# Application of Novel Techniques for Monitoring Quality Changes in Meat and Fish Products during Traditional Processing Processes: Reconciling Novelty and Tradition

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#### Abstract:

In this review, we summarize the most recent advances in monitoring changes induced in fish and other seafood, and meat and meat products, following the application of traditional processing processes by means of conventional and emerging advanced techniques. Selected examples from the literature covering relevant applications of spectroscopic methods (i.e., visible and near infrared (VIS/NIR), mid-infrared (MIR), Raman, nuclear magnetic resonance (NMR), and fluorescence) will be used to illustrate the topics covered in this review. Although a general reluctance toward using and adopting new technologies in traditional production sectors causes a relatively low interest in spectroscopic techniques, the recently published studies have pointed out that these techniques could be a powerful tool for the non-destructive monitoring and process optimization during the production of muscle food products.

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Review

# Application of Novel Techniques for Monitoring Quality Changes in Meat and Fish Products during Traditional Processing Processes: Reconciling Novelty and Tradition

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Abstract: In this review, we summarize the most recent advances in monitoring changes induced in fish and other seafood, and meat and meat products, following the application of traditional processing processes by means of conventional and emerging advanced techniques. Selected examples from the literature covering relevant applications of spectroscopic methods (i.e., visible and near infrared (VIS/NIR), mid-infrared (MIR), Raman, nuclear magnetic resonance (NMR), and fluorescence) will be used to illustrate the topics covered in this review. Although a general reluctance toward using and adopting new technologies in traditional production sectors causes a relatively low interest in spectroscopic techniques, the recently published studies have pointed out that these techniques could be a powerful tool for the non-destructive monitoring and process optimization during the production of muscle food products.

**Keywords:** control; curing; drying; fermentation; muscle foods; preservation; process optimization; spectroscopy

# 1. Introduction

Muscle foods, including meat, fish, and their products, play important roles in human nutrition due to their rich contents of high-quality proteins, vitamins, and minerals. Indeed, meat and meat products (e.g., beef, lamb, pork, chicken, sausages) as well as seafood, such as fish and shellfish, have become an integral part of the human diet [1,2]. However, it is well-known that fresh meat and fish and their products are highly perishable and have a relatively short shelf life due to their high content of water and the existence of a large number of nutrients. Therefore, quality deterioration

can occur rapidly unless adequate processing or preservation methods are employed. Examples of

these methods include thermal treatments, low-temperatures, and natural preservatives, among many others [3–8].

Traditional preservation techniques such as salting, drying, and fermenting have been widely used for centuries in order to prolong the safety, storage life, and quality of meat and fish. Despite the emergence of many novel processing technologies, these traditional products remain in the modern cuisine due to their exceptional taste, smell, and textual properties [9–11]. However, modern food production holds ever-higher standards on the safety, sensory, and physicochemical quality. On the other hand, producers aim at increasing the yield and cost-effectiveness of their processes, as well as meeting consumer demands of high product quality and consistency, alongside more sustainable and environmentally friendly productions [12,13]. This calls for process optimizations and close process monitoring to maintain and optimize quality, ensure consistency, and aid sorting and the effective removal of defected products. Traditionally, these processes are assessed using visual assessment or laboratory-based physicochemical analytical techniques, which have several limitations, such as being sample destructive, time-consuming, expensive, labor heavy, and often requiring dangerous chemicals or solvents [2,14].

Although spectroscopic analytical methods have been available for several decades and have been actively applied in various fields such as chemistry and medicine, they have only recently attracted interest within the food sector. Moreover, recent technical advances of the spectroscopic methods and the simultaneous development of powerful chemometric data processing methods have opened the possibility of applying fast, non-destructive, accurate, and precise physicochemical analyses in complex media such as meat and fish [15-18]. Spectroscopic methods (e.g., vibrational spectroscopy, fluorescence, NMR) allow a reliable and fast analysis of food quality and authenticity due to their ability to produce spectral "fingerprints" of the samples depending upon the molecular composition of the products [19,20]. Due to these characteristics, spectroscopic analytical methods can be equally applied online, inline, or in a laboratory setting to evaluate a wide range of both intrinsic and external physicochemical traits within muscle foods during processing [2]. Several methods, such as magnetic resonance imaging (MRI), X-ray, and hyperspectral imaging can also provide images of the spatial distribution of food components in addition to spectral data during processing [2,15,18,21,22], giving an even deeper insight into the effects of varying processing methods. Therefore, methods combining the advantages of both spectral and image analysis are considered as especially promising techniques in the development of automated food quality and safety inspection [23].

Despite all the aforementioned advantages of spectroscopic techniques, their utilization in real-life applications is still limited in traditional processing sectors compared with other processing industries. Indeed, a general reluctance toward implementing and adopting new technologies in traditional production sectors can be noticed. For example, new technologies, in terms of either processing or analytical techniques, can hardly find a place in a plant of lutefisk: a traditional Norwegian fish dish in which the same procedures of preparing and monitoring the quality have been followed for ages [24,25].

In the scientific literature, it is possible to find many review papers dealing with either processing processes [6,9,10] or assessment techniques [1,2,13]. However, until now, no reviews have been available focusing on both traditional processing processes and analytical assessment methods. Thus, the objective of this review paper is to give an overview of recent advances in the applications of spectroscopic analytical methods for food quality assessment and monitoring during traditional processing processes, such as the salting, drying, and fermenting of meat and fish and to discuss their advantages, limitations, and future prospects for food quality assessment. This review paper is organized as follows. A short description of the most widely applied traditional processing processes will be first presented. Then, traditional and emerging methods used to assess quality changes in meat and fish resulting from the application of traditional processes will be discussed, putting a special

focus on the spectroscopic methods. Finally, technical challenges and future application opportunities are highlighted.

