

## Review

# Current State of Milk, Dairy Products, Meat and Meat Products, Eggs, Fish and Fishery Products Authentication and Chemometrics

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**Abstract:** Food fraud is a matter of major concern as many foods and beverages do not follow their labelling. Because of economic interests, as well as consumers' health protection, the related topics, food adulteration, counterfeiting, substitution and inaccurate labelling, have become top issues and priorities in food safety and quality. In addition, globalized and complex food supply chains have increased rapidly and contribute to a growing problem affecting local, regional and global food systems. Animal origin food products such as milk, dairy products, meat and meat products, eggs and fish and fishery products are included in the most commonly adulterated food items. In order to prevent unfair competition and protect the rights of consumers, it is vital to detect any kind of adulteration to them. Geographical origin, production methods and farming systems, species identification, processing treatments and the detection of adulterants are among the important authenticity problems for these foods. The existence of accurate and automated analytical techniques in combination with available chemometric tools provides reliable information about adulteration and fraud. Therefore, the purpose of this review is to present the advances made through recent studies in terms of the analytical techniques and chemometric approaches that have been developed to address the authenticity issues in animal origin food products.

**Keywords:** animal and animal-derived matrices; authenticity; adulteration; chemometric tools; analytical techniques; milk; dairy products; meat and meat products; eggs; fish and fishery products



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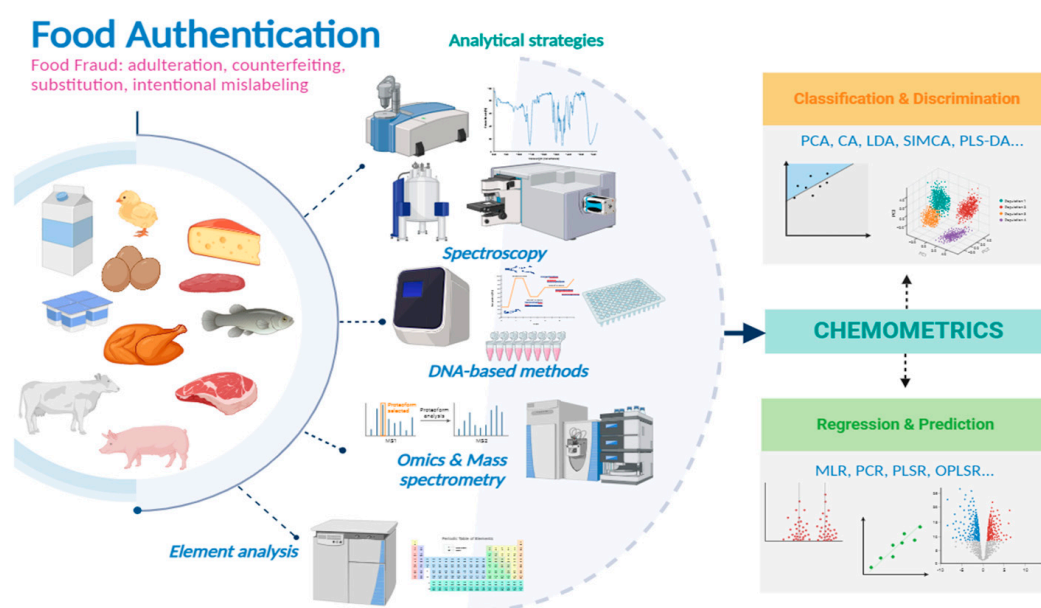


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## 1. Introduction

In the modern world, the concepts of food safety and quality have expanded. Although they are inextricably linked to the hygiene and wholesomeness of food and, by extension, to consumers' protection, they also incorporate the knowledge on food fraud and authenticity, intended as the adulteration, counterfeiting, substitution, addition and/or omission of ingredients/information on both the processing and origin of the products [1,2]. On the other hand, the consumer has become increasingly aware and sensitive to the communication/implementation of risk, and to the notions of healthy eating and nutrition as powerful tools to prevent and treat the onset of many diseases [3]. Therefore, the food system has to face the challenge of demonstrating not only the healthiness, nutritional value, safety and quality of the product, but also its sustainability, traceability and authenticity [4]. As a consequence, food scientists, assessors and managers have developed more informative, sensitive and accurate analytical methods to meet these needs.

These high-throughput and high-content methods generate a large amount of data, which in order to be best evaluated need to interface with biostatistical, bioinformatic and chemometric approaches. In particular, in the analytical branch of food authentication, complex protocols of sample preparation followed by analytical separation, identification and quantitation are “hyphenated” with advanced chemometrics [5,6]. This interdisciplinary statistical-based discipline is used to identify the relationships between many variables (multivariate analysis), and to analyse, elaborate and interpret the data. The construed models are validated and become able to substantially discriminate between and classify the food products. In these ways, the data generated from conventional analytical techniques, chromatographic, spectrophotometric, biochemical, immunochemical and histological methodologies, and omics applications are complemented [7,8]. Figure 1 presents the implementation of authenticity analysis in animal and animal-derived matrices with the help of chemometric tools.



**Figure 1.** The implementation of authenticity analysis in animal and animal-derived matrices with the help of chemometric tools.

In this framework, the aim of this review is to give a comprehensive overview of analytical methods used for food authentication purposes, with a special attention on animal and animal-derived matrices, i.e., meat and meat products, milk and dairy products, and fish and fish products. Furthermore, with this study, the authors would like to emphasize the power of chemometric protocols, discussed in depth, used for processing the datasets. These considerations may be a useful tool not only for researchers and researchers, laboratories and stakeholders but also for governments and authorities since the majority of these methods for animal-derived matrices do not meet regulatory acceptance criteria yet [9]. In fact, although the targets of the majority of detected food frauds are fish, honey, dairy products and meat, in the current worldwide legislations, there are few official and internationally accepted methods for these applications. Thus, the authentication of food products has become a major priority and concern not only for safety reasons but also for preventing economic fraud since these matrices are among the most internationally traded food commodities [10–12].

## 2. Chemometrics of Milk, Dairy Products, Meat and Meat Products, Eggs, Fish and Fishery Products

### 2.1. General Considerations: Chemometrics and Multivariate Analysis

The animal-derived matrices, discussed in this review, are characterized by very different physicochemical characteristics. Similarly, the topic of food authenticity is complex with many attributes that should be considered and assessed. Environmental and growing conditions, feeding and feed types, and the processing, handling and storage of materials are only some variables that may influence the results of the analytical controls of these matrices. If examined through the usage of classical descriptive statistics, exploring each variable separately (univariate analysis), the results often have no statistical significance and offer a partial image of food origin and global composition. For this reason, in the last decades, researchers and academics have made an effort to give more power to authentication studies by merging them with multivariate analysis. The resulting combination of analytical and chemometric studies is currently the best approach for a precise, global and multiview analysis and to fight and win the battle of food fraud.

### 2.2. Chemometrics of Milk and Dairy Products

Cheese is widely consumed worldwide and represents a well-known food product. New approaches that partially or fully replace milk fat derive from the increasing demand for nutritionally enhanced or functional dairy products from the cheese industry. This has also led to the identification of new methods of adulteration, which may result in these fully/partially substituted products being marketed as cheese [13]. There are over 4000 different types of cheeses worldwide, and dairy fat consists of approximately 400 fatty acids, making it one of the most complex dietary fats [14]. A growing issue in the dairy product market is the incorrect labelling or adulteration of high-value milk samples, such as goat's or sheep's milk, with cow's milk [15]. Adulterations in milk primarily involve water, reconstitution agents (oils, sugar from sugarcane, animal fats), thickening agents (starch, urea, glucose, salt, etc.), preservatives (formaldehyde, sodium carbonate, hydrogen peroxide, etc.), melamine and more [16].

