

<http://foodbulletin.net>

e-ISSN: 2979-9848

<https://prensip.gen.tr>

REVIEW ARTICLE

Electrophoretic Methods for Identifying the Species of Seafood and Its Derivatives

Bahar Tokur[✉] • Koray Korkmaz

Ordu University, Faculty of Marine Science, Department of Fisheries Technology Engineering, Ordu/Türkiye

ARTICLE INFO

Article History

Received: 01.12.2023

Accepted: 21.12.2023

First Published: 31.12.2023

Keywords

Electrophoretic methods

Food adulteration

Seafoods and their products

Species identification



ABSTRACT

The identification of the species of seafood and their products, whether they are fresh or cooked, is one of the key concerns of food regulations in many countries that have a significant intake of seafood. In point of fact, a commercial fraud happens when a species that is less value is intentionally substituted for a species that is more valuable, and a sanitary fraud takes place when a product that is potentially harmful is introduced into the market. A primary responsibility of veterinary inspection of seafood products is the detection of harmful species with the aim of removing them from the retail trade (Council Directive 91/493/ECC). Two efficient methods for seafood species identification are molecular biology methods and protein electrophoresis. Classic electrophoretic methods have been used for a long time to authenticate seafood; they are simple, accurate, and inexpensive compared to molecular biological methods, which are the wave of the future in food safety labs. The purpose of this article is to offer an overview of the electrophoretic methods commonly used to identify different species of seafood, including sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), native or urea–isoelectric focusing electrophoresis (IEF), two-dimensional electrophoresis (2-DE), and capillary electrophoresis (CE).

Please cite this paper as follows:

Tokur, B., & Korkmaz, K. (2023). Electrophoretic methods for identifying the species of seafood and its derivatives. *Food Bulletin*, 2(2), 61-70. <https://doi.org/10.61326/foodb.v2i2.121>

1. Introduction

Assuring proper species identification, mode of production (cultivated or wild) and geographical origin, via “seafood authentication” verifies that seafood items have been labeled correctly. The most important part of seafood authentication is species authentication, which helps keep seafood fresh, and prevents fraud by guaranteeing market transparency. By using species identification, we can be sure that the product's commercial and scientific names are accurate representations of the species (Bozariis, 2014).

Species identification of fishery and aquaculture products is essential for ensuring fair business practices and providing accurate consumer information. According to the European

Union has established European Commission Regulation (EC) 104/2000 (EC, 2000) and 2065/2001 (EC, 2001), fish products are only allowed to be sold commercially provided the label clearly states the commercial name, method of production, and catch area. When dealing with cultivated species, it is necessary to provide the appellation of the country name of the nation in which the product will be subjected to the ultimate stage of its growth. When these standards are followed, consumers are given more options to choose from, and they are provided with complete and accurate information about the product. These regulations may be successfully implemented provided comprehensive species-specific data for all fish species are accessible, in order to avoid the replacement of economically significant fish.

[✉]Correspondence

E-mail address: baharorhun@gmail.com



The challenges associated with identifying fish species in fish products are attributed to many reasons, notably (Civera, 2003):

- The process of market globalization has led to a significant increase in the number of various species that need to be studied.
- A significant quantity of processed fish products that do not possess the morphological features necessary for the conventional identification process.
- Inadequately trained individuals used in the identification of species.
- Labelling fish species is essential to prevent adulteration, as it helps to avoid fraudulent substitution of fish species with varying values and prices.

The range of fish items available on the market is quite varied. Furthermore, they provide an assortment of processed fish goods, including fish balls, fish steaks, canned fish, and other similar items. Also, given that the majority of fish are imported in the form of compressed flesh blocks or fillets, it is not feasible to identify them based on surface features. Due to the advancement of processing technology, the conventional practice of using fish appearance to identify species has become less successful, leading to a potential rise in the occurrence of substitutes. The primary approach for identifying fish species is explicitly differentiating them based on their physical traits or morphology, including skeletal structure, muscles, branching taxonomy, as well as fin characteristics, scales, and life history. Nevertheless, the visual attributes of intact fish sometimes prove inadequate for precise differentiation of fish species, and the identification of processed fish products poses an even greater challenge (Chien et al., 2022).

Protein analysis methods center on a number of physical distinctions, including size, net charge, and amino acid content, among others. In the late 1970s, the first protein markers were created by determining the water solubility of proteins and isozymes using electrophoresis in polyacrylamide or starch

gels. These markers paved the way for new understanding of the genetic diversity and structural variation across populations, species, and variations hailing from all over the world. As a result of the fact that proteins are the final product of gene expression, which can differ from tissue to tissue, developmental stages, environments, and seasons, this method has a significant drawback: it couldn't detect enough variation to distinguish between related varieties or species. (Hubalkova et al., 2007; Lago et al., 2014).

Protein electrophoresis and molecular biological methods are the two primary approaches to species identification in cases where the fish has lost its biological characteristics (Etienne et al., 2001). Classic electrophoretic methods have proven to be reliable, easy to apply by food control laboratories, and, at the moment, still less sophisticated and less expensive than molecular biological methods (Piñeiro et al., 1999a, 1999b), despite these latter methods representing the future in food control laboratories. Several electrophoretic methods are available, and the choice of methodology relies on the required level of resolution and the type of the material being analyzed (e.g., fresh, frozen, heat treated, etc.). To prevent the adulteration of fish species and protect consumer rights, fish species have been successfully identified using classic electrophoretic, such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), native or urea-isoelectric focusing electrophoresis (IEF), two-dimensional electrophoresis (2-DE), and capillary electrophoresis (CE) (Rehbein et al., 1999; Civera, 2003; Hubalkova et al., 2007). For the purpose of identifying the species of raw and heat-processed fish, Piñeiro et al. (1999a) and Etienne et al. (1999) optimized the standard operating procedures of SDS-PAGE and urea-IEF (Mackie et al., 2000; Etienne et al., 2001a, 2001b). The protein electrophoresis technology remains more cost-effective and less complex to run compared to DNA-based approaches (Piñeiro et al., 1999a, 1999b). Table 1 shows various methodologies based on protein analysis for the identification of seafood (Civera, 2003).

Table 1. Different approaches to seafood identification based on protein analysis (Civera, 2003).