#### 2. Short Description of Selected Traditional Preservation/Processing Processes

During human history, food preservation has been a fundamental tool. The first preservation techniques employed were curing, sun drying, and fermentation, among others, which allowed providing food when fresh food was not accessible [26]. As human civilization evolved, greater amounts of processed food were needed and new preservation techniques were developed to solve the limitations of the traditional ones, such as the degradation of biological compounds of great interest, long preparation times, high energy consumption, or undesirable changes in the organoleptic and palatability properties. Nevertheless, the use of these techniques (alone or in combination with other traditional processes) to preserve food products is still very common. In the paragraphs bellow, a short description of traditional techniques will be provided.

# 2.1. Curing

The curing process is one of the oldest methods to preserve meat, fish, and derivative products, dating back to 3000 BC. This process involves the use of dry salt or brines to cover or rub the surface of the food, which causes a reduction of the water activity. This term refers to the water not bound to food molecules, which can support the growth of microorganisms. Thus, reducing the availability of water prevents the microbial spoilage [27]. The osmotic phenomena cause the water content reduction, as water flows from the food (lower salt concentration) to the outside (higher salt concentration). Then, water dissolves the salt, which penetrates inside the food [28]. These products are kept at low temperatures  $(1-4 \degree C)$  during long periods [29]. In the past, sea salt was used in the curing process. This salt often contained contaminants such as sodium or potassium nitrate or nitrite. These compounds contributed to the curing and preservation processes, but their role was not identified until later. Potassium nitrate can be transformed into nitrite and later into nitric oxide, which reacts with myoglobin. This reaction leads to the formation of an attractive red color both in meat and fish products [30-32]. Nitrites present well-known antioxidant properties, and their addition prevents the development of rancid flavors during storage, and along with nitrates, they improve the overall flavor of cured foods. However, these compounds are mostly used in the food industry due to their antimicrobial properties [30,32]. Currently, there is great concern about the use of nitrites and nitrates in cured products, since some studies suggest that their consumption may have harmful effects on health. However, other studies do not support these claims, so a consensus on their use has not been reached [33]. Worldwide, there exists a great variety of meat-cured products, such as cured ham, sausages, or bacon and traditional foods such as pastrami [27]. Regarding cured fish products, salted salmon, cod, anchovy, or lakerda (a dish that employs salted bonito) are some examples [29,34].

# 2.2. Drying

Dying is considered the earliest method used to extend the shelf life of meat, fish, and derivative products. This process is usually used before applying other preserving techniques, including curing or fermentation. Similar to curing, this process aims to dehydrate foods, reducing the water content to prevent the microbial growth and enzyme spoilage. In this technique, the water reduction is achieved by evaporation, using heat and air [35,36]. Two factors should be considered to obtain dry products. First, the process should be performed properly. For example, if the drying is too fast, the final product can present a hard texture, which causes negative effects in the palatability of the product. Second, if the drying is to slow, food microbes could survive and subsequently contaminate the product [37]. Drying processes have several advantages, such as weight and volume reduction, easy food storage, packaging and transport, and they also offer characteristic flavors and odors to the food products. However, drying-based methods have been reported to present several disadvantages, such as a long drying time, high energy consumption, loss of aroma, flavor, and functional compounds

(including vitamins, proteins, and lipids), and it often causes lipid oxidation, resulting in off-flavored products [36–38]. Nowadays, several innovative drying techniques have been developed to maintain the nutritional and physicochemical characteristics of the dried foods, such as infrared drying or microwave drying [38].

# 2.3. Fermentation

Since ancient times, this process has been applied to a wide range of foods, including muscle foods (e.g., meat, fish). It has been proposed that the fermentation process was discovered by accident or by trial and error [35]. The fermentation process consists of the use of food as substrate for the growth of beneficial microorganisms, mainly lactic acid bacteria. These bacteria present a safe metabolic activity, using the food's sugar to produce organic acids and other metabolites, and they are considered as GRAS (generally recognized as safe) organisms for the elaboration of food products for human consumption [39]. In the case of meat, bacterial species such as Lactobacillus spp., Streptococcus spp. or Bifidobacterium spp. are employed. In the case of fish, Lactoccocus spp., L. brevis, and Pediococcus spp. are the most used bacterial species [40]. Traditionally. the fermentation process was carried out using the flora present naturally on the food. Currently, the food industry employs starter cultures (a microbiological culture), allowing standardization of the products and also ensuring their safety. The action of enzymes produced by the microorganism and enzymes present naturally in the product hydrolyze the polysaccharides, proteins, and lipids present in the fermented food [39]. Unlike curing and drying, which are based on reducing the water content, the fermentative metabolism of the microorganisms caused acidification, preventing the development of foodborne pathogens and enhancing the shelf life of the product [41]. Furthermore, other characteristics are improved such as digestibility, nutritional value, color, aroma, and texture, and it also decreases the undesirable compounds that may be present in the raw product [42,43]. Several examples of fermented meat and fish and derivative products include fermented sausage, fermented meat sauce, "surströmming" (herring fermented, traditional to Swedish cuisine), and different traditional fermented products made of anchovies, sardines, oysters or squids, for example [39–41].