Traditionally, goat's milk is used in the production of dairy products that have undergone fermentation, especially cheese and yogurt. The nutritional characteristics of goat's milk (less allergenic protein fraction, higher lipid content and high mineral availability) have increased its demand and production; hence, its high susceptibility to adulteration. Its easy dilution with water and cow's milk whey (from cheese processing), or even mixing with cow's milk, gives goat's milk a higher market value compared to cow's milk, which is abundant. Hence, the adulteration of goat's milk with cow's milk has become more evident nowadays, and this particularly targets many consumers with lactose intolerance and cow's milk protein allergies, leading to various allergic disorders [17]. Therefore, goat's or sheep's milk with cow's milk added alters the sensory characteristics of the final product and also poses a significant risk to consumers with intolerance or allergies to cow's milk. Similarly, adulteration with urea in goat's and cow's milk often occurs to compensate for protein content [17,18].

An illegal and significant adulteration is the deliberate addition of formaldehyde to raw milk, with the aim of extending the shelf life of milk at room temperature. Rapid deterioration of milk is caused by a high moisture content. Hence, the preservative and antiseptic properties, along with the ability to improve the appearance, including the odour of milk, need to be presented, and this is carried out by formaldehyde. However, it should be considered that formaldehyde is classified as a human carcinogen by the International Agency for Research on Cancer (IARC), being toxic at low concentrations [16,19]. Higher levels of formaldehyde in foods can lead to symptoms such as nausea, coma, abdominal pain, dermatitis, eye irritation, asthma and more [16]. Formaldehyde has been found in milk available in Brazil, Kenya, India and Pakistan, among other places. Another case of adulteration to remember took place in China in 2013 with melamine detection in milk

powder. Melamine was added to increase the apparent protein content, leading to dramatic consequences for public health. [19].

The need to include the geographical origin of foods on labels for many commercial products, such as cheeses produced in a specified region with specific physicochemical and sensory characteristics, is depicted in recent EU regulations. Consequently, geographical origin is considered a significant indicator [20]. A usual form of adulteration in dairy product production is the substitution of one type of milk with another due to the lower cost and year-round availability. Some milk products, such as milk powder, are susceptible to dilution or adulteration with exogenous fats or oils. Additionally, the incorrect declaration of geographical origin is another common form of fraud. There is a risk of adulteration due to the violation of PDO protocol specifications in the case of high-value dairy products such as some PDO (Protected Designation of Origin) cheeses.

There is a high demand for the development of portable NIR devices specifically in the production chain of goat dairy products since they are effective in distinguishing between authentic and adulterated samples, for a reduction in the economic cost and analysis time. This will target the production of high-quality products and consumer safety while simultaneously controlling the nutritional value stated on their labels [21].

### *2.3. Chemometrics of Meat and Meat Products*

The growing issue of meat fraud occurs in the increasingly globalized and complex food supply chains. In the meat industry, substitution fraud mainly concerns meat derivative products, which consist of minced meat mixed with other ingredients to manufacture sausages, salami, kebabs, burgers, meatballs and stuffed pastas. Raw meat must be correctly labelled when it comes from different animal species, otherwise it becomes fraudulent. When other parts of animals, such as fat, collagen, entrails or internal organs, are added to minced meat during the manufacturing process, fraud might occur [22]. Typical meat products including raw sausages or ham made from game species such as wild goats (chamois), red deer or chamois are consumed in certain alpine regions, such as Switzerland, Austria, Germany and Italy. Game meat is more expensive and easier to substitute with other meats that have a remarkably similar taste, colour and appearance due to its scarcity, coupled with challenges in hunting activities due to increased habitats [23].

The quantity of each ingredient must be declared in meat-derived products, and this is known as quantitative ingredient declaration. The identification of species and differentiation of animal tissues in meat products are of significant concern regarding consumer protection against illegal and/or unwanted adulteration, not only for economic and health reasons but also for religious matters. The presence of pork derivatives such as pork skin, lard, pork meat and pork gelatine in any food products is a significant issue for religions such as Islam, Judaism and Hinduism, which prohibit their followers from consuming pork and its derivatives. Furthermore, halal and kosher, which are still widely practiced by Muslims and Jews, respectively, and certified accordingly, guide the production, slaughter and preparation of meat for human consumption. To be considered kosher, an animal's meat must come from a cloven-hoofed animal. For example, halal-labelled products should not include the substitution of non-halal meat, and this is considered to be an illegal and unacceptable practice according to Islamic law. Fraud and the adulteration of halal meat products occur due to financial incentives. Food manufacturers in many countries nowadays choose to use pork meat or pork derivatives (pork fat, pork gelatine, etc.) because they are inexpensive and readily available [24,25].

The meat industry faces ongoing challenges in ensuring the authenticity and quality of its products. Fraudulent practices, mislabelling and adulteration are persistent concerns. However, the integration of chemometrics, a multidisciplinary field encompassing chemistry, mathematics and statistics, offers robust solutions to address these issues effectively. This essay explores the diverse applications of chemometrics in safeguarding the authenticity of meat and meat products across numerous studies.

#### 2.4. Chemometrics of Eggs

Data interpretation and visualization can be carried out by chemometrics, which provides powerful tools. Principal Component Analysis (PCA) helps in reducing the dimensionality of complex datasets, making it easier to identify patterns and trends in the chemical composition of eggs. This allows researchers to gain a comprehensive understanding of the factors affecting egg authenticity. Chemometric methods enable the clear differentiation between different types of eggs, such as organic and conventional eggs, based on their chemical profiles. By applying multivariate statistical techniques, researchers can establish distinct chemical fingerprints for each egg type, enhancing the ability to detect fraudulent labelling and ensure accurate product labelling.

During the long-term storage of eggs, a significant change that occurs in the egg is the reduction in the elasticity of the vitelline membrane, allowing for easier migration of water from the albumen through the weaker vitelline membrane. The result of this process is that the yolk becomes flatter, and thus, the yolk index parameter measures the thickness and diameter of the yolk. Therefore, the yolk index indicates the viscosity of the yolk, and the higher it is, the better the quality of the egg. In addition,  $\gamma$ -aminobutyric acid was found to be a good marker of the age of eggs during storage [26].

Chemometrics aids in the identification of egg production systems by analysing the chemical composition of egg components. Puertas et al. [27] employed UV-VIS-NIR spectroscopy and chemometric techniques such as SVM, LDA and QDA to identify different egg production systems. Their study focused on the analysis of yolk lipid extracts.

In addition, S-ovalbumin is a biological marker that arises from albumen (the most abundant protein found in eggs). S-ovalbumin is highly correlated with storage time, with low physical variability, and has the potential to become a common indicator for assessing egg freshness [28]. The freshness and storage time of eggs were assessed using chemometric models based on parameters such as the Haugh unit, pH of albumen and yolk height. These parameters were measured using spectroscopic techniques such as VIS-NIR spectroscopy [29]. Moreover, liquid chromatography–tandem mass spectrometry for the quantitation of lipidomic profiles in the yolk granule and yolk plasma of egg yolk was used by He et al. [30]. The recorded differences revealed by using chemometrics, particularly PCA and OPLS-DA, enlighten the need to study new functional and high-value novel egg products.