Fishery product	Methods	Compounds to be analyzed	Drawbacks	Reference
Raw fish and raw products	Electrophoretic § IEF	All sarcoplasmic proteins	Heat denaturation; polymorphism	Durand et al. (1985), Rehbein (1990), Rebhein et al. (1995), Colombo et al. (2000), Tepedino et al. (2001)
Raw and processed fish	§ IEF	Acid, calcium-binding proteins (parvalbumins)	High concentration in white muscle	Esteve-Romero et al. (1996)
	Specific enzymic detection, IEF	Enzymes in sarcoplasmic proteins (LDH and G-3-PD)	Heat denaturation	Piñeiro et al. (2001)
	Capillary zone electrophoresis	Sarcoplasmic protein	Heat denaturation	Larrain et al. (2002)
Heat-processed	SDS-PAGE	Sarcoplasmic and myofibrillar proteins	Faint variability in fish protein pattern (no identification in closely related species)	Civera and Parisi (1991), Etienne et al. (2001)
	Urea-IEF	Sarcoplasmic and myofibrillar proteins	Good identification only in species rich on parvalbumins	Etienne et al. (2001)

2. Native or Urea-Isoelectric Focusing Electrophoresis (IEF)

The method of isoelectric focusing of sarcoplasmic proteins, which make up 20 to 35% of fish muscle proteins, is well recognized for species identification of seafood, and its reliability has been evaluated in several research (Rehbein, 1995; Mackie, 1996). Arginase (ARGK), arginin kinase (CK), malate dehydrogenase (MDH), adenylate kinase (AK), aldolase, glycerol 3-phosphate dehydrogenase (G 3-PD), lactate dehydrogenase (LDH), and other proteins like parvalbumin, myoalbumin, and globulin are all part of the sarcoplasmic proteins. Because various fish species exhibit different band patterns when separated using the isoelectric focusing method, the proteins found in the sarcoplasmic fraction are ideal for this purpose (Recio et al., 2001). The process of sarcoplasmic protein separation in IEF is achieved by using their isoelectric point, employing distinct pH ranges such as 'wide range' (3-10) and 'narrow range' (3-6) (Civera, 2003). The IEF approach relies on the separation of molecules on a gel, which is accomplished by adding an ampholyte to create a pH gradient (Hubalkova et al., 2007). Specifically, the anode may be used for species identification by using the acidic proteins that are located nearby, with pI values ranging from 3.5 to 5.5. These proteins are small in size and have the feature of binding to calcium (Verrez-Bagnis et al., 2017).

Many fish species for species identification of nonheat treated fish musculature and products that have been identified using IEF technology include; snapper, catfish, *Sparidae* species, tilapia, barramundi, different Atlantic and Aegean species, spearfish and swordfish, red snapper, puffer fish, tunas, mackerels, bonitos, flat fish and perch species in combination with two-dimensional electrophoresis or *gadoid* species through the use of auxiliary enzyme detection in the detection of food fraud (Verrez-Bagnis et al., 2017). Most sarcoplasmic proteins may be dissolved in aqueous solutions and undergo denaturation when exposed to heat treatment. Rehbein et al. (1999) demonstrated that IEF approach is more effective than conventional electrophoresis in distinguishing sarcoplasmic proteins due to its greater selective capacity. The IEF method was used to construct a library of commercial fish species that were classified as either *Gadiformes* or *Pleuronectiformes* based on the sarcoplasmic proteins they contained (Tepedino et al., 2001). It was possible to differentiate gadoid fish species due to the presence of certain enzymes in the interferon-enzymatic fraction (IEF) of sarcoplasmic proteins in the pH range of 3.5-9.5. They are ARGK, LDH, G 3-PD, AK, CK, and MDH, among others. The mobility of LDH and G 3-PD on electrophoresis was used to discriminate most of the gadoid fish species investigated by Piñeiro et al. (2001), including closely related members of the *Merlucciidae* family. Ataman et al. (2006) also employed the IEF method of sarcoplasmic proteins

to distinguish between nongadoid and gadoid species; a related methodology has been developed.

Protein configuration is additionally impacted by freezing because the amount of water that is available in frozen products decreases. As a result, proteins tend to aggregate and precipitate, which makes it difficult to extract proteins and obtain reproducible results from the methodology (Shenouda, 1980).

Examinations of fish from the genera *Thunnus* or *Tetrapturus* revealed similar patterns among closely related species. Occasionally, protein polymorphism leads to variances in patterns among specimens of the same fish species, which makes it difficult to assign samples. The charge polymorphism of proteins, observable by IEF, may be affected by either genetic variables (such as the expression of different alleles of a single gene) or by post-translational metabolic processes (e.g., phosphorylation). Various protein patterns have been recorded in several species of fish and mollusks. Polymorphism poses difficulties and impedes food management efforts, although it also forms the basis for the electrophoretic separation of distinct populations of fish species. In order to accomplish this objective, a range of isoenzymes are extracted (often by electrophoresis on starch gels) and rendered detectable using staining methods specific to each enzyme. To provide reliable and accurate outcomes from IEF of fishery products, it is essential to consider several factors that might influence protein patterns (Rehbein, 1990).

Two varieties of fish may be recognized based on the color of their fillet: white-fleshed fish (such as cod and plaice) and red-fleshed fish (such as sardine, mackerel, and tuna). Fishes belonging to the latter category possess a greater amount of myoglobin and a larger proportion of red muscle compared to fishes with white meat. White and dark muscle exhibit distinct variations in their physiological function and metabolic processes. White muscle is distinguished by elevated levels of glycolytic enzymes, reduced quantities of chromoproteins, and a high concentration of parvalbumins. Hence, it is unsurprising that the protein patterns of both muscle types exhibit variations in terms of the quantity, location, and intensity of protein bands when subjected to analysis using IEF. White muscle may often be differentiated from red muscle by its prominent anodal bands, which are indicative of parvalbumins and perhaps additional unidentified acidic proteins. Both forms of muscle exhibited distinct protein patterns that were unique to each fish species. With the exception of a few cases, red muscle has not been used for the purpose of species identification. Based on the above statements, it is evident that in order to get consistent findings using electrophoresis, it is necessary to separate white muscle from blood, red muscle, or any other tissues (Rehbein, 1990).