#### 2.4. Other Traditional Processing Processes

Smoking is an ancient technique to preserve foods, and it has been applied together with other processes, especially with drying. Smoking reduced the water activity and also favors the incorporation of antimicrobial substances. Moreover, the antioxidant phenolic compounds present in smoke can delay the oxidation of lipids. This process modifies the characteristics of the food, including the color, texture, and especially the aroma. Smoked meat products include bacon, German salami, speck (smoked pork belly), and different kinds of sausages, while some common smoked fish species are mackerel, salmon, trout, or herring [29,37].

Several products have been protected from microbial spoilage due to changes in the pH of the food, such as in the case of fermentation. However, other preservation processes are focused on increment the pH. For example, the "lutefisk" is a Nordic traditional cod dish that reaches a pH of 12 during its production and is consumed at pH above 10. This high pH is achieved by soaking the fish in lye. The water content of lutefisk is also very high (more than 90%) due to the repetitive steps of soaking in water during the preparation of this product (Figure 1). This process prevents the growth of pathogenic microbes and is also conducive to a partial hydrolyzation of the muscle proteins and the characteristic smell and appearance of the product [25].



**Figure 1.** Water content (WC) and pH values of the four lutefisk brands. Reproduced in compliance with CC BY license from Ref. [24].

#### 3. Classical Methods Used to Evaluate Quality Changes

Quality evaluation of meat and fish and derivative products encompasses many techniques. Sensory assessment and microbiological analysis are among the most used methods. Sensory evaluation implies the use of the five human senses (i.e., sight, smell, taste, touch, and hearing) for evaluating various quality attributes of food products [3]. Traditional processing processes, such as salting, fermentation, etc., induce significant changes in sensory characteristics. For example, the color, springiness, and overall sensory acceptability of Russian sturgeon (*Acipenser gueldenstaedti*) were reported to be associated with different salt concentrations applied during the immersion of fish fillets in salt solutions [44]. In another study, it was indicated that the sensory attributes of lightly salted Atlantic cod can be improved by treating the fillets with a combination of NaCl and NaHCO<sub>3</sub> [45]. In addition, the curing of grass carp with either 1.6% salt or 1.6% and 0.8% sugar was reported to improve, among other factors, the sensory attributes of the treated fish [46].

Several investigations have been also carried out on meat and meat products. For example, unacceptable organoleptic characteristics of dry-cured sausages were noticed after 6 weeks of storage at 20 °C [47]. In another investigation, the effect of different salt types, including NaCl, KCl, and MgSO<sub>4</sub> with different concentrations and mixtures on bovine meat was studied [48]. A metallic flavor and bitter taste were found to be related to samples treated with MgSO<sub>4</sub>, whereas KCl induced greyer compared to meat treated with NaCl. In a more recent study, a better sensory acceptability was shown for Harbin dry sausage treated by replacing NaCl with KCl [49].

In addition to sensory analysis, numerous microbiological measurements, such as total viable count (TVC), have been widely applied and considered as standard tools used in order to validate the shelf life and demonstrate changes in the quality of meat and fish or other products. Microbial growth and microbiological analysis are especially important in the case of fermented products, such as fermented fish and seafood [11,25,50–52]. Various types of microorganisms and different counts have been reported in the literature according to traditional processing processes (i.e., fermentation, drying, smoking, etc.) and the severity of the applied treatment. For instance, the treatment of Russian sturgeon fillets with different concentrations of NaCl caused an inhibitory action on microbial growths, and the preservative action was more remarkable with increasing the concentration of the salt [44]. However, some kinds of microorganisms, called a halotolerant, can survive even at high salt concentrations [11,53–55]. Different parameters, such as water content and storage conditions, were reported to affect the microbial growth and shelf life of meat and fish [47,54,55]. For example, for muscle food products with low water activity, such as dry-cured sausages, the TVC values were

found to be low, and product spoilage during the advanced stages was attributed to lipid oxidation rather than microbial contamination [47].

In addition to sensory analysis and microbiological assessment, a wide range of physicochemical measurements has been widely tested for evaluating quality changes in meat and fish and derivative products resulting from the application of traditional processes. These include, but are not limited to, chemical composition, pH, water-holding capacity, k value, volatile compounds, lipid and protein oxidation [3,44,53,56–59]. However, most of these techniques and approaches share general limitations, including the destructiveness of the sample and the time required for the analysis, in addition to specific challenges that are applicable to each of these methods. In recent years, several targeted methods such as electronic nose, electronic eye, and electronic tongue have been proposed in order to evaluate the sensory attributes of the examined products in a more objective way [17,60]. More interestingly, non-targeted methods based on spectroscopy have gained popularity during the last few years due to several advantages and features that are going to be detailed in the next section.

#### 4. Spectroscopic Monitoring Techniques

# 4.1. Short Description of Spectroscopic Techniques

Spectroscopic techniques exploit the interaction between electromagnetic radiation and matter to characterize the samples. Due to the many possibilities offered by the electromagnetic spectrum in terms of the frequency ranges that can be explored and the various modalities that can be employed to register the signal (e.g., reflection, transmission, transflection), spectroscopic techniques are highly versatile tools for the characterization of foodstuffs. In the remainder of the present section, the main characteristics of the spectroscopic techniques most commonly adopted for the quality control of food products—and more specifically, for monitoring changes in meat and fish and derivative products—will be briefly discussed.