Furthermore, determination of the geographical origin of eggs can be carried out by chemometric approaches. In addition, chemometrics assists in predicting the freshness and storage time of eggs based on key parameters such as the Haugh unit, pH and yolk height. By establishing mathematical models, chemometrics enables the estimation of egg quality over time, ensuring that consumers receive fresh products. What is more, studies such as that of Joshi et al. [31] have demonstrated the use of chemometric models to distinguish genuine eggs from counterfeit ones based on their chemical properties. This helps in maintaining product integrity and consumer trust. Moreover, by providing accurate and objective assessment tools, chemometrics contributes to quality assurance in the egg industry. Reliable authenticity testing enhances consumer confidence, promotes fair trade practices and ensures that consumers receive the products they pay for.

In conclusion, chemometrics plays a pivotal role in addressing various aspects of egg authenticity, ranging from the differentiation of egg types to determining geographical origin and predicting freshness. Its ability to handle complex chemical data and provide actionable insights makes it an invaluable tool in ensuring the accuracy and reliability of egg quality assessments. By combining various analytical techniques with advanced data analysis methods, several studies have successfully differentiated between different egg types, identified production systems, determined geographical origins, and predicted freshness and storage times. These findings highlight the importance of chemometrics in ensuring the quality and authenticity of egg products, contributing to consumer confidence and fair-trade practices in the egg industry.



### 2.5. Chemometrics of Fish and Fishery Products

The employment of qualitative spectroscopy and chemometrics applied to authenticate fish and seafood products is developed in this section. In fact, several spectroscopic techniques have focused on fish species substitution, geographical origin misrepresentation, and the processing and production method. Using PLS and PCA, Gayo et al. [32] utilized VIS-NIRS to distinguish Atlantic blue crab mixed with the meat of blue swimmer crab. These models are better able to predict the adulteration since the standard errors of prediction (SEP) of 0.252 (PLS) and 0.244 (PCA). NIR spectroscopy reviewed seven species of freshwater fish [33]. In this study, to distinguish between fish samples, PCA, PLS and fast Fourier Transform (FFT), coupled with LDA models, were established by nine preselected spectra wavelengths, and a good prediction of the approved strategy was revealed. The PCA-LDA and FFT-LDA models were performed with high accuracy, specificity, sensitivity and precision. According to Zhang et al. [34], three fish surimi species were categorized by NIR vibration. Using PCA, a disjointed cluster related with red coat surimi species was noticed, and a full distribution rate was provided by LDA findings. Alamprese and Casiraghi [35] developed FT-NIR and FT-MIR to (i) assess the replacement of prized red mullet and plaice species with low-cost Atlantic mullet-and-flounder, and to (ii) distinguish fresh and frozen-thawed fish. These authors noted that LDA and SIMCA associated with FTIR displayed a high difference between samples, and was used to separate fresh and frozen ones. In this sense, a specificity of >95% was determined and sensitivity values were >60%.

Cozzolino et al. [36] used NIR/PLSR to authenticate the fishmeal batches from different fish species. They concluded that dummy PLSR achieved ~80% good classification; in addition, PCA had a score of >80%.

In addition to NIR, further vibrational spectroscopic tools have been extensively promised to be able to perceive fraud in fish and fish products. For instance, MIR was employed to identify fraud by replacing Atlantic salmon with rainbow trout [37]. Using PCA/PLSR, 12 formulations were effectively predicted. Similar trends were observed by Chen et al. [38] who used Raman vibration.

Rašković et al. [39] applied Raman spectroscopy for the classification of 12 different fish fillets of different species. By using HCA/Raman spectra, three separated clusters were revealed. To separate samples, belonging to cod, haddock, saithe and pollack, Standal et al. [40] evaluated and characterized their phospholipid profiles, obtained by <sup>13</sup>C NMR. Linear analysis contributed a 78% classification rate, while the Bayesian belief network (BBN) achieved a classification equal to 100%. To distinguish between wild/farmed salmon according to the degree of processing, Capuano et al. [41] utilized <sup>1</sup>H NMR. These authors reported that by employing SIMCA, a full separation was achieved on the oleic and linoleic acid levels. Similar trends were reported by Vidal et al. [42] who discriminated between farmed/wild European sea bass due to great di-unsaturated acyl groups.

By applying PNN and SVM techniques, Masoum et al. [43] applied the <sup>1</sup>H NMR to separate salmon fish oils from eight different geographical sites. These authors noted a grouping % of 98.5 and 100%, respectively. Likewise, promising results were reported by Dalle Zotte et al.'s [44] study that used the combination of <sup>1</sup>H-NMR/PCA and LDA. These authors noted a perfect separation between wild/farmed samples. In the same way, LDA variables' selection allowed a classification of 100% of the tested wild and farmed samples. Protein structures, exaggerated by thermal processing, have been examined by the application of spectroscopic tools. He et al. [45] investigated the impact of wet cooking on the myosin/ctin denaturation in false abalone by the heat transfer model. These authors concluded that immobilized water was condensed with a prolonged processing time, and the shear force was also reduced by LF-NMR and MRI. PLSR showed a great relationship among immobilized water and sensory studied features. By fluorescence microscopy joined with physicochemical changes, Cropotova et al. [46] measured the lipid oxidation in sous-vide-cooked Atlantic mackerel. At 70 °C and 80 °C during 10/20 min it was achieved with/without antioxidants. Fluorescence micrographs of extracted lipids

were acquired in  $\lambda_{\text{ex}}$  475/40 and  $\lambda_{\text{em}}$  530/50, and interrelated with TBARS. It was stated that the conjugated trienes produced by lipid polymerization throughout the storage of processed mackerel were associated and correlated with the instrumental yellowness of the fish flesh. Fluorescence at 415 nm and 347 nm for uncooked and cooked fish fillets was assessed by Tavares et al. [47], and the outcomes showed the highest intensity was observed in baked and fried samples compared to the raw and boiled ones. Xia et al. [48] employed NMR and MRI to investigate the boiling, frying and stewing of turbot. Assessments were connected with texture and colour quality. A good separation was made between cooking methods and was shown by PCA/NMR data. Moreover, NMR results were confirmed by weighted images of the MRI scans with exposed conceptions of interior structural data. Some modern spectroscopic tools for tracking thermal handlings in fish and fish products are outlined in Table 1.

**Table 1.** Some recent examples of spectroscopic techniques used for monitoring thermal treatments in fish and fish products.

