COMMERCIAL FISH SPECIES IDENTIFICATION WITH ISOELECTRIC FOCUSING: APPLICATION TO BREADED FISH PRODUCTS

[IDENTIFICATION DES ESPÈCES COMMERCIALES AVEC LA FOCALISATION ISOÉLECTRIQUE: APPLICATION AUX PRODUITS DE LA PÊCHE]

by/par

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Abstract

The globalization of fish and fishery products markets combined with the increase of consumer demand lead to a huge variety of non-endemic fish species in the marketplace. This might raise concerns about the inability of fish inspectors to distinguish fish species with similar morphological characteristics, particularly when they are processed and sold as fillets or slices in the country of origin, and to detect fish substitution, a practice where high value species are mislabeled and/or substituted in whole or in part with low value, species or products with potential toxins.

Several biochemical methods have been developed in order to identify fish species. The isoelectric focusing (IEF) of the water soluble sarcoplasmic proteins proved to be suitable, fast and reliable. A project funded by the Italian Agricultural Ministry aimed at identifying fish species with IEF has recently been concluded. The results demonstrated the suitability of the method in identifying most of the commercial fish species. The database developed contains the patterns of more than 250 fish species, and the software is able to compare the IEF patterns and identify unknown species.

In the present paper we show the application of the IEF method to semicooked products in order to demonstrate the usefulness of the method also in the identification of species used in breaded fish products (breaded fish fingers and fillets). The importance and advantages of using the IEF technique in the African fish industry exporting to Europe in the context of self-control and certification program are also highligthed.

Key words: Isoelectric focusing (IEF), Breaded fish products, Commercial fraud, Traceability

Résumé

La globalisation des marchés de poisson et produits de la pêche combinée à la demande croissante du consommateur conduit à une grande variété d'espèces non endémiques de poisson sur le marché de poisson. La conséquence directe est l'incapacité pour les inspecteurs du poisson de distinguer les espèces de poisson ayant des traits morphologiques similaires, en particulier quand elles sont transformées et vendues comme filets ou tranches dans le pays d'origine. Une identification correcte du poisson est fondamentale pour éviter une substitution frauduleuse.

Plusieurs méthodes biochimiques ont été développées dans le but d'identifier les espèces de poisson. La focalisation isoélectrique (IEF) sur les protéines sarcoplasmiques hydrosolubles a montré sa pertinence, sa rapidité et sa fiabilité. Un projet financé par le Ministère italien de l'agriculture visant à identifier les espèces de poisson avec IEF a été récemment accompli. Les résultats ont démontré la pertinence de la méthode dans l'identification de la plupart des espèces commerciales de poisson. La base de données contient les modèles de plus de 250 espèces de poisson, et le logiciel est capable de comparer les modèles IEF et identifier des espèces inconnues.

Dans le document présent nous avons appliqué la méthode IEF aux produits semi-cuits pour démontrer l'utilité de la méthode également dans l'identification des espèces utilisées dans les produits de poisson pané (bâtonnets et filets de poisson pané).

Mots clés: Focalisation isoélectrique (IEF), Produits de pêches panés, Fraude commerciale, Traçabilité

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1. INTRODUCTION

Consumers, and particularly children, like breaded fish products due to the enriched flavour, lack of bones and easiness to prepare. For their manufacture different fish species belonging to Order of Gadiformes and Pleuronectiformes are used. In the marketplace these products are labelled as breaded cod fish fingers, breaded cod medallions and breaded plaice fillets. The fish species declared on the label and mostly used for the manufacture of cod fish fingers and cod medallion are the Argentine hake (*Merluccius hubbsj*) and the shallow water Cape hake (*Merluccius capensis*), while for the plaice fillets the specie commonly used is the plaice (*Pleuronectes platessa*).

Current EU legislation does not require a compulsory indication on the label of the scientific or common denomination of the species used in the processed fish products. According to traceability and transparency requirements specified in the White Book and subsequent EU Regulations, consumers must be provided with all information regarding ingredients and species used in the manufacturing of any food products, including fish products.

In order to check if processed food products are properly labeled and to identify fraudulent use it is necessary to employ an analytic laboratory method for the identification of species.

