies across the world, particularly using methods based on DNA analysis for identification of species. EUROPOL (European Union Agency for Law Enforcement Cooperation) considers fish the third highest risk category for food fraud [4]. Oceana (an international organisation established by a number of leading foundations to focus on oceans) periodically examines restaurants and stores, finding high percentages (20-30 %) of samples mislabelled [5].

This chapter will deal with fish and invertebrates used as food: molluscs, crustaceans, jellyfish, excluding mammals and reptiles. It will not deal with fish oil.

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# 1. Product Identity

## 1.1. Definition of the product and manufacturing process

The commercial designation for seafood products is under the heading 03 in the CN code, Commission Implementing Regulation (EU) 2017/1925 [6].

**0302** is for “Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304” and includes all types of fish: Salmonidae, flat fish, tunas, herrings, cod families, tilapias, and also the offal of fish.

**0303** is for “Fish, frozen, excluding fish fillets and other fish meat of heading 0304” including again the same types of fish.

**0304** is for “Fish fillets and other fish meat (whether or not minced), fresh, chilled or frozen”

**0305** is for “Fish, dried, salted or in brine; smoked fish, whether or not cooked before or during the smoking process; flours, meals and pellets of fish, fit for human consumption”

**0306** is for “Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked crustaceans, whether in shell or not, whether or not cooked before or during the smoking process; crustaceans, in shell, cooked by steaming or by boiling in water, whether or not chilled, frozen, dried, salted or in brine; flours, meals and pellets of crustaceans, fit for human consumption” and includes lobsters, crabs, shrimps, crayfish

**0307** is for “Molluscs, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked molluscs, whether in shell or not, whether or not cooked before or during the smoking process; flours, meals and pellets of molluscs, fit for human consumption” and includes oysters, scallops, mussels, cuttle fish and squid, octopus, snails, abalone and others.

**0308** is for “Aquatic invertebrates other than crustaceans and molluscs, live, fresh, chilled, frozen, dried, salted or in brine; smoked aquatic invertebrates other than crustaceans and molluscs, whether or not cooked before or during the smoking process; flours, meals and pellets of aquatic invertebrates other than crustaceans and molluscs, fit for human consumption” like sea cucumbers, sea urchins, jellyfish.

The presence on the market of material which is in the shape of fillets or minced flesh, and material which has been subjected to curing and processing, freezing, smoking, drying, opens possibilities for fraudulent or accidental substitution and mislabelling.

## 1.2. Current standards of identity or related legislation

Though not a standard, the main reference for scientific names and common names of fish is FishBase [7]. FAO maintains the ASFIS (Aquatic Sciences and Fisheries Information System) database for fishery statistics [8].

The Coordinating Working Party on Fishery Statistics (CWP) has developed a Handbook of Fishery Statistics, published since 1990 [9], which includes the definition of the FAO fishing areas.

Codex Alimentarius has a fairly recent Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003 [10]), incorporating good manufacturing practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) system.

The European Union has a legislation on seafood labelling, Regulation EU 1379/2013, requiring indication of commercial designation, scientific name, method of production (caught, farmed), geographical origin (catch area, body of water, country), fishing-gear category [11]. This is associated to the traceability requirements of the General Food Law Regulation 178/2002 [12]. Other voluntary information is allowed about dates of catching, environmental or social information, and nutritional content.

In the USA, the U.S. Food and Drug Administration has produced and maintains a list of Acceptable Market Names which are allowed for seafood species [13].

# 2. Authenticity issues

## 2.1. Identification of current authenticity issues

The main problem for seafood authenticity is mislabelling for the species name, or species substitution [14]. Indication of the species is an obligation in most labelling requirements. However, particularly in processed products where visual recognition is not possible, the identity of the animal can be counterfeited. Usually, there is an economic motivation, substituting expensive and valued material with other species of lesser value or from illegal fishing. A further problem is the fact that many seafood species are marketed under a shared name ("umbrella" term) encompassing different species and/or genera; translation into local languages adds more problems.

