IDENTIFICATION OF FRAUD IN TRADE IN PROCESSED FISH PRODUCTS BY DNA ANALYSIS

Federica Corrado[1], Anna Cutarelli[1], Daniela Criscuolo[1], Antonella De Roma[1], Bianca Cecere[1],Daniele Simonetti[1], Giorgio Galiero[1], Elena Coccia[2], Carmina Viola[2], Ettore Varricchio[2], Marina Paolucci[2]

[1]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli, federica.corrado@cert.izsmportici.it
[2]Department of Sciences and Technologies (University of Sannio), Benevento

Abstract – Institutions working for food safety have the main goal to guarantee consumers about the security of what they eat. In seafood field a key aspect of food safety is represented by the possibility to correctly identify the species of fish especially when the product has been already processed and therefore no more morphologically identifiable. In the present study we analysed 20 seafood products with the aim to properly classify their fish species composition. The analysis was carried out by sequencing a region of cytochrome oxidase subunit I gene. Results revealed ability of DNA sequencing to recognize fish species in very transformed seafood preparations, being able to reveal wrong labelling of the product.

Keywords: *Fraud, DNA, COI, Bold*

1. Introduction

In the last decade, the increase in the consumption of fishery products and, in particular, of cephalopods and their handling of goods has increased the spread of food fraud to the detriment of the consumer. To confirm this, the control activities carried out by the competent authority has detected a significant increase in commercial fraud in foodstuffs intended for human consumption. The large number of seizures accounted for about 40% of non-compliant labeling. A sector very impressed from fraud is related to fishing industry. The most common cases of fraud in official controls in this area are represented by the replacement, partial or total, of a fish species with another less valuable. The correct identification of species is therefore a crucial step in the quality control of foods in order to avoid fraud (1). The identification of species, carried out by evaluating the morphological characteristics of the whole fish, (Law No. 125 of 25.03.1959), it becomes very difficult, if not impossible, to carry out in some products (fillets, sticks, nuggets) as the morphological features are partially or completely lost during the technological transformation to which the food product is subject (1). In this context, it is essential to have an analytical method able to identify, unequivocally and rapidly, the species object of fraud for both health and commercial implications, related to incorrect labeling. In this regard, in recent years it has significantly developed the use of molecular investigations based on the analysis of polymorphic DNA sequences in the mitochondrial genome. These techniques are already widely used for the identification of species in many fields: medical, forensic and food (2). In this paper, we analyzed 21 food products fish based by analyzing the sequence of a fragment (655 bp) of the cytochrome oxidase subunit I gene (COI). The results, very encouraging, have allowed us to reveal, for cod based products, the presence of species different from that stated on the label of food product. Fraud, which involved the use of a species of lesser value than that declared and permitted by law, would never be detectable by morphological analysis, since the product was greatly transformed.

2. MATERIALS AND METHODS

2.2. Materials

Food preparations based on fish and cephalopod species were analyzed. Among these samples, 17 were frozen commercial products and 4 were fish products undergo various technological treatments (salting, cooking, smoking, put in oil). All samples we have been provided by the Harbourmaster as part of routine investigations. The type of sample and the fish species identified are reported in Table 1.

2.2. DNA extraction and PCR amplification

THE DNA was extracted from test samples (200 mg) using the "QIAGEN" kit according to the manual instructions. The mitochondrial DNA by COI was amplified by the following primers (3):
FishF: 5'-TCAACCAACCACAAAGACATTGGCAC -3 '
Fish R: 5'-TAGACTTCTGGGTGGCCAAAGAATCA -3 '

The reaction mixture (50µL) included: 25µL of 2x Master Mix (Applera), 1µL of each primer (10µM), 18µL of water DNAse / RNAse free and 100 ng of DNA extracted. The reaction temperature profile was the following: 1 ° denaturing step of 15 min at 95 ° C, 35 cycles of: 30 sec at 94 ° C, 40 sec at 52 ° C, and 1 min at 72 ° C, a step of final elongation of 10 min at 72 ° C. The PCR products have been displayed on capillary electrophoresis Qiaxel.