The Isoelectric focusing (IEF) on polyacrylamide gels of the sarcoplasmic proteins proved to be particularly effective for the identification of species on fresh and frozen fish products (Lundstrom, 1979; Rehbein,1990; Tepedino, 2001). In processed and thermal treated products, proteins can be denatured and might lose their own characteristics. This often leads to an IEF pattern altered compared to the standard, and therefore not identifiable. However, most breaded products (fish fingers, medallions and fillets) are quickly pre-fried in oil and such heat treatment does not seem to alter the proteins.

2. OBJECTIVES

The purpose of this research was to use the IEF technique to identify fish species used to prepare breaded cod fish fingers, and breaded plaice fillets. A preliminary market investigation was carried out in Milan (Italy) with the purchase of fifteen packages of processed and quick frozen fish products of different brands with the selling denomination of “Breaded Cod fish fingers”, “Breaded Cod medallions” and “Breaded Plaice fillets”.

3. METHODOLOGY

Frozen breaded fish fingers, medallions and fillets were cut in the central part in order to sample the muscular tissue. Special care was used to avoid sampling the breaded parts. 0.2–0.5 g of tissue were collected using a scalpel, and suspended in H_2O to obtain a concentration of 1 g/ml. The protein extraction was carried out as previously described (Berrini, 2005; Tepedino, 2004; Tepedino, 2003).

The extracted proteins were analyzed with the IEF technique described earlier. Briefly, 30 μg of extracted proteins were loaded on a gel for IEF (GelBond PAG pH 3.5–9.5, GE Healthcare). The separation was performed at 10 °C, applying constantly 30W, with a maximum voltage of 1500V. The run time was 90 min. At the end of the run, the gel was fixed on TCA, acetylsalicylic acid for 60 min and stained with Coomassie Brilliant blue. The gel image was acquired using a scanner with a resolution of 300 points/cm and inserted into the data-base to perform the pattern analysis (Gel Compar II, AppliedMath, Saint-Martens, Belgium).

4. RESULTS

The IEF patterns obtained from breaded products were perfectly identifiable in comparison with the standard patterns obtained from the fresh products. This confirms that the quick pre-fry does not alter sarcoplasmic proteins of the inner part of breaded fish.

Figure 1 shows an example of the standard patterns of Argentine hake (*Merluccius hubbsi*), Cape hake (*Merluccius capensis*), plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*) and the patterns obtained from breaded fish fingers and fillets. The patterns from unknown breaded samples are perfectly comparable with the patterns from standard species. This allows a certain identification of the species used in the preparations.
Table 1 reports the results obtained from the 15 products analysed.

As far as cod-based products are concerned, the species identified were the Argentine hake (*Merluccius hubbsi*) (n. 5), the Alaska pollack (*Theragra chalcogramma*) (n. 2), the Cape hake (*Merluccius capensis*) (n. 2) and the blue whiting (*Micromesistius poutassou*) (n. 1). The results showed that for the manufacture of breaded products, different fish species with diverse commercial value and quality are commonly used. In fact, the generic term "cod" includes different species belonging to either the Gadidae family or Merluccidae family. Since the indication on the label of the scientific denomination of fish species used in processed fish products is not compulsory, the majority of the analysed samples complied with current legislation. The only products not complying with the legislation were those containing Alaska pollack and Blue whiting, that cannot be labelled with the generic term "cod".

Regarding the samples of breaded plaice fillets, only 1 out of 5 was manufactured using the species *Pleuronectes platessa*, while the others were made with the Dab (*Limanda limanda*). It has to be noted that for all these commercial products the species reported on the label was *Pleuronectes platessa*. This is an illegal labelling.

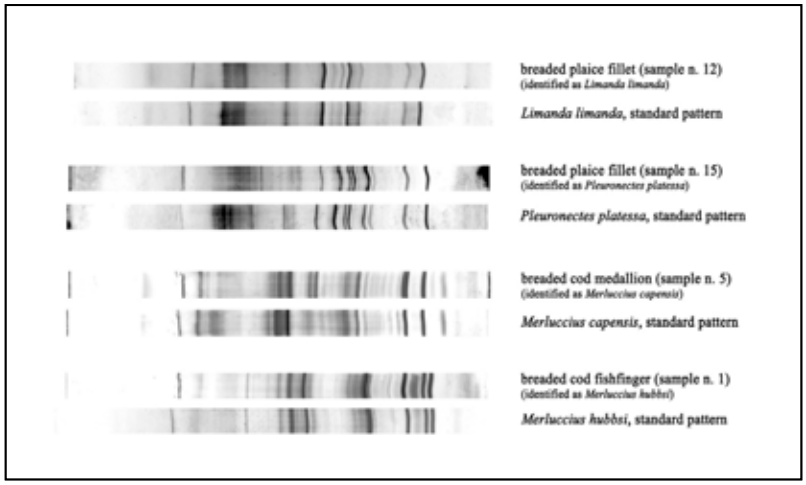

Figure 1. Standard patterns of some fish species and the patterns of breaded fish fingers and fillets