| Genus/Species of Fish                                 | Applied Technique/Wavelength Range                            | Model               | Main Findings                                                                                                                                                                                          | References |
|-------------------------------------------------------|---------------------------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| False abalone ( <i>Volutharpa ampullacea perryi</i> ) | NMR, MRI/21.3 MHz                                             | PLSR                | Quantitative descriptions of actin and myosin protein denaturation and water distribution                                                                                                              | [45]       |
| Atlantic mackerel                                     | Fluorescence:<br>Excitation = 475 nm<br>Emission = 530 nm     | Univariate analysis | -Fluorescence/lipid oxidation products are highly correlated                                                                                                                                           | [46]       |
| Alaska pollock surimi                                 | FTIR/4000–400 $\text{cm}^{-1}$                                | PCA                 | The reduction in the gel strength was consequence of modifications in protein secondary structures                                                                                                     | [49]       |
| Atlantic salmon                                       | FTIR/4000–400 $\text{cm}^{-1}$                                | PCA                 | Cooking/Electrolyzed water:<br>-significantly reduced <i>Listeria monocytogenes</i><br>-developed protein denaturation                                                                                 | [50]       |
| Bighead carp ( <i>Aristichthys nobilis</i> )          | Raman/400–3500 $\text{cm}^{-1}$ and 22.6 MHz                  | PCA                 | -A decline in $\alpha$ -helix structures<br>-With the increase in heat treatment, a modification in myosin secondary structures                                                                        | [51]       |
| Hairtail ( <i>Thichiurus lepturus</i> ) fillets       | Fluorescence:<br>Excitation = 347 nm<br>Emission = 415 nm     | Univariate analysis | -In cooked fish, an increase in fluorescence was observed<br>-As compared to boiled ones, more fluorescence was noted from baked and fried fillets                                                     | [47]       |
| Sturgeon ( <i>Acipenser gueldenstaedtii</i> )         | Fluorescence:<br>Excitation = 360 nm<br>Emission = 380–600 nm | Univariate analysis | -Compared to fluorescence of samples before digestion, the fluorescence was increased after digestion<br>-With roasting times, changes in spectral patters (shape and intensity) were observed         | [52]       |
| Turbot                                                | NMR, MRI/21.2 MHz                                             | PCA                 | Correlation between NMR relaxation parameters/texture and colour measurements                                                                                                                          | [52]       |
| Atlantic salmon                                       | FTIR/4000–400 $\text{cm}^{-1}$                                | PCA                 | With high cooking temperature and cooking time, the amid I region exposed an increase in aggregation and protein denaturation                                                                          | [53]       |
| Fish cakes                                            | NIR/760–1040 nm                                               | PLSR                | -In fish cakes, prediction core temperatures were 2.3 °C and 4.5 °C for NIR point system and imaging system, respectively<br>-In the NIR system, $T^{\circ}$ changes till 11–13 mm depth in fish cakes | [45]       |

### 3. Current Analytical Methods for Milk, Dairy Products, Meat and Meat Products, Eggs, Fish and Fishery Products Authentication and Chemometrics

#### 3.1. Overview of Analytical Methods

As shown in Figure 1, the analytical techniques used for food authentication protocols are very diverse. Each technique can have different configurations, applications, advantages and disadvantages.

The spectroscopic techniques, although extremely diversified, are united by the principle of the emission or absorption of electromagnetic radiation with the consequent generation of continuous spectra, band spectra or line spectra. Each substance is capable of emitting or absorbing particular radiations with intensity dependent on the concentration of the substance itself. Among the most used are Nuclear Magnetic Resonance spectroscopy, Infrared and Near-Infrared spectroscopy, and Raman spectroscopy, based, respectively, on the measurement of paramagnetic spin transitions, vibrational transitions and the inelastic scattering of photons. These techniques are able to provide a fingerprint of the substance and are particularly useful for geographical traceability studies of food products.

Similarly, mass spectrometry, which can be used for both inorganic and organic and hyphenate analysis with different types of chromatographic separation, can provide the characterization, identification and precise and sensitive quantification of all small molecules, metabolites, macromolecules and trace elements, which have ionizability as their characteristic.

Finally, to establish the species and the genomic and transcriptomic characteristics, the techniques based on DNA analysis, which today have found their maximum use in the

next generation sequencing (NGS) techniques preceded by the construction of complex libraries, are today the most studied and investigated.

### 3.2. Authenticity of Feed Materials towards Egg Authenticity

Determination of the geographical origin of food products is a crucial step; however, it might not be sufficient on its own to guarantee authenticity. Mapping and controlling the entire supply chain, including raw feed materials, could be essential for precise control over the various factors influencing their quality and authenticity.

For example, diet variations among hens even within the same geographical area might introduce complexities in ensuring consistent quality and authenticity. Mapping the raw feed materials becomes crucial in ensuring precise control over various aspects impacting egg authenticity. It is not just the location where hens are raised that matters but also the source and quality of their feed. Differences in feed can significantly affect the nutritional composition and even the chemical markers present in eggs.

Formulation of an effective feed should consider the right cost and nutritional quality to cover the essentials of poultry as reported by Belkhanchi et al. [54].

For livestock, the feed must also provide enough nutrients to meet production needs (eggs or meat). Different forms can comprise the feed such as: raw materials, compound feed (a mixture of at least two raw materials), complete feed (compound feed with sufficient composition, to cover the daily requirements) or supplementary feed (for example, cereals to supplement the raw materials given to the animal) [55,56].

The increase in the needs of poultry arises from the growth of global demand for animal protein [57], thus creating many challenges [58–65]. Tremendous changes in the growth of all phases have been historically observed for the commercial poultry industry from the hatchery to broiler and layer farm practices across meat and egg processing technological advances [66]. Hence, the volume of poultry meat and eggs produced has also expanded to match this rise in retail and consumer demand [67–69]. This rise still depends on advances in bird genetics, nutritional management, processing technologies and food safety [70–76].

The success of a quality feed formulation depends on the physicochemical characteristics of raw materials [77] and the production efficiency and meat quality in broiler chickens derived from the effect of the partial replacement of raw materials with others [78–82].

According to the European Union (EU)-funded project MARLON, the organization and characteristics of specific livestock and feed production chains (conventional, organic, GM-free) within the EU, with an emphasis on controls, regulations, traceability and common production practices, have been studied. Moreover, the origin of animal feed used in the EU as well as an examination of the use of genetically modified organisms (GMOs) in feed is provided according to Kleter et al. [83]. They showed that livestock is traceable at the herd or individual level, depending on the species. Geography and animal species affect husbandry practices, which can vary. For feeds, only coarse estimates could be made for the amount of GM feed ingredients that an animal is exposed to.

The approach followed by EU risk assessors is described in a detailed guidance developed by the European Food Safety Authority's panel of experts on genetically modified organisms (EFSA GMO Panel) and incorporated into EU legislation [84,85].

The authenticity of native eggs was detected by combining near-infrared (NIR) spectroscopy with data-driven-based class modelling (DDCM) and model-independent variable selection, i.e., joint mutual information (JMI) as reported by Chen et al. [86]. A total of 122 eggs of three types were collected. Principal Component Analysis (PCA) was utilized for exploratory analysis. Near-infrared (NIR) spectroscopy has become increasingly important in food field as a powerful analytical technique [87–92]. NIR spectroscopy can characterize multiple chemical components of samples showing great advantages such as A lower sample preparation requirement, reduced analysis time and cost, multicomponent analysis and the potential for online analysis. NIR spectral information is hardly selective due to the NIR spectrum corresponding to overtones and combinations of the fundamen-



tal molecular vibrations. NIR-based quantitative and qualitative analyses need the help of chemometrics.

Another study by Rogers et al. [93] used stable isotopes to develop authentication criteria for eggs laid under cage, barn, free range and organic farming regimens from the Netherlands and New Zealand. Commercial poultry feeds and egg albumen from 49 poultry farms across the Netherlands were used to determine the isotopic variability in organic and conventional feeds. Trophic effects of these corresponding feeds and barn, free range and organic farming regimens on corresponding egg albumen were also assessed. This study suggested that nitrogen showed particular promise as a screening and authentication tool for organically farmed eggs. They proposed that Dutch organic egg whites should have a minimum  $\delta^{15}\text{N}$  value of 4.8‰ to account for an organic plant-derived diet. Regarding New Zealand egg isotopes over the past 7 years suggested that organic eggs should have a minimum  $\delta^{15}\text{N}$  value of 6.0‰, a higher value due to the use of fishmeal or meat and bone meal (MBM), restricted in the EU.