Absorption spectroscopy in the UV and visible range (190–800 nm) [61] is a relatively widespread approach due to its simplicity and wide availability. It is based on the transitions between molecular electronic levels that are induced by the absorption of a photon with the proper frequency by the sample's constituents. Since electronic transitions are usually accompanied by unresolved vibro-rotational transitions, UV-Vis bands are normally rather broad, and they seldom allow the univocal identification of the absorbing species. On the other hand, being high-energy transitions, the corresponding sensitivity is rather good.

Considering the same spectral range, selectivity/specificity can be improved, whenever possible, by moving from absorption to emission spectroscopy, i.e., considering fluorescence techniques. Molecular fluorescence [62] is based on recording the intensity of the light emitted by a molecule when radiatively relaxing from an excited state to the ground state; in this context, the promotion of a molecule to the excited state is also achieved by the absorption of a photon with a specific wavelength. Since not all the chromophores are fluorescent, the technique has a higher selectivity with respect to its absorption counterpart; however, this can also be a disadvantage, as not all the samples may contain fluorescent molecules, so that, in order to be able to use the technique, a pre-derivatization with fluorescent molecules could be needed. On the other hand, since detection of the fluorescence signal is carried out along a direction that is not colinear with the incident light, the signal to noise ratio is higher, and significantly lower limits of detection can be obtained.

Another family of techniques that are widely used for the characterization of food products is that of vibrational spectroscopies, i.e., the ones that involve transitions between the vibrational levels of the molecules. This family includes mid-infrared (MIR), near-infrared (NIR), and Raman spectroscopy.

Mid-infrared spectroscopy (400–4000 cm<sup>-1</sup>) [63] deals with the fundamental vibrational transitions and results in relatively narrow bands, which can be associated either to the presence of specific functional groups or to the movement of the whole molecular backbone. In this respect, MIR spectroscopy can be advantageously used for qualitative characterization. On the other hand,

near-infrared spectroscopy (800–2500 nm corresponding to 12,500–4000 cm<sup>-1</sup>) [64] exploits transitions corresponding to overtones and combinations of fundamental vibrations; due to the highly anharmonic nature of such transitions, it mostly involves X–H bonds, where X can be carbon, nitrogen, oxygen, and sulfur. Overtones correspond to transition vibration frequency, while combination bands arise from the simultaneous occurrence of two or more transitions and occur at sums of multiples of the fundamental transition vibration frequency, while combination bands arise from the simultaneous occurrence of two or more transitions and occur at sums of multiples of the frequencies of each interacting mode. Based on the nature of the transitions involved, which have a lower probability of occurrence than their fundamental counterparts, for this reason, NIR bands are very weak and typically broad and highly overlapping. As a consequence, the assignment of bands to sample components is not straightforward, and the use of chemometrics to extract useful information from the spectra is even more necessary than with other spectroscopies. On the other hand, these same characteristics are also responsible for the main advantages of NIR with respect to other spectroscopic techniques: indeed, the low absorptivity allows a higher penetration depth, which in turn results in the possibility of analyzing thicker, strongly absorbing or, even, highly scattering samples, without any need of sample pre-treatment.

Raman scattering [65] is another effect of light interacting with molecular vibration, and it involves a change in molecular polarizability, so that even molecules that are symmetrical and not IR-active can produce a signal. Raman spectroscopy couples the straightforward interpretability of the bands associated to fundamental molecular vibration (just as in MIR) with some characteristic features that make it particularly suitable for the analysis of complex matrices: the use of lasers in the visible/near-visible region allow the direct analysis of packed samples, provided that the packaging is transparent to the radiation; moreover, the low polarizability of water results in the possibility of analyzing aqueous solutions without any interference of the solvent.

All the above-mentioned techniques can be coupled with imaging or microscopic devices in order to provide hyperspectral images [66]. The use of hyperspectral imaging, i.e., the possibility of recording images in which a whole spectrum is acquired for each pixel, allows obtaining at the same time the spatial and spectral characterization of the samples under investigation. This is particularly useful when samples may be highly inhomogeneous or, in general, when the knowledge of the spatial distribution of the sample constituents is fundamental.

Lastly, nuclear magnetic resonance (NMR) spectroscopy [67], which is based on the magnetic properties of specific atomic nuclei, can be used to obtain not only qualitative and quantitative information about the foods, but also to investigate their structural characteristics. In particular, while high-resolution spectroscopy, in particular <sup>1</sup>H-NMR, can be considered as a fingerprinting technique allowing elucidating the various sources of chemical variability within the samples and the various bi-dimensional spectroscopy can shed light on the molecular structure of the samples' constituents, information about the water distribution and mobility or about the texture can be obtained from low-resolution experiments.

#### 4.2. Examples of Applications of Spectroscopic Techniques

As mentioned before, spectroscopic techniques represent a powerful tool for monitoring and optimizing the traditional processes finalized to the production of meat and fish and their products. Various quality parameters are investigated by diverse techniques; a brief overview of the purposes that the different spectroscopic tools aim at is reported in the related subparagraph below. In the literature, it is possible to find several papers that focus on the evaluation of quality of meat and fish during or after the curing/drying processes. The main investigated topics are chemical composition, which is strictly linked to the water content and the water activity, the effects of sodium reduction/additives adjunction, and sodium monitoring. Additionally, being traditional processes often carried out on typical high-added valued foods, also the characterization and the authentication of these products play a relevant role in this context. A brief overview of applications where spectroscopic techniques are exploited to investigate the mentioned topics is reported in the sub-section below; additional research

works are reported in Table 1. Moreover, a further survey of possible non-destructive approaches for quality assessment (focusing on dry-cured hams) can be found in [68].