A second important issue concerns geographical origin, connected to the FAO fishing zones. When this is declared on the label, it might be a fraudulent declaration to cover for IUU fishery or to mask a species substitution. Similarly, a declaration about the fishing gear may raise the price of the food product and be a fraud.

Processing or treatment can be falsely declared on the label, as in the case of freeze/thaw process to sell fresh fish.

Additives can also be fraudulent, as in the case of tuna added with vegetables extracts, salts or carbon monoxide to change the colour and make it look fresher.

Sustainability is a new issue which generates opportunities for fraud, when declarations about place and way of fishing are untrue.

## 2.2. Potential threat to public health

Some fish or seafood species are toxic, and mislabelling can cause poisoning: puffer fish, escolar, ciguatoxic species are examples of fish which contain toxins, like tetrodotoxin or histamine. Farmed fish can contain higher levels of contaminants, and organic compounds: also, in this case a fraudulent declaration about the origin of fish or production method can have health effects through exposure to environmental contaminants.

Scombroid syndrome is an allergic reaction caused by some fish species which contain histamine. Substitution and mislabelling can expose allergic consumers to health risks, leading them to consume seafood they would normally avoid.

False declaration about the cold chain, or the freezing and thawing of products, may be hazardous due to development of microbes and possible infections.

The recent Minamata Mercury Convention has highlighted the problem of mercury pollution in fish and seafood. Mercury is transformed into the neurotoxic form methylmercury (MeHg) mainly in aquatic environments, and from animal to animal it accumulates along the food chain. Humans are exposed to MeHg through consumption of predator fish like tuna and swordfish, therefore a correct labelling of the species name is important for an informed choice. The area of origin might also be important in determining the levels of MeHg, but in this case it is hardly expected that consumers might recognize the issue when purchasing fish [15,16].

Mislabelling for the geographical origin could become a health threat in case the seafood comes from polluted areas due to radioactivity, or for the use of veterinary drugs allowed in some countries and not in others.

# 3. Analytical methods used to test for authenticity

## 3.1. Officially recognised methods

Chemical analyses can be used to detect addition of: (i) salt or phosphates, used to increase weight by attracting water; (ii) benzoic acid, used to increase shelf life; (iii) citric acid or other compounds as preservatives and to change the colour; (iv) carbon monoxide to increase the red colour; (v) proteins to increase weight; (vi) excess water or brine, overglazing, to increase weight. Standard methods are provided by the Association of Agricultural Chemists (AOAC), European Committee for Standardization and others. A recent review reports about traditional and non-destructive methods for seafood quality analysis [17].

Traditionally, the identification of animal species, also for fish and seafood, was performed through protein analysis, with electrophoresis, chromatography, or immunological methods [18]. The Regulatory Fish Encyclopedia hosted by the U.S. FDA was a repository of information on protein analyses for fish identification, mostly IEF patterns [19]. A possible advantage of protein analytical methods is to address the presence of some specific allergens, which is relevant also for food safety purposes.

However, proteins can be degraded or destroyed by processing, making these methods ineffective. Methods based on analysis of DNA are more effective because of higher specificity and sensitivity, and because DNA can be amplified from few molecules also in degraded samples [20].

A standard method for establishing if the fish has been thawed from frozen is based on microscopy analysis of muscle, by the Italian accreditation body ACCREDIA [21]. Other methods based on physical and chemical parameters are being developed [22–24].

A COMET test on DNA integrity can provide indication to detect foodstuff which has been irradiated, and the method is standardized (EN 13784:2002, [25]).

## 3.2. Other commonly used methods

### 3.2.1. DNA-based techniques

DNA-based techniques [2,26] make use of different markers, amplified fragments or restriction profiling: sequencing, AFLP (amplified fragment length polymorphism), FINS (forensically informative nucleotide sequencing), RAPD (random amplified polymorphic DNA), RFLP (restriction length polymorphism), SSCP (single-stranded conformational polymorphism), multiplex PCR and real time PCR for diagnostic fragments [27,28]. An important resource is the Reference Standard Sequence Library for Seafood Identification including over 1000 sequences from seafood vertebrates and invertebrates [29]. The D-loop region in mitochondrial DNA can be a good target for species differentiation because of high polymorphism and mutation rate [30]. A recent survey [31] has singled out the most common methods used by laboratories for identification of species: (i) Forensically Informative Nucleotide Sequencing (FINS), (ii) Restriction Fragment Length Polymorphisms (RFLP) and (iii) Isoelectric Focusing (IEF).