Identification of species is done by analyzing the parvalbumin pattern or the whole protein pattern. In the white

muscles of several fish species, including *Pleuronectiformes* and *Gadiformes*, you may find parvalbumins, which are acidic, calcium-binding, heat-stable sarcoplasmic proteins with a low molecular mass (12 kDa) (Esteve-Romero et al., 1996). Identifying a species of fish is as simple as comparing its pattern to a standard. The USFDA has produced a Regulatory Fish Encyclopedia that includes IEF patterns of sarcoplasmic proteins from 76 species that are widely sold in North America (Available online at <https://wayback.archive-it.org/7993/20170406002931/https://www.fda.gov/Food/FoodScienceResearch/RFE/ucm219129.htm>). Unfortunately, there are currently no protein patterns for toxic seafood. Not only has IEF been used effectively on raw fish, but it has also been used to cooked fish, smoked fish products, and sturgeon eggs. The proteins are dissolved using urea or SDS, and the electrophoretic pattern is stained with silver (Etienne et al., 1999; Piñeiro et al., 1999a; Rehbein et al., 1999).

Based on their research, Renon et al. (2005) concluded that IEF is an easy and dependable way to spot blue marlin steaks that have been interchanged for Mediterranean spearfish (*Tetrapturus belone*) and cold-smoked swordfish (*Xiphias gladius*) fillets that have been fraudulently switched out for lower-value blue marlin (*Makaira mazara*).

Regarding the application of IEF to shrimp authentication, a study with sarcoplasmic proteins succeeded to differentiate generic shrimp from fish and lobster (Rehbein, 1995; Ortea et al., 2010). The specific proteins, which belonged to the class of calcium-binding polypeptides, were in the 4-5 pI range, and resulted to be heat stable. For this reason, such proteins provided species-specific patterns for cooked products as well as in raw samples. In the same work, a similar method based on IEF in urea gels was applied to the analysis of products in which the sarcoplasmic proteins had been previously removed by washing steps during processing, this strategy allowed the differentiation of shrimp from fish and cephalopod meat. Although specific bands were detected in heated samples, the shrimp species could not be differentiated due to protein loss and band clustering (Ortea et al., 2012).

Proteins are denatured and precipitated during heat treatments. Due to the difficulty in identifying species using electropherograms of the sarcoplasmic proteins, this method is only useful for analyzing foods that are either slightly cooked, frozen, or very fresh. To employ electrophoretic methods in cooked foods, one must first extract the precipitated proteins using urea, which enables denaturing-condition protein extraction (Bozariis, 2014). According to Ortea et al. (2012), several studies have shown that IEF is not appropriate for heated or marinated food because it causes the breakdown of some muscle proteins under these settings.

Urea-isoelectric focusing (An et al., 1988) can be used to analyze heat-denatured muscle protein that has been extracted using denaturing solvents containing urea. Rehbein et al. (1999)

and Etienne et al. (1999) optimized the urea-IEF methods for identifying cooked fish species. The IEF approach can only be used for heated products of fish species that exhibit a distinct pattern with the heat-stable parvalbumins (Rehbein, 1992). In general, the process of heating may cause the denaturation of myofibrillar and sarcoplasmic proteins in fish muscle. To solubilize these proteins, a chaotropic agent like urea or a detergent like sodium dodecyl sulfate (SDS) can be used. The proteins generated using this method may be further examined using urea-IEF (An et al., 1988).

Etienne et al. (2001) conducted a comparative analysis of *Gadus morhua*, *Theragra chalcogramma*, *Pollachius virens*, *Macruronus novaezelandiae*, and other fish species in terms of their electrophoretic distinguishability using the urea-IEF, SDS-PAGE, and native IEF procedures. Each of the three methods effectively distinguished between different types of processed fish. However, SDS-PAGE and only urea-IEF were shown to be useful in identifying fish species in products treated with high pressure. However, it was also shown that urea-IEF is less precise than SDS-PAGE in distinguishing between salmonoid fish and tuna fish, since the proteins of the latter exhibit neutral or alkaline properties. SDS-PAGE failed to differentiate between *Gadus morhua* and *Theragra chalcogramma*, despite its ability to differentiate other gadoid fish species. According to authors, if proteins remain in their original structure (not denatured), native IEF may be used.

Denaturing urea-IEF has shown better efficacy in identifying species in high pressure-treated products (Etienne et al., 2001). It may also serve as an alternate method for determining the species of fried fish, depending on the specific product (Rehbein et al., 1999; Etienne et al., 2001; Verrez-Bagnis et al., 2017).

According to Etienne et al. (1999), proteins undergo denaturation and degradation in various processing techniques as cooking, frying, and smoking. As a consequence, the distinctive bands seen in IEF are lost. Regarding the products provided, we provide gravad and smoked salmonid items, as well as smoked eels., the findings of a collective investigation indicate that urea IEF is more efficient than SDS-PAGE in distinguishing between different species. (Mackie et al., 2000). The relevant research group generally suggested a mix of native IEF, urea IEF, and SDS-PAGE, with the specific ratio varying by product and species. Protein band patterns were sufficiently similar that IEF alone could not distinguish several closely related species (Verrez-Bagnis et al., 2017).

Lago et al. (2014) stated that IEF and urea-IEF provide some benefits over conventional electrophoretic techniques:

- Electrophoresis results in the proteins being confined to small zones, leading to enhanced resolution and sensitivity.
- After electrophoresis, the system reaches equilibrium, reducing the impact of any changes in the experimental

settings on the resulting protein pattern. The bands of the sample can then be compared to the bands of the reference patterns using images.

- Utilizing polyacrylamide gels produced by companies in conjunction with semi-automated machinery enhances the consistency of the outcomes and reduces the duration of analysis.

Additionally, they indicated that these IEF approaches exhibit significant disadvantages as well:

- The resulting protein profiles include several bands, making their interpretation sometimes challenging.
- This arduous approach requires skilled operators and appropriate equipment.
- The sample procedure is intricate and the technology is costly, complicating its use in laboratories that conduct food analyses (Lago et al., 2014).

3. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE is the most often used method for achieving precise separation of protein mixtures with high resolution. The process comprises the first denaturation of constituent proteins using an anionic detergent that also binds to them, resulting in all proteins acquiring a negative charge that is directly proportionate to their molecular mass. Following this, electrophoresis is conducted using a permeable acrylamide gel matrix, which effectively separates proteins based on their molecular mass with high precision (Walker, 1996). The SDS detergent is appropriate for extracting denatured proteins in studied materials, making it a good approach for determining species that have undergone heat treatment (Članjak–Kudra et al., 2021).