Aim of the Study	Analytical Technique	Chemometric Tool	Reference
	Meat		
Evaluation of quality deterioration index in vacuum packaged dry-cured sausages	HSI (380–1000 nm)	DFA, PLSR	[47]
Color of sausages in modified casings	HSI (380–1000 nm)	PLSR	[69]
Monitoring Parma ham during processing	Fluorescence: Excitation from 270 to 550 nm; emission from 310 to 590 nm	PARAFAC; PLSR	[70]
Quantification and spatial characterization of moisture and NaCl content of Iberian dry-cured ham slices	HSI (900–1700 nm)	PLSR	[71]
Determining water distribution within beef during dehydration	HSI (380–1700 nm)	PLSR, MLR, LS-SVM	[72]
Impact of crystal size on salt (NaCl) uptake and water activity (aw) of dry-cured pork	HSI (400–1000 nm)	PLSR, Si-PLS, CARS-PLSR	[73]
Monitoring composition and digestibility of ripened bresaola	size exclusion HPLC, HPLC/MS, <sup>1</sup> H NMR	ANOVA, LSD	[74]
	Fish		
NaCl concentration assessment during the herring marinating process	NIR	PCA, PLSR	[75]
Monitoring secondary structural changes in salted and smoked salmon muscle myofiber proteins	FT-IR Microspectroscopy (4000–1000 cm <sup>-1</sup> )	PCA, PLSR	[76]
Monitoring sodium chloride during cod fish desalting process	FT-IR spectroscopy	PLSR	[77]
Fat distribution in salmon fillets	HSI (760–1040 nm)	PLSR	[78]
Salting process monitoring on cold-smoked salmon	HSI (760–1040 nm); X-ray computed tomography	PLSR	[79]
Assessment of mobility of water during storage of salted fish	<sup>1</sup> H NMR	One-way analysis of variance, post-hoc tests with Bonferroni adjustment	[80]
Effects of sodium tripolyphosphate on frozen shrimp	<sup>1</sup> H NMR	Levene and Mauchly tests, one-way analysis of variance, Pearson correlations test, post-hoc tests with Bonferroni adjustment, Dunnett's C test	[81]
Water distribution in brine salted cod	<sup>1</sup> H NMR technique	PCA	[82]
Determine water profiles in fish exposed to different temperature, moisture, and water activity	Pulse <sup>1</sup> H NMR technique	-	[83]

Aim of the Study	Analytical Technique	Chemometric Tool	Reference
Effects of phosphates and salt on water-holding capacity in ground raw and cooked farmed cod	<sup>31</sup> P-NMR	ANOVA, Fischer's pairwise comparison test	[84]
Evaluation of the effects of salting and sugaring on water distribution and quality of grass carp during storing	LF-NMR	LSD	[46]

Table 1. Cont.

HSI: Hyperspectral imaging, PARAFAC: Parallel factor analysis, PLSR: Partial least squares regression, Si-PLS: Synergy interval partial least squares, LSD: Least significant difference, DFA: Discriminant factor analysis, MLR: Multiple linear regression, LS-SVM: Least squares support vector machines, CARS-PLS: Competitive adaptive reweighted sampling partial least squares, <sup>1</sup>H NMR: <sup>1</sup>H Nuclear magnetic resonance, LF-NMR: Low field nuclear magnetic resonance, HPLC/MS, High-performance liquid chromatography/Mass spectrometry, PCA: Principal component analysis, FT-IR: Fourier transform infrared, LSD: Least significant difference.

*Quantification of Chemical Composition*: In order to protect the health of consumers and to ensure the specific organoleptic characteristics of the final product, it is important to constantly check the chemical composition of meat and fish and their derivative products, either during the productive processes they undergo or before they are introduced on the market. Generally, the most commonly investigated substances are proteins, fats, and water. Different techniques can be used to quantify their content, and various approaches have been proposed for the spectroscopic quantification of these compounds. In this regard, NIR (and to a lesser extent, MIR) spectroscopy is probably one of the preferred techniques for the detection and quantification of fats, proteins, and water. A successful application of NIR coupled with chemometrics for the quantification of the aforementioned compounds has been reported by Kartakoullis and collaborators [85]. In more detail, in their work, the authors quantified proteins, fats, and moisture in salted minced ham meats at different temperatures (between -14 and 25 °C). The analyses were run by a benchtop NIR spectrometer and a portable NIR (smartphone) working in a reduced spectral range (740–1070 nm). Quantification was achieved by partial least squares (PLS) and random forest (RF) regression methods. The most accurate results (in terms of  $R^2$  and root mean square error in prediction (RMSEP) on an external set of samples) were provided by the latter approach. On the other hand, the PLS model achieved remarkable results, but it was a bit less accurate when calculated on the spectra collected by the portable device on frozen meat. Similarly, the chemical composition of fish can be also investigated in the NIR spectral range, as shown by Wold et al., who quantified moisture in dried salted coalfish by hyperspectral imaging (HSI) with a spectral range of 760–1040 nm [86]. In this study, measurements were collected on portions of fleshes and on homogenized samples. Eventually, the water content was estimated by PLS; the best results were obtained when PLS models were calculated on the data collected on the homogenized samples. This approach led to an R<sup>2</sup> of 0.92 and to a root mean square error in cross-validation (RMSECV) of 70%. The authors highlighted this latter achievement as particularly remarkable since it was more accurate than the traditional approaches.