2.2. PCR sequencing

The PCR products were purified using QIAquick PCR purification kit (Qiagen) and subsequently subjected to bi-directional sequencing PCR using the ABI PRISM Big Dye3.1 Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manual instructions. The sequences were then purified by dye terminator DyeEX 2.0 spin kit (Qiagen) and then subjected to capillary electrophoresis instrument with the ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed by software SeqScape v.2.5 (Applied Biosystems) and multiple alignment software CLUSTAL W version 1.5. The sequences obtained from the COI gene were analyzed through BOLD Identification System (IDS) (www.boldsystems.org).

3. RESULTS

The Food NucleoSpin kit used for extraction of DNA allowed us to obtain a good quality DNA. The analysis of the COI gene sequence has allowed to identify the species of fish in most of the tested food products, despite the strong technological transformations suffered. In all food products containing only one species of fish it has been possible to identify the species present. In some cases, the sequence analysis has confirmed to the label (of battered cod fillets, slice of smoked tuna, anchovies, smoked marlin). For other samples where in the ingredients was reported only the words "fish", it was possible to provide the species. An example is the sea pancakes and steaks from the sea (see Table 1) which is evidenced to be made both by *Gadus chalcogrammus*. The most significant results concern the cod fillets (sample 1), which were found to be comprised of a different species from that indicated on the label. As showed from the alignment results of gene sequences (Table 2) the species identified was Pollachius virens instead of the Gadus morhua species. This result was obtained for 3 similar food samples with different commercial brands. Likewise, Pollachius virens has been identified as the species also present in the sample 17 (dried cod morsels) on which label is only reported the words " 60% cod".

Table 1: analyzed samples and fish species identified

	Nome commerciale*	**Indicazione etichetta**	**Specie rilevata**
1	Battered*cod fillets	*Cod (Gadus Morhua)*	*Pollachius virens*
2	Battered*cod fillets	*Cod (Gadus macrocephalus)*	*Gadus macrocephalus*
3	Fish cutlets *	*Fish 30%*	*Gadus chalcogrammus*
4	Seafood pancakes *[1]	*Fish 51%*	*Gadus chalcogrammus*
5	Claws surimi *	*fish flesh*	*Mixture of fish*
6	fish sticks *[1]	*Fish*	*Merluccius productus*

7	Fish cutlets *	*Fish 53%*	*Merluccius spp.*
8	Ocean fish slice*	*fish flesh 38%*	*Gadus chalcogrammus*
9	Precooked fish sticks *[1]	*Fish 50%*	*Merluccius gayiagay*
10	Sea food cutlets*	*Fish*	*Gadus chalcogrammus*
11	Surimi*	*fish flesh 38%*	*Gadus chalcogrammus*
12	Fish sticks*	*fish flesh 38%*	*Miscele di più specie*
13	Surimi stocks *	*Surimi (fish pulp and squid)*	*Miscele di più specie*
14	Marinated anchovies	*Engraulis Encrasicolus*	*Engraulis Encrasicolus*
15	Anchovies fillets	*Engraulis Encrasicolus*	*Engraulis Encrasicolus*
16	Surimi law *[1]	*Fish flesh*	*Fish mixtures*
17	Cod cutlets *[1]	*Cod 60%*	*Pollachius virens*
18	Smoked tuna	*Thunnus albacares*	*Thunnus albacares*
19	Smoked macaira	*Makaira indica*	*Makaira indica*
20	Octopus spp*	*Octopus spp*	*Octopus spp*
21	Dosidicus gigas	*squid*	*squid*

* frozen foods

[1] Breaded product

Table 2: Scores alignment obtained by CLUSTAL

Multiple Sequence Alignments
Sequences (1:2) Aligned. Score: 97
Sequences (1:3) Aligned. Score: 89
Sequences (1:4) Aligned. Score: 89
Sequences (2:3) Aligned. Score: 92
Sequences (2:4) Aligned. Score: 91
Sequences (3:4) Aligned. Score: 97