Myofibrillar proteins, sarcoplasmic proteins, and total muscle homogenates are the three types of muscle extracts that SDS-PAGE has been proposed by many authors as an alternative to IEF methods. Certain items, such as imitation crab sticks, crab claws, and lobster tails, were specifically required to undergo SDS-extraction. These products are crafted from minced fish flesh (surimi), which has been extensively washed to remove the majority of water-soluble proteins. Peptide mapping of the myosin heavy chain employing restricted proteolysis for fish identification was suggested as a solution to tackle the most challenging cases (Esteve-Romero et al., 1996).

Gel electrophoresis with sodium dodecylsulfate is a useful tool for analyzing heat-denatured muscle protein that has been extracted with denaturing solvents including urea or SDS. Solution methods like 2% sodium dodecyl sulphate (SDS) allow for the dissolution of heat-denatured proteins, which may then be separated using SDS-PAGE. Methods for identifying species of cooked fish using SDS-PAGE were improved by Rehbein et al. (1999) and Etienne et al. (1999). When

employing the urea-IEF and SDS-PAGE methods for fish species differentiation, proteins with a low molecular weight, such as myosins (14-23 kDa), troponins (19-30 kDa), and parvalbumins (12 kDa) are the most suitable options (Etienne et al., 1999; Piñeiro et al., 1999a; Rehbein et al., 1999). Additionally, SDS-PAGE has shown improved species identification in high pressure-treated products (Etienne et al., 2001), and there may be different methods for cooking fish species depending on the product type (Rehbein et al., 1999; Etienne et al., 2001; Verrez-Bagnis et al., 2017). But it is inefficient for differentiating between canned fish species (Mackie et al., 1999).

While Civera and Parisi (1991) did find some subtle variation in SDS-PAGE patterns across generic shrimp and crabs, they were unable to distinguish between individual species within either group. Pink shrimp (*Farfantepenaeus duorarum*), white shrimp (*Litopenaeus setiferus*), and rock shrimp (*Sicyonia brevirostris*) were successfully distinguished using SDS-PAGE, as shown by An et al. (1988). Specific electrophoretic patterns for each species were observed in the water-extractable protein fraction of the raw specimens. Protein precipitation made species identification impossible when water-soluble samples were heated; under these conditions, only an extraction with SDS allowed for the separation of the two genera (Ortea et al., 2012).

Using SDS-PAGE protein patterns, Rouxel et al. (2001) determined that four different types of seaweeds could be identified. Species such as *C. crispus* and *G. verrucosa* stand out as having quite different characteristics when compared. That notwithstanding, SDS-PAGE seems to be a practical way to identify red seaweeds that are approved for usage as sea veggies or food components. Additives like carrageenans and agar are also made from bacteria like *C. crispus* and *G. verrucosa*. It is possible to regularly utilize electrophoresis on raw materials for food phycocolloid manufacturing. However, Fleurence et al. (1995) demonstrated that the technology was specifically tested on raw material SDS-PAGE and effectively used under certain circumstances to differentiate between two *Ulva sp.* that are often eaten as sea vegetables.

The procedures for protein extraction from the SDS solution and SDS-PAGE electrophoretic analysis were detailed by Piñeiro et al. (1999a). Salmonid fish that had been smoked or marinated could not be positively identified using the SDS-PAGE method. There was hard to distinguish the Atlantic salmon (*Salmo salar*) from the smoked sea trout (*Salmo trutta*) since they seemed so identical as well. The rainbow trout (*Oncorhynchus mykiss*) did show some variation, but the differences found were not strong enough to be identified with certainty. Differentiating smoked arctic char (*Salvelinus alpinus*) from other processed fish was a breeze. But we were able to identify every single raw reference species. The SDS-PAGE method was ill-suited for detecting treated substances

because the changes in protein band separation were very minute. There is little effect of smoking and marinating on the protein separation achieved by SDS-PAGE and IEF using urea methods. Because of this, it is somewhat more difficult to distinguish between closely related species.

The species of salmon and trout may be identified by native electrophoretic, SDS-PAGE, and IEF, as stated by Mackie et al. (2000). Although there are more protein bands produced by the SDS-PAGE technique compared to the IEF method for processed items, there are still not enough unique bands to reliably identify them. Therefore, when trying to differentiate between salmon and trout, the IEF method is widely regarded as the most effective. Arctic char and rainbow trout, on the other hand, were determined to be more suited to the SDS-PAGE method. Nevertheless, the previous studies have shown that eel species may be identified using both SDS-PAGE and IEF with urea procedures. In contrast, the urea-based IEF technique works better for distinguishing between the two Atlantic eel species, *Anguilla rostrata* and *Anguilla anguilla*. The indigenous IEF method is another option for differentiating the Atlantic short-finned eel (*Anguilla australis*) from its southern version. However, given the many situations surrounding smoking in the workplace, this approach just provides supplementary data (Mackie et al., 2000).

The SDS-PAGE method was used by Martinez et al. (2001) to determine the species of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), minke whale (*Balanoptera acutorostrata*), Arctic char (*Salvelinus alpinus*), and harp seal (*Phoca groenlandica*). They used a variety of gels in order to conduct an analysis on samples that had not been treated in addition to those that had been processed (cold and hot smoked, cooked, and marinated). The researchers observed that the Anderson gel exhibited remarkable proficiency in distinguishing Atlantic salmon and rainbow trout by displaying six clearly identifiable diagnostic bands. The ExcelGel exhibited three clearly distinguishable bands, but the Tris-Tricine gel showed two bands and the NuPAGE Bis-Tris gel exhibited just one band. All experimental gels, except for the ExcelGel, exhibited similar profiles when subjected to testing using the same species in both their raw and cooked forms. Additionally, it was observed that the distinctions between the marinated and smoked samples were notably different from the raw samples, resulting in increased difficulty in their identification. The achieved differentiations were inadequate to discern between the smoked and marinated rainbow trout and salmon. Furthermore, the bands that migrated the quickest exhibited blurring and warped paths on most of the gels. Estimation is an important procedure, but it should be noted that the findings may vary somewhat for the same bands based on factors such as migration lengths, temperature, buffer pH, etc. For accurate assessment of a product's authenticity, it is crucial to examine samples on the same gel, under same conditions.