Finally, Bandoniene et al. [94] developed a method for labelling poultry products by the selective enrichment of two rare earth elements (REE), namely, terbium and thulium, in the feed for laying hens to discriminate labelled from unlabelled poultry products. Analysis was varied by using either conventional or laser ablation inductively coupled plasma mass spectrometry. This was found to be a good methodology to detect authenticity.

### 3.3. Current Analytical Methods for Milk and Dairy Products Authentication and Chemometrics

Li et al. [95] as well as Huang et al. [96] noted that Raman spectroscopy is gaining more and more attention in food quality control in combination with chemometrics due to it being fast, portable and non-destructive. Furthermore, this technology allows the measurement of intact samples (while inside the packaging), while the water content of the samples does not affect measurements.

One of the primary applications of chemometrics in the dairy industry is the detection of adulteration. Numerous studies in the provided table demonstrate how chemometric techniques can effectively identify various forms of adulteration, including the addition of water, non-dairy substances and contaminants such as melamine. For example, FT-NIR spectroscopy combined with chemometric methods such as PCA, PLS-DA and iPLS was employed by da Paixao Teixeira et al. [21] to detect the adulteration of yogurt and cheese made with goat's milk using bovine milk. Such techniques provide rapid and accurate detection, bolstering consumer trust and safety. Differentiation of dairy products based on their geographic and seasonal origin can be carried out by chemometrics, and this is particularly valuable for products with Protected Designation of Origin (PDO) status. Studies such as Pellegrino et al. [97] and Tarapoulouzi and Theocharis [98] employed chemometric methods to distinguish between different types of cheese. This verification is vital for protecting traditional cheese-making practices and ensuring consumers receive genuine products. More recently, Tarapoulouzi and Theocharis [99] discriminated Halloumi and Anari cheese in two classes, thus per cheese type. In addition, they discriminated samples based on milk species, i.e., cow and goat-sheep origins for each cheese type. They combined Fourier Transform Infrared (FTIR) spectroscopy with Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). The success of this study highlighted the importance of FTIR spectroscopy in combination with chemometrics in food authenticity. Furthermore, beyond authenticity, chemometrics aids in quality control and process verification. It facilitates the monitoring of dairy product quality by analysing various chemical and physical parameters. For instance, using rheology and FT-NIR spectroscopy, combined with chemometric analysis, Strani et al. [100] assessed the impact of physicochemical parameters and the use of skimmed milk powder in milk thickening. This approach enables producers to maintain product consistency and quality.

The authenticity of milk and dairy products is ensured by the pivotal role played by chemometrics applications. They serve as powerful tools for detecting adulteration, verifying the origin of products, identifying species, confirming cheese types and maintaining

product quality. These applications not only enhance consumer confidence but also help protect the integrity of traditional dairy products and support quality control efforts within the dairy industry. As technology continues to advance, the role of chemometrics in the dairy sector is poised to become even more critical, guaranteeing that consumers receive authentic and high-quality dairy products. Table 2 presents recent studies of chemometrics and the authenticity of milk and dairy products.

**Table 2.** Recent studies of chemometrics and authenticity of milk and dairy products.

| Type of Matrix                                                           | Purpose of Study                                                                                                                                                                      | Method of Analysis                                       | Chemometric Method                 | References |
|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|------------------------------------|------------|
| Goat milk                                                                | Detection of water, urea, cow's whey, cow's milk                                                                                                                                      | NIRS                                                     | PCA, k-NN, PLS-DA, SIMCA           | [17]       |
| Fresh milk                                                               | Formaldehyde detection                                                                                                                                                                | TD-NMR                                                   | PCA, PLS, SIMCA                    | [19]       |
| Fresh cow's milk                                                         | Formaldehyde detection                                                                                                                                                                | ATR-FTIR                                                 | PCA, SIMCA, PLS, PCR               | [16]       |
| Milk coagulation using rennet                                            | Effect of physicochemical parameters and use of skimmed milk powder                                                                                                                   | Rheology, FT-NIR                                         | MCR-ALS                            | [100]      |
| Skimmed milk powder                                                      | Detection of vegetable protein powder, whey powder, starch, lactose, glucose, fructose as well as non-protein nitrogen such as ammonium chloride, ammonium nitrate, melamine and urea | Multiple optical sensors (UV-Vis, fluorescence and NIRS) | Algorithm one class classification | [18]       |
| Milk                                                                     | Milk powder detection                                                                                                                                                                 | UPLC-QTOF-MS                                             | PCA                                | [101]      |
| Milk                                                                     | Geographical and seasonal origin                                                                                                                                                      | IRMS, EDXRF, ICP-MS                                      | OPLS-DA, SIMCA                     | [102]      |
| Milk                                                                     | Discrimination based on seasonal and animal origin                                                                                                                                    | IRMS                                                     | One-way ANOVA                      | [103]      |
| Fresh milk                                                               | Geographical origin                                                                                                                                                                   | IRMS                                                     | PCA                                | [104]      |
| Milk                                                                     | Geographical origin                                                                                                                                                                   | IRMS                                                     | OPLS-DA                            | [105]      |
| Milk and halloumi cheese                                                 | Discrimination based on animal origin                                                                                                                                                 | FTIR                                                     | PCA, OPLS-DA                       | [106]      |
| Milk                                                                     | Formaldehyde detection                                                                                                                                                                | ATR-FTIR                                                 | PCA, SIMCA, PLSR, PCR              | [16]       |
| Milk                                                                     | Detection and quantification of skimmed milk powder in fresh milk                                                                                                                     | FE-SEM, cyclic voltammetry                               | PCA, SIMCA, PLS                    | [107]      |
| Milk and mature cheese                                                   | Control of breeding system (agricultural production system) of animals                                                                                                                | FTIR, GC, PTR-ToF-MS, sensor analysis                    | LDA                                | [108]      |
| White cheese                                                             | Adulteration with vegetable fats                                                                                                                                                      | Spectroscopy Raman                                       | PLS-DA, PLS                        | [13]       |
| PDO-Fontina cheese and traditional Fontal cheese                         | Discrimination according to the type of cheese                                                                                                                                        | GC-IMS, CZE, chromatography                              | PCA                                | [97]       |
| Graviere cheese                                                          | Geographical origin                                                                                                                                                                   | GC-MS, ICP-OES                                           | LDA                                | [20]       |
| Fresh and pasteurized milk                                               | Detection of animal origin and heat treatment                                                                                                                                         | Spectroscopy Raman                                       | PLS-DA                             | [15]       |
| Cheese                                                                   | Study of ripening and ripening type based on fatty acid content                                                                                                                       | Gravimetric GC-MS, Ag <sup>+</sup> -HPLC-DAD             | CA, PCA, LDA                       | [14]       |
| Yogurt and cheese made with goat's milk                                  | Detection of cow's milk adulteration                                                                                                                                                  | FT-NIR                                                   | PCA, PLS-DA, iPLS                  | [109]      |
| PDO grated cheese Parmigiano Reggiano                                    | Study of authenticity based on crust percentage (maximum allowed rind content percentage)                                                                                             | Spectroscopy Raman                                       | SIMCA PLS                          | [110]      |
| Cheese PDO-Pecorino Romano, PDO-Pecorino Sardo and Pecorino di Farindola | Discrimination of the type of cheese based on volatile compounds                                                                                                                      | HS-SPME-GC-MS                                            | PCA, LDA, PLS-DA                   | [111]      |
| Kefalotiri and cheddar cheese                                            | Discrimination by type of cheese                                                                                                                                                      | <sup>1</sup> H-NMR, FTIR                                 | OPLS-DA                            | [112]      |
| Dairy products                                                           | Detection of adulteration with vegetable fats                                                                                                                                         | <sup>1</sup> H-NMR                                       | Orthogonal projection              | [113]      |
| Yogurt                                                                   | Detection of adulteration with vegetable fats                                                                                                                                         | FT-NIR, FT-MIR                                           | SIMCA, PLSR                        | [114]      |
| Milk powder                                                              | Milk powder                                                                                                                                                                           | ATR-FTIR                                                 | PCA, SIMCA                         | [115]      |
| Classification of Halloumi and Anari cheese                              | Discrimination according to the origin of the cheeses                                                                                                                                 | FTIR                                                     | OPLS SIMCA                         | [99]       |
| Halloumi cheese, Kefalotiri and cheddar                                  | Discrimination according to the type of cheese                                                                                                                                        | FTIR, <sup>1</sup> H-NMR                                 | OPLS-DA, MOCA                      | [116]      |
| Prato and mozzarella cheeses                                             | Verification the authenticity of commercial samples of prato cheese                                                                                                                   | MIR                                                      | PLS-DA                             | [117]      |