Sequ 1: campione

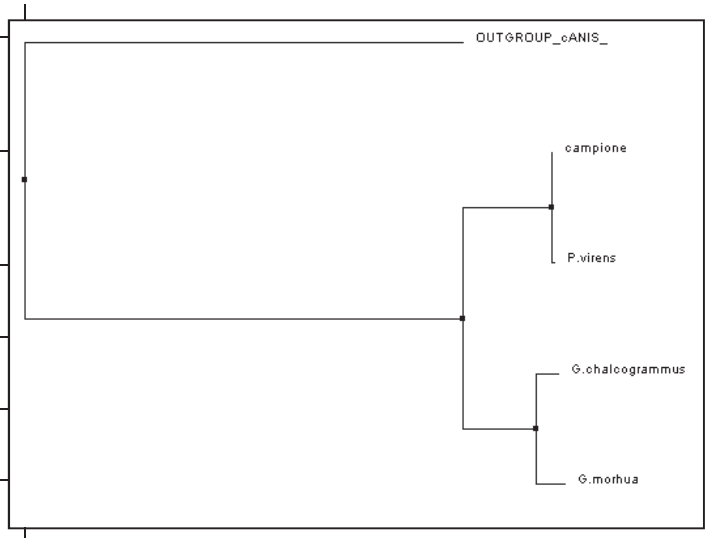
Sequ 2: *P. virens*

Sequ 3: *G.chalcogrammus*

Sequ 4: *G.morhua*

For samples mentioned above (1.17), commercial fraud is detected as by law the cod includes only two species: Gadus morhua and Gadus macrocephalus. The species identified (Pollachius virens) is filogenetically similar to these species (Fig. 1) , also belongs to the Gadidae family, but it has a lesser commercial value.

Moreover, in some cases it has been found the replacement of Octopus vulgaris with other species belonging to the family of cephalopods but of lesser value (in this case the samples were provided by UNISANNIO only for research purposes).



Phylogenetic tree obtained by Neighbor-Joining method.

Fig. 1 Phylogenetic tree

In conclusion, we were able to perform species identification for products subject to many different technological treatments: filleting, cooking, freezing, smoking, salting, marinating, breading. None of these processes has tainted DNA analysis allowing to reveal, where present, important commercial fraud. Currently, in Italy the only valid technique on a legal level, the morphological identification, does not apply to processed fish products and processed foods, which are nevertheless preferred by consumers because more practical to use. The use of biomolecular methods can solve the problem related to the identification

of species in handled products and processed, as the DNA allows a very specific discrimination between species and is stable to numerous treatments that take place during the processing of foods (4); also, the automation associated with molecular techniques makes them suitable to carry out large-scale analysis in an automated way, quickly and economically. It should be emphasized that a limit of the analysis of the DNA sequence is represented by the presence in the food product of more species. In this case, the sequencing has not allowed the identification of the contained species. This is because, since the primers are universal, each species has provided, during the sequencing reaction, the specific electropherogram, leading to an overlap of the same. However, the only information that is obtained for these samples is the presence of the ingredient "fish". The products in question generally do not have a label indicating the species contained but only the words "fish or fish flesh": the analysis then provides the information requested, or the presence of fish tissue in the food.

REFERENCES

[1] Pepe T, Trotta M, Di Marco I, Cennamo P, Anastasio A et al. (2005) Mitochondrial Cytochrome b DNA Sequence Varations: An Approach to Fish Species Identification in Processed Fish Products. Journal of Food Protection 68: 421-425

[2] Botti S, Giuffra E (2010) Oligonucleotide indexing of DNA barcodes: identification of tuna and other scombrid species in food products. BMC Biotechnology 10: 60.

[3] Ward RD, Zemlak TS, Innes BH., Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B. 360: 1847-1857.

[4] Hajibabaei M, Smith MA, Janzen D H, Rodriguez JJ, Whitfield J B, Hebert PDN (2006) A minimalist barcode can identify a specimen whose DNA is degraded. Molecular Ecology 6: 959–964.

[5] Espineira, M., and Vieites, J.M. 2012. Rapid method for controlling the correct labeiling of products containing common Octopus (Octopus vulgaris) and main substitute species (Eledone crrhosa and *Dosidicus gigas*) by fast real time PCR. Food Chem. 135 (2012) 2439-2444