Huang et al. (2010) conducted a study to determine the specific species of moray eel responsible for cases of food poisoning. The moray eel meat, along with eight other raw commercial moray eel meats (*Gymnothorax favagineus*, *G. fimbriatus*, *G. flavimarginatus*, *G. meleagris*, *G. pseudothyrsoides*, *G. undulates*, *G. albimarginatus*, and *G. javanicus*), were subjected to a causative process by heating them at 100 °C for 30 minutes. The samples were then analyzed using the electrophoresis method known as SDS-PAGE. The SDS-PAGE analysis of eight processed commercial moray eel meats revealed distinct protein bands with a molecular weight of less than 30 kD, which were peculiar to each species. The specific causative species of moray eel involved in the food poisoning case was determined to be *G. javanicus*. This conclusion was based on a comparison of the protein extracted using 2% SDS and 8 M urea, as evidenced by the SDS-PAGE patterns.

Lin et al. (2012) identified the poisonous sample as *L. bohar* based on the low molecular weight region (<30.0 kD) of both the myofibrillar and sarcoplasmic patterns. Ordinary SDS-PAGE analysis proved effective in achieving species identification, as indicated by the results.

4. Two Dimensional Electrophoresis (2DE)

2-DE enables the simultaneous separation of a large number of proteins in a single gel. In the first dimension, proteins are separated using the native technique, typically IEF, based on their PI. In the second dimension, proteins are separated using SDS-PAGE based on their MW. This method offers several advantages. Firstly, it has the capability to separate a large number of proteins simultaneously. Additionally, it can effectively separate proteins in the sample from unwanted substances through protein precipitation. Moreover, it enables the examination of similarities and differences between proteins extracted from muscles of various animal species. (Hofmann, 1997; Alikord et al., 2018).

The study conducted by Piñeiro et al. (1998) examined the profiles of water-soluble proteins in eight species of gadoid fish, including five hakes (*Merluccius spp.*), cod (*Gadus morhua*), pollack (*Pollachius pollachius*), and blue whiting (*Micromesistius poutassou*), were examined using 2D electrophoresis. Using the findings from the 2D mixed-extract analysis, they identified that among the species of *Microcystis*, one parvalbumin (10.4 kDa, pI 3.9) was shared with *M. australis* and *M. hubbsi*, while two parvalbumins (10.3 kDa, pI 4.1; 10.4 kDa, pI 3.9) were shared with *M. capensis*. They concluded that 2D electrophoresis could provide a wealth of information about the molecular weights and isoelectric points of the primary water-soluble proteins, which would be useful for distinguishing between closely related species of *Merluccius*.

Piñeiro et al. (1999b) used two-dimensional (2D) electrophoresis, gradient SDS-PAGE in the 12-14% range, nondenaturing isoelectric focusing (IEF) in the 3.5-9.5 pH range and to examine the water-soluble proteins of nine economically valuable flatfish species from the families *Pleuronectidae*, *Scophthalmidae*, and *Soleidae*. Between 3.5 and 6.9 pH, the majority of the major proteins were found. Out of all of these, the acidic fraction contained the proteins with the highest specificity (MW<16 kDa; pI<5.2). In all nine species, they found 2D protein patterns that were specific to that species, and we were able to personalize over 25 proteins. Nondenaturing IEF and gradient SDS-PAGE, performed in a urea-free environment, provided an effective method for differentiating between flatfish species under study by allowing for the precise determination of molecular weights and isoelectric points of the main water-soluble proteins.

The *Merluccidae* family is comprised of seafood species, some of which have a significant economic worth while others have a lower value. The commercialization of the *Merluccidae* family in Europe under the generic name "hake" is extremely remarkable, as was studied by Piñeiro et al. (2001). In order to achieve the goal of achieving the differential characterization of five different species of hake, the potential of proteomics was utilized in this study. These species include *Merluccius australis* (Southern hake), *Merluccius merluccius* (European hake), *Merluccius gayi* (Chilean hake), *Merluccius hubbsi* (Argentinian hake), and *Merluccius capensis* (Cape hake), some of which are intimately associated with one another. Four of the five species of hake that were investigated using IEF and/or 2-DE high-resolution gels were found to contain polypeptides that were specific to their species (Rodríguez & Ortea, 2017).

Berrini et al. (2006) used two-dimensional electrophoresis (2-DE) to differentiate between different commercial values of four freshwater fish species that are sold under the generic name "perch" and are easily "interchangeable" because they are already filleted: *Perca fluviatilis*, *Lates niloticus*, *Stizostedion lucioperca*, and *Morone chrysops x saxatilis*. Multiple species-specific spots on the proteins were shown by the 2-DE maps. Oddly enough, all four species shared multiple noticeable 2-DE spots, but none of them shared IEF bands. Analysis by 2-DE, a more expensive and time-consuming method with better resolution power, was determined to be an effective means of extracting more information from the proteomes of understudied species.

Two-dimensional electrophoresis (2DE) is a technique that requires specialized staff and is characterized by the complexity of the obtained profiles. Additionally, it is a time-consuming and labor-intensive process. Therefore, it has not been employed for the identification of numerous species. Classification of species in raw and cooked aquatic foods has been accomplished through the utilization of the 2DE method,

in conjunction with its constituent techniques. (Valenzuela et al., 1999; Chen et al., 2004; Berrini et al., 2006).

5. Capillary Electrophoresis (CE)

CE, is a method that combines electrophoresis and chromatography and it has been utilized for the purpose of species differentiation. The variation in electrophoretic mobilities of the compounds under investigation within a high-potential-exposed narrow-silica capillary column (50-150 μm in diameter) determines the outcome. While the mass-to-charge ratio does not change, the molecules are categorized based on their different masses (Članjak–Kudra et al., 2021). According to Lago et al. (2014), this automated method can be utilized for commercial purposes due to its relatively fast analysis time of only about 10 minutes. Recio et al. (2001) utilized various variations of CE in their investigation of animal protein analysis. One approach used sarcoplasmic protein analysis in fish musculature to rule out gadoid species. The methods described above were used to identify all species of salmonid fish and smoked eels (Mackie et al., 2000).

When compared to various other methods of analysis, this method has the following advantages:

- It can detect and quantify multiple molecules at once thanks to a system that automatically replaces the column's filling buffer with another one, eliminating the need to manually handle each component of a sample during analysis.
- The entire analysis process takes less than 10 minutes, with just 3 minutes needed to clean and rebalance the column in between each set of analyses.
- Minimal volumes of samples are necessary. Although this method was insensitive at first, that is no longer an issue thanks to enhanced detection systems.