Table 1. Market research

Sample n.	Selling denomination of the product	Fish species indicated on the label (where present)	Fish species actually used and detected with IEF
1	Breaded cod fishfingers	*Merluccius hubbsi*	*Merluccius hubbsi*
2	Breaded cod fishfingers	*Merluccius hubbsi*	*Merluccius hubbsi*
3	Breaded cod fishfingers	*Merluccius hubbsi*	*Merluccius hubbsi*
4	Breaded cod medallions		*Theragra chalcogramma*
5	Breaded cod medallions		*Merluccius capensis*
6	Breaded cod medallions		*Theragra chalcogramma*
7	Cod slices in crunchy breading		*Merluccius capensis*
8	Cod slices in crunchy breading		*Micromesistius poutassou*
9	Cod slices in crunchy breading		*Merluccius hubbsi*
10	Cod slices in crunchy breading		*Merluccius hubbsi*
11	Breaded plaice fillets	*Pleuronectes platessa*	*Limanda limanda*
12	Breaded plaice fillets	*Pleuronectes platessa*	*Limanda limanda*
13	Breaded plaice fillets	*Pleuronectes platessa*	*Limanda limanda*
14	Breaded plaice fillets	*Pleuronectes platessa*	*Limanda limanda*
15	Breaded plaice fillets	*Pleuronectes platessa*	*Pleuronectes platessa*

5. CONCLUSIONS

Electrophoretic methods are widely employed in investigations of food products. IEF is a particularly well known technique used for species identification in fish products, even in those partially processed (breaded preparations). The correct fish identification is fundamental to avoid fraudulent substitution as in the case of Plaice fraud described above, and is a challenge faced by consumers and regulators. The direct advantages of using the IEF technique are to:

- help the inspectors identify fish products that, due to processing, are not easily identified macroscopically, and also detect species substitution that could result in potential adverse health consequences or could be a source of economic fraud; and
- provide consumers with true information about the product and its origin. This is particularly true nowdays with a growing appreciation of these "ready to cook" products by consumers, which requires a proper label indication of fish species used and their origin.

6. SPECIAL RECOMMENDATION FOR DEVELOPING COUNTRIES

Protein IEF, which is the most popular official method for fish species identification in the United States of America, can be of great interest to developing countries, in particular the African region where, in the last decade, artisanal and industrial processing plants increased their export to the European market of fish products mostly in the form of fillets. Species commonly used for fillets production belong to *Merluccius capensis* (Cape hake), *Solea senegalensis* (Senegalese soley), *Pseudupeneus prayensis* (West African goatfish), *Lates niloticus* (Nile perch) and *Zenopsis conchifer* (Slevery John Dory). These fillets are used in the European post-processing industry to manufacture breaded fish fingers, breaded fish medallions and other products.

The economy of IEF (which with around US$15/IEF pattern is cheaper than DNA method), its practicability (no need for sophisticated laboratory and expertise) and rapidity (results obtained in less than 2 days), make this technique a practical and friendly method to use for developing countries affected by economic and laboratory infrastructure constraints. The African fish industry in the context of self-control programs, might apply IEF techniques as pre-verification tests by randomly checking batches of fillets and other fish products destined for export and, consequently, certify what is declared on the label. In Italy the IEF is being positively evaluted as a potential official method and this will help mutual verification between exporter and importer.

IEF techniques will definitely allow the African fish industry to comply with transparency and traceability requirements specified under EU legislation, add value to their products and offer better guarantees to European and worldwide consumers, providing them with correct information on the origin of, and species utilized in, processed fish products.

Ultimately the technical cooperation and information exchange between countries will pave the way for transferring this technology and make it available to African research institutions involved in fish quality and safety.

7. REFERENCES

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