A different approach in DNA-based analyses, the DNA barcoding technique, is a well-known standard to detect species of seafood in food samples, also after extreme processing: for instance, it is used by the Canadian Food Inspection Agency. The initiative Barcode of Life Data System [32] with the FISH-BOL, fish barcode [33], is the main source of data for species identification. The marker of choice is cytochrome b (cyt-b) or cytochrome *c* oxidase I gene (COI) located on the mitochondrial DNA; other markers are 16S or 18S ribosomal DNA (16S-rDNA, 18S-rDNA), the internal transcribed spacer type I-ribosomal DNA or type II (ITS1-rDNA, ITS2-rDNA) [34]. The markers are amplified with PCR from universal primers, and the amplicons are then sequenced for comparison with the data base [35]. A comparison of different DNA methods has been shown to lead to 100 % differentiation in *Merluccius* species [36].

Several research projects funded by the European Commission have produced databases, protocols and standard operating procedures for molecular analyses in seafood identification: recent examples include FishTrace, SEAFOODplus, CHILL-ON, FoodIntegrity, AuthentNet and PrimeFish.

Analyses which can be of use in ascertaining the geographical origin can be based on DNA markers, if the local populations of fish have distinctive features. Otherwise, chemical analyses for elements and trace elements, stable isotopes, fatty acids can be used [37–39].

### 3.2.2. Stable isotope ratio analyses

Methods for establishing the compliance with declarations about wild or farmed fish have been developed in order to fight frauds connected with provenance and processing which could also impact on health. Following on from early studies that had shown that the content of stable isotopes reflects both the environment in which the fish is grown and the composition of its diet, a major project known as COFAWS[1] was set up to further develop these techniques.

There are several correlations between the content of isotopes and the geo/climatic environment of a food product. The content in $^{13}C$ and $^{15}N$ are related to diet; $^{18}O$ and $^{2}H$ are influenced by the origin of the water in the product. To differentiate the farmed and wild origin of salmon, isotope ratios $^{18}O/^{16}O$ (expressed as $\delta^{18}O$) and $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) are measured by IRMS (isotope ratio mass spectrometry) on the fish oil and choline from the lipid fraction extracted from the fish

[1] COFAWS – Confirmation of the Origin of Farmed and Wild Salmon and other fish. Part funded by the European Commission under the "Fight against Fraud" action and by the UK Food Standards Agency.

muscle [37]. These parameters successfully separated wild and farmed salmon both from known origins and unknown market samples. The technique has since been used to check mislabelling in the UK market. It has since been extended to other fish such as bream, cod, bass.

Other studies have been reported in the literature including a chemometrics approach addressing the global chemical composition (trace elements, stable isotopes, fatty acids) has been recently suggested [40,41]. Stable isotope ratios for carbon, nitrogen and oxygen have also been suggested as a means for discriminating wild from farmed fish, and organic from intensive production, based on differences in the feed origin [38,42]. A combination of isotope determination and other profiling methods, e.g. trace elements or fatty acids, could be more effective. Isotopes of Strontium could be indicative of geographic provenance, since this element is present together with calcium in bones and calcified materials of seafood [43].

## 3.3. Future analytical perspectives

New methods or improvements of existing methods should make the analyses for species identification easier to perform, and sufficiently rugged to be executed on board vessels for instance, requiring no DNA extraction and no electrophoresis, for example, lab-on-chips approaches, or ultra-fast Real Time PCR [44]. Multiplexing the amplification [45,46] or using DNA chips could increase analysis throughput. Also developing methods for rapidly detecting gene variants without sequencing could be beneficial, such as the application of High Resolution Melting (HRM) analysis after amplification of marker genes [47]. For the same reason, handheld devices for non-destructive analyses will also be highly appreciated on board vessels and for controls on line [48]. Quantification of species composition could become a necessity in some cases, for example when verifying the fish content of complex foods. Molecular markers can be employed in quantitative PCR for the purpose [49], but the use of mitochondrial gene markers require sophisticated considerations, due to the fact that multiple copies of the mitochondrial DNA exist in cells [50].