In the recent years, HSI has started playing a key role in food analysis; therefore, it has been widely applied for the chemical composition assessment of the traditional preparation of muscle foods (e.g., meat, fish). An example regarding seafoods is the research proposed by Wu and collaborators on dehydrated prawns [87]. Spectral images were collected on the entire dehydrated crustaceans, and then PLS and least-squares support vector machines (LS-SVM) were used to predict the moisture content. Eventually, a successive projections algorithm (SPA) was used to reduce the number of variables (going from 482 down to 12 wavelengths) and further regression models (exploiting PLS, LS-SVM, and multiple linear regression, MLR) were built on the reduced set of features. The most accurate results were achieved by the combination of SPA and MLR, which led to an R<sup>2</sup> of 0.962. Similar results were obtained on meat and meat products. For example, in a recent study, Yu et al. monitored moisture content (characterized by traditional methods), free fatty acid composition (measured by gas chromatography–mass spectrometry; GC-MS), and water-soluble low molecular weight compounds

(identified by high-resolution <sup>1</sup>H NMR) of dry-cured beef of Yunnan province (China), during different stages of the processing chain (raw meat, after salting for 20 days, after drying for 1–2–3 months, and after 4 months as a final product) [88]. The authors used PLS discriminant analysis (PLS-DA) on NMR data and succeeded in distinguishing the different processing stages. A similar study has been carried out by Liu and collaborators, where NMR coupled with GC-MS has been exploited for water-soluble low molecular weight compounds and fatty acids quantification, respectively, with the aim of differentiating five kinds of traditional Chinese dry-cured hams [89]. As in the previous work, the authors performed a PLS-DA analysis on <sup>1</sup>H-NMR data including the determination of variable importance in projection (VIP) scores. *Saba ham* group was clearly separated from the other four types of hams, while *Nuodeng, Xuanwei*, and *Heqing* hams were partially overlapped. VIP analysis highlighted 15 metabolites as significantly different, giving a high contribution for the discrimination of samples. As expected, the same compounds can be investigated/quantified also by HSI in different spectral ranges, as in the work published by Ma and collaborators, where different HSI devices working in different ranges—Vis-NIR (400–1000 nm) and short wave NIR (1000–2500 nm)—were successfully used to quantify the moisture, proteins, and fats content in cured pork meat [90].

*Water Activity*: Beside the estimation of water in meat and fish and their products, i.e., the quantification of moisture, the water activity  $(a_w)$ /mobility is a relevant parameter for the evaluation of the quality of processed meat and fish. Spectroscopy, in particular NIR and MIR, is especially suitable for assessing this parameter; consequently, different applications of these techniques in the field of traditionally processed foods can be found in the literature. One valuable example is the study proposed by Collell and collaborators, where three different NIR devices were used in order to estimate the water activity in fermented sausages (with or without *Penicillium* spp. and presenting diverse salt concentrations) during the drying stage [91]. Eventually,  $a_w$  was estimated by means of PLS; the best results (R<sup>2</sup> of 0.984, and RMSEP of 0.007%) were achieved when spectra were collected by an instrument scanning the spectral range between 830 and 2500 nm. A similar study, aiming at  $a_w$  evaluation during sausage drying, was carried out by Stawczyk et al. [92]. Again, in this case, NIR spectroscopy was exploited and coupled with PLS, and the authors concluded that the technique can be used as a control system of drying conditions during the pork sausage drying process.

HSI was used to monitor the meat-salting process for the time by Liu and collaborators who investigated whether it was possible to estimate water activity in porcine meat slices using this technique in the spectral domain between 400 and 1000 nm [93]. PLS was found to be able to predict water activity and NaCl content, providing satisfying results ( $R^2$  of 0.909 and 0.928, for water activity and NaCl content, respectively). Eventually, further regression models were built on a reduced set of spectral variables, and slightly more accurate results were achieved. Furthermore, NMR (magnetic resonance imaging (MRI), and to a lesser extent, the low-field) has been used to monitor water molecular mobility/availability and distribution, as it was illustrated by two similar works conducted on pacific oyster [94] and on another bivalve mollusk called Sur clam [95] during drying and rehydration processes. These approaches also aimed at correlating moisture content and NMR parameters ( $T_{22}/A_{22}$  and  $A_2$ , respectively), and high  $R^2$  and low values of RMSE were obtained. In addition, in [95], NMR relaxation data collected on hot-air-dried and sun-dried samples were analyzed by PCA to highlight the possibility of differentiating the influence of diverse drying methods and drying/rehydration times (analysis performed every 1 h) on water dynamics.

Oxidation Processes and Volatile Organic Compounds: The investigation of oxidative processes and the quantification of volatile organic compounds provide relevant information about quality changes occurring in food products during the application of different traditional processing processes. In fact, the oxidation of specific compounds (*in primis* fats) and the release of volatile substances (for instance, the total volatile basic nitrogen) are significant indicators for quality control during the monitoring of productive processes. Additionally, the oxidation grade is a suitable tool for quality checks on the final product and assessing the degree of ripening during the storage time.

