Successful quantification of Atlantic salmon in mixed food products using Droplet Digital Polymerase Chain Reaction (ddPCR) to identify fraud

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Seafood plays an important role in the human diet, and as fish stocks are on the decline, more fraudulent practices are prone to happen. Salmonids are important fish species in large parts of the world, both commercially and as game fish, with Atlantic salmon (*Salmo salar*) being the most farmed marine fish. Cheaper salmonid species are potentially used as substitutes for the more expensive salmon species. Up till now, no tool existed to reliably quantify Atlantic salmon in mixed food products. The most used technique for seafood identification is DNA barcoding through Sanger sequencing, which does not allow quantification and only works when the sample contains one single species.

By using Droplet Digital Polymerase Chain Reaction or ddPCR, we developed a quantification tool to assess the percentage of Atlantic salmon in mixed food products to allow for a quick identification of seafood fraud. A *Salmo salar* specific ddPCR assay was designed using the nuclear rhodopsin gene of Atlantic salmon and nine closely related salmonid species, i.e. pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*O. keta*), Coho salmon (*O. kisutch*), rainbow trout (*O. mykiss*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tshawytscha*), brown trout (*S. trutta*) and Arctic char (*Salvelinus alpinus*). No cross-amplification was detected and the limit of detection was set at 0.37 copies per µl (0.024 ng total DNA) and a limit of quantification at 5.51 copies per µl (3 ng total DNA). A linear relation (R^2 = 0.96) was found between total DNA added to the PCR mix and the measured target copies. This is in agreement with other qPCR studies, where multi-copy genes were used instead of the nuclear rhodopsin.

Subsequently, the accuracy of quantification was tested in multiple species samples by mixing both extracted DNA and tissues from different salmonid species with Atlantic salmon tissue. The ddPCR method was able to reliably estimate the percentage of Atlantic salmon DNA, even when mixing salmon tissues in different weight percentages. A linear relationship (R^2 = 0.89) was found between the DNA concentration of Atlantic salmon in the extract (i.e. DNA added to the PCR mix) and the concentration measured by the ddPCR. This confirms that the real percentage of Atlantic salmon in a mixed food product can be successfully deduced by the developed ddPCR assay.

We further tested the impact of four common processing techniques, since food processing can heavily impact the quality and quantity of DNA. Freezing samples led to significantly lower DNA copy numbers. Smoking, Bellevue boiling or gravad lax did not significantly impact the DNA copy number quantification. Finally, the ddPCR method was validated by testing 46 retail products (31 Belgian and 15 Polish) containing Atlantic salmon (30 samples) or Pacific salmon (16 samples) according to the labels. No Atlantic salmon was detected in any of the Pacific salmon products. In contrast, Atlantic salmon was detected in 27 out of 30 Atlantic salmon samples. No Atlantic salmon could be detected in three canned fully homogenised Polish products, although this was mentioned on the label. In conclusion, the developed ddPCR assay can reliably and accurately quantify Atlantic salmon DNA in complex food matrices, even in extremely low concentrations and in the presence of closely related salmonid species. The method may be used to quickly detect fraud through verification of the percentage of Atlantic salmon mentioned on the food product labels, with the proviso that the processing (especially freezing) and the ingredients mentioned on the label are taken into account.

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