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## DNA sequencing for salmon filet certification

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### Abstract

Salmon is a popular fish with high nutritional and economic value due to its delicious and tender pink flesh rich in omega-3. However, there is a problem with mislabeling other types of fish as salmon in the market, which is misleading for consumers. A study used molecular methods to identify fish species sold as salmon in commercial centers and found that out of the 35 samples analyzed, 33 were indeed salmon, but two were trout. The study highlights the need for strict enforcement of food trade and labeling regulations.

**Keywords:** Salmon; DNA sequencing; Food legislation; DNA barcoding

### 1. Introduction

Salmon is a common name for several species of fish, including Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus* spp.), which comprise several sub-species such as chinook, coho, sockeye, pink, and chum salmon. A prevalent issue in the market is the fraudulent substitution of other types of fish for salmon in filets, unbeknownst to consumers. While fish can be identified based on morphological characteristics such as size, fins, teeth, and scales, these characteristics are typically removed during the filleting process, necessitating the use of molecular tools for species identification. The certification of fish filets requires molecular methods, including DNA sequencing. Smith et al. (1) have reported that protein electrophoresis was the first official method for identifying fish filets, and DNA sequencing has now become a valuable tool for this purpose. DNA sequencing involves determining the order of nucleotides in a specific gene segment under examination. Molecular identification has been widely used to determine the authenticity of fishery products. Recent studies have shown that DNA barcoding is a reliable and efficient technique for identifying fish species in processed products (2, 3, 4, among others).

The sale of packaged food in Brazil is subject to regulations that require proper registration and labeling of the product. The label must contain clear and accurate information about the product so that the consumer can easily identify it. The regulations that indicate how this information should be presented are diverse and have been established over the years. Establishments that sell fish and aquaculture products must prove, at the time of inspection, the scientific name of the species, as well as the information provided to the consumer about the commercial name of the species, the production method, and the capture zone. In addition, pre-packaged fish and aquaculture products in the absence of the consumer must include on the label the scientific name and commercial name of the species, the production method, and the capture zone. In summary, proper labeling of packaged food is important to ensure safety and correct information for the consumer.

The objective of this study was to use molecular methods to identify the species of salmon present in filets collected from different commercial centers in several Brazilian cities and verify compliance with current food legislation.

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The fish filet samples studied in this work were acquired by PROTESTE (Brazilian Association of Consumer Defense) and through personal purchase from supermarkets, fish markets, and municipal markets in several cities in Brazil, all marketed under the name salmon, except for one sample (marketed as trout), totaling 35 samples. DNA extraction was performed using the INVITEK Kit, following the manufacturer's instructions. For polymerase chain reactions (PCRs), the primer pairs ATP8.2-L8331 (5'-AAAGCRTTRGCCTTTAACG-3') and CO3.2-H9236 (5'-GTTAGTGGTCAGGGCTTGGRTC-3') were used (5).

The amplification reactions were performed with a total volume of 25 µL containing 15 ng of template DNA, Tris-KCl (20 mM Tris-HCl pH 8.4 and 50 mM KCl), 1.5 mM MgCl<sub>2</sub>, 2.5 µM of each primer, 0.1 mM of each dNTP, and 2.5 U Taq polymerase. The reaction conditions were: initial denaturation at 94°C for 4 minutes, annealing at 56°C for 30 seconds, and elongation at 72°C for 2 minutes, followed by 40 cycles of 15 seconds at 94°C, 30 seconds at 56°C, and 2 minutes at 72°C, and a final extension step of 10 minutes at 72°C. After checking amplification using a 1% agarose gel, the reactions were sent for sequencing to a third-party company. The obtained sequences were visualized and edited using Chromas Lite v2.01 and verified in GenBank (<http://www.ncbi.nlm.nih.gov>) through the "Blast n" program to confirm their similarity with mitochondrial sequences from other fishes. To avoid artifacts, sequences that did not show high quality were excluded from the analysis.

We found that the specimens referred to as "salmon" in most cases belong to the group of fish classified as salmon, which includes species such as *Salmo salar* (28 samples), *Oncorhynchus keta* (three samples), and *Oncorhynchus kisutch* (one sample). Nevertheless, it is noteworthy that three of the analyzed samples were identified as *Oncorhynchus mykiss*, also known as the rainbow trout, but only one was really referred to as trout in the supermarket.

The *Salmo salar* species, commonly known as Atlantic salmon, is found in the northern Atlantic Ocean, from the Arctic Circle region to the north of the Iberian Peninsula. This species is at risk of extinction due to overfishing and the construction of hydraulic works that prevent these fish from swimming up rivers to reproduce. Despite this, its trade is not prohibited, and many companies process this type of fish for commercialization. The *Oncorhynchus keta* and *Oncorhynchus kisutch* species, corresponding to Pacific salmon, are not endangered species and can replace Atlantic salmon consumption until this species can be consciously exploited without the risk of extinction.

*Oncorhynchus mykiss* species, commonly known as rainbow trout, although not a salmon species, has nutritional characteristics similar to salmon. Rainbow trout is widely cultivated in cold-water rivers and fish farms. To obtain a salmon-like coloration, producers have been using a process called salmonization, which involves adding natural or artificial pigments to the fish's diet, resulting in a more orange-colored meat, similar to that of salmon (6).

One of the issues with salmon and trout is that the cost of producing and commercializing salmon is higher than that of producing and commercializing trout, which can be easily cultivated in fish farms with lower costs. In addition, consumers should be correctly informed about the type of product contained in the package.

Based on food labeling regulations, it can be confirmed that two samples sold under the name of salmon are mislabeled once they are actually rainbow trout, which violates the regulations. The Wong and Hanner (2) study revealed that 25% of the samples of sea food were possibly mislabeled. Almerón-Souza et al (3) focuses on the use of DNA barcoding to identify shark and ray species in the Brazilian shark meat market, where the use of imprecise nomenclature has made it difficult to accurately identify the species being sold. The study found that 63 samples of shark meat traded as "cação" corresponded to 20 different species, including some that are threatened or critically endangered according to IUCN criteria. The results highlight the need for more accurate labeling practices to monitor catch allocations and promote conservation efforts.

The use of gene sequencing technique has been proven to be a viable tool for identifying species of commercial fish filets, such as salmon. Through this technique, it becomes possible to verify the species present in a particular sample of fish filet.

The results obtained from the application of this technique in the analysis of fish filet samples showed the presence of the *Oncorhynchus mykiss* species, indicating a type of fraud related to food labeling. In this context, it is possible to infer that there is a failure on the part of traders to comply with current laws, which reinforces the need for stricter enforcement and the implementation of a standard of identity and quality for this type of product - fish filets in general. The adoption of these measures aims to prevent mislabeling or fraud and ensure the integrity of the consumer.

## 2. Conclusion

The study found that the majority of the specimens referred to as salmon belonged to the group of fish classified as salmon, including *Salmo salar*, *Oncorhynchus keta*, and *Oncorhynchus kisutch* species. However, three samples were identified as *Oncorhynchus mykiss*, also known as rainbow trout, but only one was referred to as trout in the supermarket. Proper labeling of packaged food is crucial to ensure the safety and correct information for consumers. The study emphasizes the importance of molecular tools, including DNA sequencing and DNA barcoding, for species identification and certification of fish fillets.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

No conflict of interest.

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