### 3.4. Current Analytical Methods for Meat and Meat Products Authentication and Chemometrics

Khan et al. [118] used Fourier Transform Infrared Spectroscopy (FTIR) along with Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR), combined with chemometrics, enabling the analysis of a complex multitype of meat blends. This approach ensures the maintenance of desired physicochemical characteristics in beef, pork, chicken and turkey meat products. This chemometric approach ensures the reliable identification of adulterants, enhancing product quality and consumer trust.

Varrà et al. [119] monitored the impact of radiation processing on sausages. NIR spectroscopy, employing the chemometric tools of Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) provided the necessary insights to maintain product safety and quality. Achata et al. [120] monitored bacterial growth in beef muscle under various storage conditions, which is a critical study for food safety. They used Vis-NIR hyperspectral imaging along with the total viable count (TVC) and Partial Least Squares Regression (PLSR). Rebellato et al. [121] worked

to determine the sodium content in hamburgers by applying Near-Infrared spectroscopy (NIR). Chemometrics, including Partial Least Squares (PLS) and PLS Discriminant Analysis (PLS-DA), supported this assessment effectively. Leng et al. [122] identified adulteration with pork and duck meat in ground beef products. NIR spectroscopy, in conjunction with Discriminant Analysis and Partial Least Squares (PLS), helped to achieve this goal. A very recent study performed by Cozzolino et al. [123] took place to differentiate between traditional and wild meat species. PCA, LDA and the similarity index (SI) were applied. The authors concluded that SI is a quite simple method for comparing two spectra. Comparison of SI to classical chemometric methods (e.g., LDA, PCA), showed that SI can be more easily understood, has a low cost and can be applied by only using software such as Excel® (2013). Another study based on NIR spectroscopy was implemented by Hoffman et al. [124]. The adulteration of exotic meat species (emu and camel) with traditional or commercial species (beef and lamb) in binary mixtures of minced meat was monitored by chemometric methods, such as PCA and PLS-DA. It was concluded that the level or ratio of adulteration can be determined by NIR spectroscopy.

Based on the aforementioned research studies, chemometrics stands as an indispensable asset in the realm of meat and meat product authenticity studies. Its capabilities span from precise adulteration detection to the characterization of multi-meat blends, authentication of halal products, differentiation of meat types, monitoring of processing effects, and assessment of biochemical and physicochemical properties. Furthermore, chemometrics plays a pivotal role in food safety by monitoring microbial flora and ensuring compliance with dietary recommendations. By embracing chemometrics, the meat industry can ensure that consumers receive products of the utmost authenticity and quality. Table 3 presents recent studies of chemometrics and the authenticity of meat and meat products.

**Table 3.** Recent studies of chemometrics and authenticity of and meat and meat products.

| Type of Matrix                      | Purpose of Study                                                        | Method of Analysis | Chemometric Method                  | References |
|-------------------------------------|-------------------------------------------------------------------------|--------------------|-------------------------------------|------------|
| Chicken                             | Status identification: fresh or frozen                                  | NIR                | PLS-DA, CPANN, SVM                  | [125]      |
| Sausages (pork and beef mix)        | Soy protein adulteration                                                | E-nose             | PCA, PNN                            | [126]      |
| Beef meat preparation               | Wild boar meat adulteration                                             | FTIR               | PCA, PLS                            | [127]      |
| Beef, lamb and venison              | Discrimination red meats                                                | Spectroscopy Raman | PCA, PLS-DA $\kappa \alpha t$ SVM   | [128]      |
| Beef mixture                        | Chicken adulteration                                                    | ATR- FTIR          | PCA, PLSR, ANN                      | [129]      |
| Beef meat preparation               | Adulteration with another type of meat                                  | FTIR               | PCA, PLS-DA $\kappa \alpha t$ SIMCA | [130]      |
| Minced pork                         | Aging and wear, characterization of changes during storage and spoilage | HS-SPME-GC-MS      | PCA, OPLS-DA                        | [131]      |
| Beef meat                           | Adulteration with maltodextrin                                          | MIR                | PLS-DA                              | [132]      |
| Beef, pork, chicken and turkey meat | Physicochemical characteristics                                         | FTIR               | PCA, PLSR                           | [118]      |
| Sausages                            | Radiation treatment process                                             | NIR                | PCA, OPLS-DA                        | [119]      |
| Beef muscle                         | Bacterial growth at two storage temperatures                            | Vis-NIR HIS        | TVC, PLSR                           | [120]      |
| Hamburger                           | Sodium content                                                          | NIR                | PLS, PLS-DA                         | [121]      |
| Ground beef                         | Adulteration with pork and duck meat                                    | NIR                | DA, PLS                             | [122]      |
| Beef sausage                        | Adulteration with pork                                                  | LC-HRMS            | PLS-DA                              | [133]      |
| Sausage products                    | Adulteration with pork                                                  | GC-MS, FTIR        | PCA                                 | [134]      |
| Pork meat                           | Authenticity of pork fat according to the rearing system                | NIR                | DD-SIMCA                            | [135]      |
| Chicken                             | Geographical origin                                                     | ICP-OES, ICP-MS    | OPLS-DA, CDA                        | [136]      |

### 3.5. Current Analytical Methods for Fish and Fishery Products Authentication and Chemometrics

#### 3.5.1. DNA-Based Methods

In order to identify fish species, several DNA-based methods have been employed. Their realization involved multiple similar preparative steps, viz. the isolation of DNA and in silico investigation applying convenient databases (such as specific primers). The most noteworthy for fish species identification are methods using restriction cleavage RFLP and AFLP (Amplified Fragment Length Polymorphism), DNA barcoding, FINS (Forensically Informative Nucleotide Sequencing), HRM (High-Resolution Melting), PCR (Polymerase Chain Reaction), RAPD (Random Amplified Polymorphic DNA) and SSCP (Single-Stranded Conformational Polymorphism) [137,138]. In addition, the Loop-Mediated

Isothermal Amplification (LAMP) technique has been newly employed for fish species identification. Several methodologies have been carried out using either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA), but recently, LAMP (Loop-mediated isothermal amplification) method has been applied for fish species [139,140]. Nuclear (nDNA) or mitochondrial mtDNA have been used in several lines. Databases of genome sequences/nucleic acid sequences could simplify the choice of appropriate target molecules; on the other hand, identification markers can support these methodologies through their complexity and efficiency.