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In this regard, the most commonly used spectroscopic techniques are HSI and NMR. In fact, different applications of HSI to estimate the oxidation degree of lipids in traditionally processed meat and fish and their derivative products can be found in the literature. An example is a recent study carried out by Aheto and collaborators, who exploited the potential of HSI to quantify the oxidation degree of fats in dry-cured pork belly [56]. In particular, the authors focused on the estimation of a specific family of by-products linked to the oxidative process of fats, namely the thiobarbituric acid reactive substances (TBARS). The quantification of TBARS was made by means of PLS, and it provided acceptable results ( $R^2p = 0.77$ ), indicating that HSI coupled with PLS can definitely be used for the quantification of TBARS as an index of lipids oxidation during dry-curing meat processes. On the other hand, the actual oxidation of lipids can be estimated by <sup>1</sup>H NMR, as it was investigated by Guillén and Ruiz in an early study on salted and unsalted salmon fillets [96]. In this study, salmon samples were dry-salted for one day, and then oxidation conditions were mimed in a fan oven (at 50 °C), and fats were sampled and analyzed periodically by <sup>1</sup>H NMR. The authors concluded the inspection of acyl groups allows not only the detection of the oxidation but also its extent.

Total volatile basic nitrogen (TVB-N) is generally quantified by destructive analytical methodologies, which cannot be applied for online monitoring. For this reason, spectroscopic-based solutions have been proposed. For instance, Yang and collaborators developed an HSI-based procedure (400–1000 nm) for TVB-N monitoring in pork cured meats [97]. Once collected, experimental variables were selected by inspection of the PLS regression coefficients, and a further predictive model was built. Diverse regression approaches were tested, and the best results were achieved by MLR (R<sup>2</sup>p of 0.861). Furthermore, in the literature, it is possible to find several methodologies for the estimation of TVB-N that are based on a multi-platform approach [98], as in the research carried out by Khulal and collaborators, where data from a colorimetric sensor and HSI were jointly elaborated by data-fusion approaches to estimate the TVB-N concentration in chicken meats [99].

*Sodium Monitoring/Reduction and Effects of Additives*: As discussed above, a suitable presence of salt allows a greater shelf life of meat, fish, and derivative products. In order to ensure the quality and safety of salted aliments, NaCl content is constantly evaluated. In general, this parameter is assessed by means of destructive approaches; nevertheless, in the last years, efforts have been put toward proposing alternative, non-destructive methodologies. Among the others, impedance spectroscopy, exploiting the correlation between conductance, and the concentration of ions in the samples [100,101] have been often used for this purpose. For instance, the efficiency of this approach is testified by the study proposed by Masot and collaborators [102]. In this work, impedance spectroscopy, coupled with PLS regression, has been successfully used to estimate salt contents in salted minced pork meat; in fact, this approach led to an R<sup>2</sup> of 0.934 and a : predicted residual error sum of squares (PRESS) of 75.60 on an external sample set.

Nevertheless, for health-related reasons, there has been an increasing tendency in recent years to evaluate the possibility of reducing the concentration of NaCl in food products. Consequently, the advantages and the disadvantages of the inclusion of sodium substitutes has been investigated. A valuable study in this regard has been proposed by Cheng and collaborators, who have exploited NMR to investigate the effect of sodium substitutes for the preparation of Harbin dry sausage [49]. In particular, sausage samples were prepared following three recipes: (1) 100% NaCl; (2) 70% NaCl and 30% KCl; and (3) 70% NaCl, 20% KCl, 3.5% maltodextrin, 4% L-Lys, 1% L-Ala, 0.5% citric acid, and 1% Ca-lactate. After a fermentation stage (12 days), Na and K content were measured by atomic absorption spectrophotometer, whereas the moisture, water activity, and distribution were assessed by NMR, and ANOVA was used to assess the effect of the different formulations. It was found that odium substitute using the third formulation could replace 30% of the NaCl in the sausages. Similarly, Campos et al. recently used NIR spectroscopy to investigate the properties of ham meats produced by applying two different salting techniques (one involving salting replacements) [103]. The results showed that the technique could be useful for the control and monitoring of the stages in the ham curing process.

*Storage Time/Ripening*: For several reasons (either hygienic or related to the organoleptic quality of foodstuffs), it is necessary to control the degree of ripening of processed foods. Ideally, quality checks on the final products should be carried out by non-destructive approaches, in order to avoid/minimize the loss of these commodities; consequently, spectroscopy is widely used for this purpose. An example of a spectroscopic methodology proposed for assessing the ripening degree of a traditional (and typical) aliment is the work proposed by Svensson and collaborators [104]. In their valuable study, the authors estimated ripening in barrel salted herrings. In particular, they demonstrated that the variation of protein concentration (total nitrogen) in brine indicates variation in the composition of this product during the storage time. The analysis was carried out by NIR spectroscopy (signals were collected on the brine), and the data were analyzed by PLS. The results indicated that NIR spectroscopy has a promising potential for assessing the protein content in brine from barrel-salted herring rapidly and non-destructively.