But, its primary drawback is that:

- Each reagent requires its own unique detection system, which, because of the extremely small volumes used, must be extremely sensitive (Westermeyer, 2016). Using CE for protein analysis is a fully automated process. Compared to other electrophoretic methods that are currently available, this one has a significant advantage because it doesn't require specialized technicians and is easier to perform (Lago et al., 2014).

6. Conclusion

There is no doubt that there will be an increase in the number of instances of food fraud all over the world. This is due to a number of factors, including the rise in population, the shifting trends in nutrition, the desire to make a profit, and the growing demand for fish as a source of protein. It will be necessary to the detection of fish mislabeling in order to protect the health and wellbeing of consumers, as well as their financial interests.

The use of electrophoretic techniques is widespread in the field of research pertaining to food items. Protein electrophoresis is a well-known technique that is utilized to ascertain the species of fish. Additionally, it is performed in order to validate the authenticity of marine products. The techniques of electrophoresis have a number of applications, including the identification of species and the research of proteomes in relation to the evaluation of product freshness, verifying the authenticity of food products, and the evaluation of processing conditions for both plant and animal products.

Ultimately, studies investigating the application of electrophoresis for fish species identification have demonstrated that the treatment of the fish, such as its state of being raw or cooked, might potentially influence the most suitable identification methods.

Conflict of Interest

The authors have no conflict of interest to declare.

References

- Alikord, M., Momtaz, H., Keramat, J., Kadivar, M., & Rad, A. H. (2018). Species identification and animal authentication in meat products: A review. *Journal of Food Measurement and Characterization*, 12, 145-155. <https://doi.org/10.1007/s11694-017-9625-z>
- An, H., Marshall, M. R., Otwell, W. S., & Wei, C. I. (1988). Electrophoretic identification of raw and cooked shrimp using various protein extraction systems. *Journal of Food Science*, 53(2), 313-318. <https://doi.org/10.1111/j.1365-2621.1988.tb07696.x>
- Ataman, C., Celik, U., & Rehbein, H. (2006). Identification of some Aegean fish species by native isoelectric focusing. *European Food Research and Technology*, 222, 99-104. <https://doi.org/10.1007/s00217-005-0149-0>
- Berrini, A., Tepedino, V., Borromeo, V., & Secchi, C. (2006). Identification of freshwater fish commercially labelled "perch" by isoelectric focusing and two-dimensional electrophoresis. *Food Chemistry*, 96(1), 163-168. <https://doi.org/10.1016/j.foodchem.2005.04.007>
- Bozariis, I. S. (2014). *Seafood processing: Technology, quality and safety*. John Wiley & Sons.
- Chen, T. Y., Shiau, C. Y., Wei, C. I., & Hwang, D. F. (2004). Preliminary study on puffer fish proteome species identification of puffer fish by two-dimensional electrophoresis. *Journal of Agricultural and Food Chemistry*, 52(8), 2236-2241. <https://doi.org/10.1021/jf035033n>
- Chien, H. J., Huang, Y. H., Zheng, Y. F., Wang, W. C., Kuo, C. Y., Wei, G. J., & Lai, C. C. (2022). Proteomics for species authentication of cod and corresponding fishery products. *Food Chemistry*, 374, 131631. <https://doi.org/10.1016/j.foodchem.2021.131631>
- Civera, T. (2003). Species identification and safety of fish products. *Veterinary Research Communications*, 27, 481-489. <https://doi.org/10.1023/B:VERC.0000014205.87859.ab>
- Civera, T., & Parisi, E. (1991). Use of sodium dodecyl sulphate polyacrylamide gel electrophoresis for the identification of raw and cooked shrimps and crabs. *Italian Journal of Food Science*, 3, 149-157.
- Članjak-Kudra, E., Fazlović, N., Alagić, D., Smajlović, M., Čaklović, K., & Smajlović, A. (2021). An overview of most commonly used methods for detection of fish mislabeling. *Veterinaria*, 70(2), 169-184. <https://doi.org/10.51607/22331360.2021.70.2.169>
- Colombo, M. M., Colombo, F., Biondi, P. A., Malandra, R., & Renon, P. (2000). Substitution of fish species detected by thin-layer isoelectric focusing and a computer-assisted method for the evaluation of gels. *Journal of Chromatography A*, 880(1-2), 303-309. [https://doi.org/10.1016/S0021-9673\(00\)00331-9](https://doi.org/10.1016/S0021-9673(00)00331-9)
- Durand, P., Landrein, A., & Quero, J. C. (1985). *Catalogue e'lectrophoretique des poissons commerciaux*. IFREMER, Nantes.
- EC. (2000). *Council Regulation (EC) No. 104/2000 of 17 december 1999 on the common organization of the markets in fishery and aquaculture products*. Official Journal of the European Communities. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:017:0022:0052:EN:PDF>
- EC. (2001). *Commission Regulation (EC) No. 2065/2001, of 22 october 2001 laying down detailed rules for the application of the Council Regulation (EC) No. 104/2000 as regards informing consumers about fishery and aquaculture products*. Official Journal of the European Communities, L 278/6. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSL:EG:2001R2065:20070101:EN:PDF>
- Esteve-Romero, J. S., Yman, I. M., Bossi, A., & Righetti, P. G. (1996). Fish species identification by isoelectric focusing of parvalbumins in immobilized pH gradients. *Electrophoresis*, 17(8), 1380-1385.
- Esteve-Romero, J. S., Yman, I. M., Bossi, A., & Righetti, P. G. (1996). Fish species identification by isoelectric focusing of parvalbumins in immobilized pH gradients. *Electrophoresis*, 17(8), 1380-1385. <https://doi.org/10.1002/elps.1150170817>
- Etienne, M., Jérôme, M., Fleurence, J., Rehbein, H., Kundiger, R., Yman, I. M., Ferm, M., Craig, A., Mackie, I., Jessen, F., Smelt, A., & Luten, J. (1999). A standardized method of identification of raw and heat-processed fish by urea isoelectric focusing: A collaborative study. *Electrophoresis*, 20(10), 1923-1933. [https://doi.org/10.1002/\(sici\)1522-2683\(19990701\)20:10%3C1923::aid-elps1923%3E3.0.co:2-j](https://doi.org/10.1002/(sici)1522-2683(19990701)20:10%3C1923::aid-elps1923%3E3.0.co:2-j)