The most important ones are: EMBL (European Molecular Biology Laboratory, <http://www.ebi.ac.uk>); DDBJ (DNA Data Bank of Japan, <http://www.ddbj.nig.ac.jp>); and the NCBI (Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). The EU database FishTrace (<https://fishtrace.jrc.ec.europa.eu/>) employs databases based on fish nucleotide sequences. Recently, the development of DNA-based identification methods has been shortened by the recognition of the fish genome sequence. For instance, up to 2020, more than 900 whole-genome sequences of fish species were published [141]. mtDNA sequence data can also be employed to identify several fish species in concurrence with the whole-genome sequences, and >3300 mtDNA sequences of fish species have been put in the NCBI. On the other hand, for fish species identification, mtDNA can be used to elucidate the difference in nearly linked taxa and even species, and its aptitude to distinguish the geographical origin of the fish species [142]. According to the literature, for fish species identification, the commonest mitochondrial markers are: the COI (gene for cytochrome-c oxidase subunit I) [143,144] and cytb (cytochrome b) [145]. Their incidence is expected in treated fish products based on the very high mtDNA copy number in the cell [146]. The non-feasibility of DNA computing, since mtDNA levels could vary on the tissue type, is the major drawback of mitochondrial markers compared to nuclear ones [147]. In addition,  $\beta$ -actin is the most regularly used nuclear marker for fish authentication, and is also employed as an internal regulator for mRNA quantification [148] and parvalbumin [149]. Additional regularly used markers for fish species identification are: short tandem repeats (STRs) and simple sequence repeats (SSR) [150]. These nuclear microsatellites have the benefits of being extremely species-specific and being utilized for analyses at the intra-species level; meanwhile, their abundance across the genome requires a small amount of DNA to assemble data [151].

The PCR-RFLP has been fruitfully useful for fish species and their products' identification due to its ease and low cost. AFLP progresses the species-specific SCAR (sequence characterized amplified region) marker for detection of the fresh Atlantic salmon's adulteration against the less expensive rainbow trout [149]. Maldini et al. [150] applied AFLP to assess the distinctiveness of fish species in processed commercial products, while this approach could quickly classify nine species of cod fish as concluded by Akasaki et al. [151]. Espiñeira et al. [152] used PCR-RFLP to distinguish seven species of anglerfish, and Yu and Guo [153] also appraised the genetic difference of four strains and one wild population of the eastern oyster by AFLP/microsatellite markers. Lin and Hwang [154] utilized PCR-RFLP, and identified the species in eighteen commercial canned tuna products by differentiating albacore, yellowfin, bigeye and Atlantic bluefin tuna. Recently, the investigation of Yao et al. [155] established and validated 39 commercial tuna sashimi samples. RFLP is advantageous due to it being precise, reliable, operative, moderately polymorphic, with great genomic richness and with random dissemination, and extremely reproducible. However, it has some disadvantages such as being expansive, not entirely robotic, and demanding large quantities of purified and great molecular weight DNA for each digestion.

FINS uses a comparable standard to DNA barcoding and is allied with DNA sequencing/phylogenetic analyses. An informative nucleotide sequence is subsequently identified by phylogenetic analysis succeeding the sequencing of an amplified specific DNA fragment [154]. Remarkably, FINS allowed the detection of new unexplored species [155]. For instance, the adulteration of 40% of processed Cyprinidae commercial samples by *Oreochromis* spp. was noticed using the FINS method [156]. FINS was suitable for extremely

treated products and powerful when employed to define the interspecific/intraspecific variability. However, it is expensive, and highly skilled operators are required. Genetic markers employed to identify fish species are summarized in Table 4.

**Table 4.** Recent overview of genetic markers employed for fish species identification.

| Genus/Species of Fish                  | Detection Method             | Markers                      | Objective of Study                                                                                                                                                                                                               | References |
|----------------------------------------|------------------------------|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Anglerfish ( <i>Lophius</i> )          | real-time PCR                | Pvb/nDNA                     | Identification and quantitation of two European anglerfish, <i>L. piscatorius</i> and <i>L. budegassa</i>                                                                                                                        | [157]      |
| Salmon and trout                       | real-time LAMP               | cytb/mtDNA                   | Individually and simultaneously specific detection of <i>S. salar</i> and <i>O. mykiss</i>                                                                                                                                       | [145]      |
| Salmon and trout                       | real-time PCR                | COI/cytb mtDNA               | Identification of <i>S. salar</i> and <i>O. mykiss</i> in processed fish products                                                                                                                                                | [158]      |
| Atlantic salmon ( <i>Salmo salar</i> ) | real-time LAMP, PCR          | Cytb/mtDNA                   | Detection of <i>S. salar</i> in processed fish products                                                                                                                                                                          | [159]      |
| Sardina                                | DNA barcoding, real-time PCR | COI/mtDNA                    | Screen the species of <i>S. pilchardus</i> species in varied products                                                                                                                                                            | [160]      |
| Codfish species                        | HRM                          | 12S rRNA/mtDNA               | Discrimination between <i>Gadus</i> species and the other five <i>Gadiformes</i> species. Nineteen commercial codfish products were included in the <i>Gadus</i> cluster, cross-confirmed by the real-time PCR and DNA barcoding | [161]      |
| Salmon                                 | LAMP, PCR                    | cytb (LAMP), COI (PCR)/mtDNA | Identification of Atlantic salmon in processed fish product                                                                                                                                                                      | [162–165]  |
| Trout                                  | PCR, LAMP                    | COI (PCR)/ cytb (LAMP) mtDNA | Identification of rainbow trout in processed fish products                                                                                                                                                                       | [163–166]  |
| Salmon                                 | real-time PCR                | GH1, 18S rDNA/nDNA           | Detection of <i>S. salar</i> in processed fish products                                                                                                                                                                          | [167]      |
| Fish genus                             | real-time PCR                | 16S rRNA/mtDNA               | Validation and applicability to model mixtures with spiked fish                                                                                                                                                                  | [168]      |
| Salmon                                 | SNPs 94                      | SNPs loci/ Genome            | Discriminate between farmed salmon populations of several origins                                                                                                                                                                | [169]      |
| Sardina                                | PCR-RFLP                     | Cytb/mtDNA                   | The authenticity of sardines commercialized in Rio de Janeiro state                                                                                                                                                              | [170]      |

### 3.5.2. Novel Detection Methods

Recently, novel techniques for assessing fish and fishery products' features have resulted in the progress of non-invasive/non-destructive instrumental methods, such as biosensors and e-sensors.