More recently, methods based on proteomics or high throughput protein analyses are envisaged, which at times avoid extraction of proteins or digestion [18,51–54]. A new chemotaxonomic approach could add new tools for species identification in a rugged context [55].

# 4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

| Analytical technique | Indicative data or analyte | Authenticity issue / information |
|---|---|---|
| Gel electrophoresis, isoelectric focusing, capillary electrophoresis, immunoassay | Proteins | Species identification |
| Multiplex PCR | Mitochondrial 16S rDNA | Identification of species |
| PCR-RFLP | Mitochondrial DNA D loop<br>Cytochrome oxidase COI | Identification of snapper species<br>Identification of *Merluccius* species |
| PCR-RFLP, FINS | 16S mitochondrial rDNA | Species identification for sea cucumber |
| Real Time PCR | Nuclear and mitocohondrial genes | Detection and quantification of *Mytilus* species |
| DNA mini-barcoding followed by High Resolution Melting (HRM) analysis | COI, cyt b marker genes | Discrimination of species |
| MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) Mass spectrometry | Proteins and peptides patterns | Species identification<br>Trout species identification |
| Front face fluorescence spectroscopy (FFFS) | Several compounds with double conjugated bonds (vitamins, amino acids, etc.) | Fresh and frozen fish |
| NIR spectroscopy, 780-2500 nm, with chemometrics | Whole product | Freshness, frozen/thawed material |
| Hyperspectral imaging, 380-1100 nm, with chemometrics | Whole product | Freshness of fish, frozen/thawed fillets |
| Isotope ratio mass spectrometry (IRMS) | Stable isotope ratio | Geographic origin |
| Multi-element profiling<br>Stable isotope analysis | Different chemical elements | Identification of species, geographic origin and method of production |
| Stable isotope analysis, gas chromatography/mass spectrometry (GC/MS) | Stable isotopes ratio and fatty acids profiling | Wild and farmed salmon<br>Geographic origin |
| Tri-step infrared spectroscopy and chemometrics: Fourier Transform Infrared Spectroscopy (FT-IR), Second Derivative Infrared Spectroscopy (SD-IR), Two Dimensional Correlation Spectroscopy 2DCOS-IR) | Nutrients fingerprints | Species discrimination in surimi |

# 5. Conclusion

FAO [14] has identified the main needs to combat food fraud in the seafood sector: (i) reaching agreements on names of products and species; (ii) introducing mandatory labelling; (iii) improving the systems for official control of food; (iv) improving systems for food safety in production; (v) adding new Codex guidelines.

It is widely recognised [1] that seafood is essential for healthy nutrition, providing nutrients, micronutrients, vitamins. The steadily increasing consumption shows how public awareness has grown. For pregnant women and children, particularly in low/middle income countries, seafood contributes to development of the nervous system and is an accessible source of animal protein. This can increase the exposure to methylmercury leading to risks for neurotoxicity [15].

Since fish and seafood are highly perishable, the transportation to consumers, in long supply chains, provides logistic challenges and risks for health. Consumers nowadays require innovative ways for chilling, preserving, delivering seafood, and in this area authenticity or fraud issues might arise. Control of the cold chain and traceability with Universal Identifiers will be an area for development, e.g. by blockchain technology [1].

Pollution will surely become more relevant, particularly considering abandoned, lost, discarded fishing gear (ALDFG) and microplastics, on which knowledge is still missing. Fishery will also be impacted by climate change and extreme weather events, requiring adaptation measures. Aquaculture is included in the strategy for Climate Smart Agriculture, aiming to increase or maintain production and mitigating impacts. Climate change will affect stocks worldwide, opening the possibility for fraudulent behaviour in declarations on species or geographic origin. Sustainability of fishing is also connected to climate change and geographical origin.

Considering the commercialisation of transgenic salmon in Canada, a possible additional requirement for analytical methods will concern the traceability of transgenic material [56].

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