- Etienne, M., Jérôme, M., Fleurence, J., Rehbein, H., Kündiger, R., Mendes, R., Costa, H., Pérez-Martín, R., Piñeiro-González, C., Craig, A., Mackie, I., Yman, I. M., Ferm, M., Martínez, I., Jessen, F., Smelt, A., & Luten, J. (2000). Identification of fish species after cooking by SDS-PAGE and urea IEF: A collaborative study. *Journal of Agricultural and Food Chemistry*, 48(7), 2653-2658. <https://doi.org/10.1021/jf990907k>
- Etienne, M., Jérôme, M., Fleurence, J., Rehbein, H., Kündiger, R., Mendes, R., Costa, H., & Martínez, I. (2001). Species identification of formed fishery products and high pressure-treated fish by electrophoresis: A collaborative study. *Food Chemistry*, 72(1), 105-112. [https://doi.org/10.1016/S0308-8146\(00\)00205-3](https://doi.org/10.1016/S0308-8146(00)00205-3)
- Fleurence, J., Le Coeur, C., Mabeau, S., Maurice, M., & Landrein, A. (1995). Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. *Journal of Applied Phycology*, 7, 577-582. <https://doi.org/10.1007/BF00003945>
- Hofmann, K. (1997). Nachweis der tierart bei fleisch und fleischerzeugnissen. 2. Mitteilung. *Fleischwirtschaft (Frankfurt)*, 77(2), 151-154. (In German)
- Huang, Y. C., Chen, T. Y., Jeng, S. S., Chen, H. M., & Hwang, D. F. (2010). Species identification of a causative moray eel meat by SDS-PAGE. *Journal of Food and Drug Analysis*, 18(4), 232-238. <https://doi.org/10.38212/2224-6614.2238>
- Hubalkova, Z., Kralik, P., Tremlova, B., & Rencova, E. (2007). Methods of gadoid fish species identification in food and their economic impact in the Czech Republic: A review. *Veterinarni Medicina*, 52(7), 273-292. <https://doi.org/10.17221/2044-VETMED>
- Lago, F. C., Alonso, M., Vieites, J. M., & Espiñeira, M. (2014). Fish and seafood authenticity - Species identification. In I. S. Bozaris (Ed.), *Seafood processing: Technology, quality and safety* (pp. 419-452). Wiley Blackwell. <https://doi.org/10.1002/9781118346174.ch16>
- Larrain, M. A., Abugoch, L., Quitral, V., Vinagre, J., & Segovia, C. (2002). Capillary zone electrophoresis as a method for identification of golden kinglip (*Genypterus blacodes*) species during frozen storage. *Food Chemistry*, 76(3), 377-384. [https://doi.org/10.1016/S0308-8146\(01\)00263-1](https://doi.org/10.1016/S0308-8146(01)00263-1)
- Lin, W. F., Lyu, Y. C., Wu, Y. J., Lu, C. H., & Hwang, D. F. (2012). Species identification of snapper: A food poisoning incident in Taiwan. *Food Control*, 25(2), 511-515. <https://doi.org/10.1016/j.foodcont.2011.11.028>
- Mackie, I. M. (1996). Authenticity of fish. In P. R. Ashurst & M. J. Dennis (Eds.), *Food authentication* (pp. 140-170). Springer. https://doi.org/10.1007/978-1-4613-1119-5_5
- Mackie, I. M., Pryde, S. E., Gonzales-Sotelo, C., Medina, I., Perez-Martín, R., Quinteiro, J., Rey-Mendez, M., & Rehbein, H. (1999). Challenges in the identification of species of canned fish. *Trends in Food Science and Technology*, 10(1), 9-14. [https://doi.org/10.1016/S0924-2244\(99\)00013-8](https://doi.org/10.1016/S0924-2244(99)00013-8)
- Mackie, I., Craig, A., Etienne, M., Jerome, M., Fleurence, J., Jessen, F., Smelt A., Kruijt, A., Yman, I. M., Ferm, M., Martinez, I., Pérez-Martín, R., Piñeiro, C., Rehbein, H., & Kündiger, R. (2000). Species identification of smoked and gravad fish products by sodium dodecylsulphate polyacrylamide gel electrophoresis, urea isoelectric focusing and native isoelectric focusing: A collaborative study. *Food Chemistry*, 71(1), 1-7. [https://doi.org/10.1016/S0308-8146\(00\)00147-3](https://doi.org/10.1016/S0308-8146(00)00147-3)
- Martinez, I., Friis, T. J., & Seppola, M. (2001). Requirements for the application of protein sodium dodecyl sulfate-polyacrylamide gel electrophoresis and randomly amplified polymorphic DNA analyses to product speciation. *Electrophoresis*, 22(8), 1526-1533. [https://doi.org/10.1002/1522-2683\(200105\)22:8%3C1526::AID-ELPS1526%3E3.0.CO;2-3](https://doi.org/10.1002/1522-2683(200105)22:8%3C1526::AID-ELPS1526%3E3.0.CO;2-3)
- Ortea, I., Cañas, B., Calo-Mata, P., Barros-Velázquez, J., & Gallardo, J. M. (2010). Identification of commercial prawn and shrimp species of food interest by native isoelectric focusing. *Food Chemistry*, 121(2), 569-574. <https://doi.org/10.1016/j.foodchem.2009.12.049>
- Ortea, I., Pascoal, A., Canas, B., Gallardo, J. M., Barros-Velázquez, J., & Calo-Mata, P. (2012). Food authentication of commercially-relevant shrimp and prawn species: From classical methods to foodomics. *Electrophoresis*, 33(15), 2201-2211. <https://doi.org/10.1002/elps.201100576>
- Piñeiro, C., Barros-Velázquez, J., Sotelo, C. G., Pérez-Martín, R. I., & Gallardo, J. M. (1998). Two-dimensional electrophoretic study of the water-soluble protein fraction in white muscle of gadoid fish species. *Journal of Agricultural and Food Chemistry*, 46(10), 3991-3997. <https://doi.org/10.1021/jf9800965>
- Piñeiro, C., Barros-Velázquez, J., Sotelo, C. G., & Gallardo, J. M. (1999a). The use of two-dimensional electrophoresis in the characterization of the water-soluble protein fraction of commercial flat fish species. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, 208, 342-348. <https://doi.org/10.1007/s002170050427>
- Piñeiro, C., Barros-Velázquez, J., Pérez-Martín, R. I., Martínez, I., Jacobsen, T., Rehbein, H., Kündiger, R., Mendes, R., Etienne, M., Jerome, M., Craig, A., Mackie, I. M., & Jessen, F. (1999b). Development of a sodium dodecyl sulfate-polyacrylamide gel electrophoresis reference method for the analysis and identification of fish species in raw and heat-processed samples: A collaborative study. *Electrophoresis: An International Journal*, 20(7), 1425-1432. [https://doi.org/10.1002/\(sici\)1522-2683\(19990601\)20:7%3C1425::aid-elps1425%3E3.0.co;2-r](https://doi.org/10.1002/(sici)1522-2683(19990601)20:7%3C1425::aid-elps1425%3E3.0.co;2-r)
- Piñeiro, C., Vázquez, J., Marina, A. I., Barros-Velázquez, J., & Gallardo, J. M. (2001). Characterization and partial