#### Biosensor Techniques

Biosensors are able to appraise a biological or chemical reaction and to adapt the answer into an electrical signal [171]. Tang et al. [172] concluded that a sensitive amperometric sensor, based on carbon nanofibers, has a great affinity to the oxidation of Xanthine in crucian carp samples. The correlation coefficient and the limit of detection were 0.99 and 20 nM, respectively. Heising et al. [173] used electrode sensors to monitor pH variation in and conductivity of the aqueous phase, which was associated with the increase in volatile amines in fish. From raw fish flesh, Chang et al. [174] established an amine gas sensor qualified to perceive volatile amine gases such as ammonia, TMA and DMA. Li et al. [175] reviewed the impact of different triethylamine concentrations on copper nitrate–benzenetricarboxylic acid (Cu-BTC) on the concurrent assessment of Xa and HxA in fish samples. The results showed that TMA had a great influence on the electron transfer ability of Cu-BTC, improving the sensibility. A linear performance between the levels and the oxidation peak of Xa and HxA was performed on fish samples. Enzymatic biosensors, based on the evaluation of the response substance/enzyme, were created. Thandavan et al. [176] found a biosensor based on nanoparticles of iron oxide linked with Xanthine oxidase (XOD), provoking the electroreduction of H<sub>2</sub>O<sub>2</sub>. Narang et al. [177] and Borisova et al. [178] launched a system-based nanocomposite of TiO<sub>2</sub> nanoparticles/carbon nanotubes and [polydopamine/platinum, which immobilized the enzyme of XOD. In these studies, labeo and hake fish samples were examined, and these novel biosensors proved to have high reproducibility and repeatability. Similarly, Apetrei et al. [179] proposed a sensor for histamine biosensing, where the enzyme diamine oxidase was immobilized on a carbon blended with grapheme/platinum nanoparticles. The LOD was equal to 25 mM at a linear array between 0.1 and 300 µM.

Torre et al. [180] developed a biosensor based on a carbon electrode with the immobilized diamine oxidase in tuna and mackerel samples. The LOD was low (0.97 mg/L) and the accuracy, as well as recovery value were high [180,181]. To define HXA, Xa and uric acid in four different fish species, Pierini et al. [182] proposed an edge plane pyrolytic graphite electrode with immobilized nucleoside phosphorylase and XOD. Likewise, to define ATP-



related compounds, alkaline phosphatase and adenosine deaminase were concurrently co-immobilized onto alkylamine glass beads [183].

#### Sensory Bionics Technology

This technique comprises an electronic nose/tongue, a colorimetric sensor array and computer vision. In fish products, this technology was developed based on the senses of smell, taste and vision.

##### - *E-nose*

Numerous systems have been employed based on electrochemical gas, metal oxide and conducting polymer sensors joined with various extraction/data processing methods.

Guohua et al. [184] launched an electronic nose system that consisted of eight metal oxides for envisaging the freshness of grass carp. These authors employed PCA to separate all samples according to the freshness; so, with the storage time, the response intensities of sensors increased. In this study, all samples were separated into three different groups. To discriminate channel catfish according to a good/or not flavoursome aroma, Wilson et al. (2013) applied an e-nose covering an organic matrix-coated polymer-type 32-sensor array. To track the spoilage of tilapia, Semeano et al. [185] recently developed an optical electronic nose linked to the microorganism's growth. Haugen et al. [186] monitored the smoked salmon process using a gas-sensor array system. The total viable content (TVC) and lactic acid bacteria (LAB) loads were controlled, and a precise classification rate ranging from 93 to 95% was achieved. In the same way, TVC was detected by four metal oxide microsensors to separate sardines according to freshness [187]. Tian et al. [188] used metal oxide sensors to assess TVB-N and TVC in hairtail fish, and PCA was employed for the compensation of humidity and temperature effects. All these established results prove that e-noses have many benefits in monitoring the authenticity of fish freshness including their ease of operation, rapidity, reliability and precision, which could substitute for other expensive or time-consuming analysis techniques.

##### - *E-tongues*

To evaluate sea bream freshness, Gil et al. [189] developed a method based on electrodes of several kinds of metals. An artificial neural network was conducted to classify samples; the correct % was equal to 90. Barat et al. [190] employed an e-tongue composed of gold/silver wires. A high correlation was obtained between the sum of inosine + HXa and the sum of all other ATP breakdown products. Miao et al. [191] applied a combination of the e-nose and -tongue for a post-cooking sensory evaluation of canned tuna. A PCA was employed for the creation of a K-value calibration model, gaining an acceptable distribution of the samples by the first two principal components. Pattarapon et al. [191] used an e-tongue, in conjunction with an e-nose and several chemical parameters, to investigate the variations in grass carp quality between vacuum and non-vacuum packing. The outcomes showed that these techs could distinguish between the three different storage conditions, sustained by PCA/LDA analysis [191,192].

##### - *Computer vision technique*

This technique has been employed to attain and examine the image of a real scene using computers. It has the potential to track the quality of food products in an automated and non-destructive way [193,194]. To assess the colour variations in the pupils and gills of tilapia, Shi et al. [195] used a computer vision system, and the MLR was investigated to predict TVB-N, TVC and TBA values with high  $R^2$  (~0.999). Similar trends were observed by Balaban and Alçiçek [196] and Issac et al. [197], who studied fish of gilthead sea bream, and gills of Indian rohu, respectively. It should be noted that, compared with traditional evaluation approaches, computer vision displays the capability to be speedy and has a low-cost for envisaging the freshness and authenticity of fish and fish products.

#### 4. Challenge and Future Perspectives

In spite of extended investigations focused on the detection of fraud and authenticity via on-site and real-time advances, numerous pivotal challenges continue to prevail regarding both technique concerns and the framework for the validation of models. In this line, several approaches comprise non-targeted methods that detect diverse, small modifications in the studied food product; furthermore, these extracted data were investigated by a multivariate statistic approach. In this regard, it should be noted that the authentication of food samples should define the unit/number and variability in samples, and a sustained maintenance of the food database is required to guarantee its long-term ability to provide suitable results. On the other hand, various experimental conditions can impact the acquired results and contribute to analytical deviation that is not linked to the authentication issue. These deviations should be decreased to the lowest values and be well monitored to guarantee that they do not confuse the results of the analysis.

In spectroscopic techs, the results of which are reproducible and just influenced by variations in sensitivity, sample development and preparation was not normally required. This is true for liquid foods, whereas solids (viz. meat, fish, egg) are heterogeneous matrixes and may involve moderate and/or multiple preparations. Furthermore, the choice of a proper acquisition mode was crucial to attain authentic spectroscopic results. Based on the food product's nature, the kind of radiation, the type of sample performance, the versatile sample holder and the employed temperature should be tracked.

Recently, hyperspectral imaging tools have been an effective and useful alternative to point spectral scanning. In heterogeneous food product samples such as meat and fish products, hyperspectral imaging can control the large spatial distribution of components. In these two kinds of animal products, hyperspectral imaging technologies are linked to NIR radiation spectroscopy to determine the quality and the corresponding authenticity.

Generally, from any developed detection system result, multivariate data analysis is the ultimate phase that is skilled at categorizing samples as authentic or not. In this situation, a suitable algorithm and powerful validation of the model are needed to assure reproducible results that favour the agreement of these practices in legislation. Practically, several works have been established and developed at the laboratory scale, while few have been conducted at the plant level, which continues to be challenging. Thus, to meet these challenges, a collaborative effort of all actors involved, including regulatory agencies, industries, stakeholders, academics and researchers, is undoubtedly needed.

#### 5. Conclusions

Analytical techniques together with chemometrics are undoubtedly reliable techniques for predicting fraud and the authenticity of animal and animal-derived foods. It is important to find in each case a rapid and non-invasive method, together with the appropriate model of chemometric processing and validation, in order to extract a reliable tool for the rapid identification and quantification of adulteration. This is an important requirement at the current time due to the increasing number of processed animal products in which the treatments applied can cover a possible adulteration between species, against the rights of consumers. A high number of analysed samples and validation systems are needed to demonstrate that the models can accurately predict not only adulteration levels but also simple adulteration identification.

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