- sequencing of species-specific sarcoplasmic polypeptides from commercial hake species by mass spectrometry following two-dimensional electrophoresis. *Electrophoresis*, 22(8), 1545-1552. [https://doi.org/10.1002/1522-2683\(200105\)22:8%3C1545::aid-elps1545%3E3.0.co;2-5](https://doi.org/10.1002/1522-2683(200105)22:8%3C1545::aid-elps1545%3E3.0.co;2-5)
- Recio, I., Ramos, M., & López-Fandiño, R. (2001). Capillary electrophoresis for the analysis of food proteins of animal origin. *Electrophoresis*, 22(8), 1489-1502. [https://doi.org/10.1002/1522-2683\(200105\)22:8%3C1489::aid-elps1489%3E3.0.co;2-g](https://doi.org/10.1002/1522-2683(200105)22:8%3C1489::aid-elps1489%3E3.0.co;2-g)
- Rehbein, H. (1990). Electrophoretic techniques for species identification of fishery products. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 191, 1-10. <https://doi.org/10.1007/BF01202356>
- Rehbein, H. (1992). Parvalbumins as marker proteins for the fish species in fishery products. In H. H. Huss, M. Jakobsen & J. Liston (Eds.), *Quality assurance in the fish industry* (pp. 399-405). Elsevier.
- Rehbein, H. (1995). Food control by applied biochemistry of marine organisms: Comparison of proteins and metabolites from fish and invertebrate muscle. *Helgoländer Meeresuntersuchungen*, 49, 747-757. <https://doi.org/10.1007/BF02368398>
- Rehbein, H., Etienne, M., Jerome, M., Hattula, T., Knudsen, L. B., Jessen, F., Luten, J. B., Bouquet, W., Mackie, I. M., Ritchie, A. H., Marti'n, R., & Mendes, R. (1995). Influence of variation in methodology on the reliability of the isoelectric focusing method of fish species identification. *Food Chemistry*, 52(2), 193-197. [https://doi.org/10.1016/0308-8146\(94\)P4203-R](https://doi.org/10.1016/0308-8146(94)P4203-R)
- Rehbein, H., Kündiger, I., Yman, I. M., Ferm, M., Etienne, M., Jerome, M., Craig, A., Mackie, I., Jessen, F., Martinez, I., Mendes, R., Smelt, A., Luten, J., Piñeiro, C., & Pérez-Martín, R. (1999). Species identification of cooked fish by urea isoelectric focusing and sodium dodecylsulfate polyacrylamide gel electrophoresis: A collaborative study. *Food Chemistry*, 67(4), 333-339. [https://doi.org/10.1016/S0308-8146\(99\)00175-2](https://doi.org/10.1016/S0308-8146(99)00175-2)
- Renon, P., Bernardi, C., Malandra, R., & Biondi, P. A. (2005). Isoelectric focusing of sarcoplasmic proteins to distinguish swordfish, blue marlin and Mediterranean spearfish. *Food Control*, 16(5), 473-477. <https://doi.org/10.1016/j.foodcont.2004.05.009>
- Rodríguez, E. M., & Ortea, I. (2017). Food authentication of seafood species. In M. L. Colgrave (Ed.), *Proteomics in food science* (pp. 331-342). Academic Press. <https://doi.org/10.1016/B978-0-12-804007-2.00020-5>
- Rouxel, C., Bonnabeze, E., Daniel, A., Jérôme, M., Etienne, M., & Fleurence, J. (2001). Identification by SDS PAGE of green seaweeds (*Ulva* and *Enteromorpha*) used in the food industry. *Journal of Applied Phycology*, 13, 215-218. <https://doi.org/10.1023/A:1011161505839>
- Shenouda, S. Y. K. (1980). Theories of protein denaturation during frozen storage of fish flesh. *Advances in Food Research*, 26, 275-311. [https://doi.org/10.1016/S0065-2628\(08\)60320-1](https://doi.org/10.1016/S0065-2628(08)60320-1)
- Tepedino, V., Berrini, A., Borromeo, V., Gaggioli, D., Cantoni, C., Manzoni, P., & Secchi, C. (2001). Identification of commercial fish species belonging to the order *Pleuronectiformes* and *Gadiformes*: Library of isoelectric focusing patterns. *Journal of AOAC International*, 84(5), 1600-1607. <https://doi.org/10.1093/jaoac/84.5.1600>
- Valenzuela, M. A., Gamarra, N., Gómez, L., Kettlun, A. M., Sardón, M., Pérez, L. M., Vinagre, J., & Guzman, N. A. (1999). A comparative study of fish species identification by gel isoelectrofocusing two-dimensional gel electrophoresis, and capillary zone electrophoresis. *Journal of Capillary Electrophoresis and Microchip Technology*, 6(3-4), 85-91.
- Verrez-Bagnis, V., Sotelo, C. G., Mendes, R., Silva, H., Kappel, K., & Schröder, U. (2017). Methods for seafood authenticity testing in Europe. In J. M. Mérillon & K. G. Ramawat (Eds.), *Bioactive molecules in food* (pp. 2063-2117). Springer. https://doi.org/10.1007/978-3-319-78030-6_69
- Walker, J. M. (1996). *The protein protocols handbook*. Springer Science & Business Media.
- Westermeier, R. (2016). *Electrophoresis in practice: A guide to methods and applications of DNA and protein separations*. John Wiley & Sons.