It should be stressed that compared to other preservation and processing processes (e.g., freezing, modified atmosphere packaging, thermal processing, etc.), a limited number of spectroscopic studies have been conducted on traditional processing processes (e.g., salting, fermentation, etc.). This is particularly true for fluorescence spectroscopy, as fluorescence spectroscopic studies dealing with traditional processing processes are exceedingly rare. One of the scarce studies related to this research area was conducted by Svensson and Andersen who used EEM in the 260–650 nm to assess the brine composition of salted herring (*Clupea harengus*) during the ripening process [105]. The decomposition of the fluorescence landscapes using parallel factor analysis (PARAFAC) revealed the presence of four fluorophores in the brine (two states of tryptophan, vitamin B6, and riboflavin). PLS regression and N-PLS applied on unfold landscapes and raw fluorescence landscapes, respectively, allowed the authors to predict protein concentrations in brine with a high accuracy.

Characterization of Traditional Products: Traditional processes are often carried out on typical, high value food products, which are sometimes awarded quality marks such as the protected designation of origin (PDO) or protected geographical indication (PGI) label. Due to their special characteristics, which are often strictly related to a particular procedure used for their production, it is relevant to characterize these types of products. This characterization can be carried out by different approaches, which are generally based on a combination of chemical and chemometric analysis. In the literature, a wide number of papers aiming at authenticating and characterizing typical food products can be found, and chemometric classification tools play a key role in this field. Moreover, in the last years, a lot of effort has been put into developing non-destructive spectroscopic methodologies for the characterization of traditional food products [106–112]. Concerning meat and fish, several applications can be found as well. For instance, a valuable work focusing on the characterization of a traditional Nordic fish product called *lutefisk* was conducted by Hassoun and collaborators, who exploited a multi-platform approach in order to achieve this purpose [24]. Lutefisk is dried cod prepared following a traditional Norwegian procedure. In this study, samples of lutefisk (commercialized by four different brands) were investigated by fluorescence and HSI and by traditional methods (texture, water content, and pH). Eventually, samples were classified (according to the four brands) by PLS-DA. As shown in Figure 2 (corresponding to Figure 5a in [24]), the proposed methodology provided successful results, indicating that the proposed non-destructive approach can be used as a suitable tool for the characterization of this product.



**Figure 2.** PLS-DA results obtained from spectroscopic measurements of the 4 brands of lutefisk. Reproduced in compliance with CC BY license from Ref. [24].

# 5. Current Limitations and Future Directions

This review has summarized most of the research studies reporting on the use of spectroscopic techniques for monitoring changes occurring in meat, fish, and their derivative products during the application of traditional processing processes. Our overview of the literature shows that several spectroscopic techniques including mainly vibrational spectroscopy, fluorescence, and NMR have promising potential for monitoring changes in the quality of fish, meat, and other muscle foods that result from the application of salting, fermentation, drying, and smoking, among other processes. These spectroscopic methods have several advantages compared to other traditional methods such as sensory, microbiological, and physicochemical approaches. Indeed, there are mainly three features that make spectroscopic analysis an interesting alternative solution to the usually used conventional techniques. First, the techniques are non-targeted, meaning that the obtained spectra can be used to evaluate several quality parameters simultaneously. Second, the techniques are non-destructive, providing real-time, on-site measurements, and monitoring online or even inline during the production process. Third, most of the spectroscopic measurements are rapid and can be performed directly on the sample without any preparation. Thus, the opportunities offered by the spectroscopic techniques make them more suitable for industrial applications. In particular, hyperspectral imaging (HSI) seems

to be the most suitable technique, as it provides spatial information in addition to the spectral data that are usually obtained from traditional spectroscopic techniques. The technique is widely used within the visible and near-infrared regions, while the expansion of its application range to include other spectroscopic techniques (e.g., fluorescence and Raman) can be expected in future works.

Despite all the above-discussed advantages of spectroscopic techniques, their utilization in the process optimization or quality evaluation of traditional processed meat, fish, and related products is still limited, especially at industrial scales. This is because several challenges are still ahead before more applications can be implemented in a real industrial environment. Some of these challenges are related to the analytical techniques (e.g., spectroscopic methods), while others are associated to the muscle food matrices themselves. In more detail, the cost of some spectroscopic systems, the limited penetration of light into the sample for some spectroscopic methods, the large amount of obtained data (especially when using HSI), and the challenging statistical data treatment are among the main limitations of the application of spectroscopic techniques. Some of these obstacles can be addressed by developing affordable multispectral imaging systems (that generate less data) instead of HSI and using multi-platform approaches or a combination of several spectroscopic configurations, such as diffuse reflectance and interactance in order to get information from both the surface and inside the scanned product, respectively. Indeed, multi-platform approaches have become more interesting in recent years thanks to the development of data fusion techniques, among other advances.

Another challenging aspect of monitoring quality changes in meat, fish, and derivative products includes heterogeneity and variation in the raw material in terms of size, shape, and chemical composition (such as content of protein, fat, etc.). This makes it difficult, if not impossible, to choose only a few samples and assume that they perfectly represent the complete product volume. One solution can be to use HSI or multispectral imaging that allows investigating the whole production volume and providing a whole spectrum from each point of the scanned samples. Other challenges are rather related to traditional processing processes, including the long time required for the process and the multiple factors that often affect such kinds of processes. The majority of reviewed studies lack exact information about these factors, making a direct comparison between the results of these studies a challenging task.

Finally, it should be stressed that one of the main obstacles facing a wider range of applications of spectroscopic techniques is the reluctance of certain industries and research organizations to exploit and adopt new technologies. This negative attitude (*silo* mentality) toward the application of new technologies is especially true in the sector of traditional processing processes. In fact, the prevailing mindset in this industry sector can be typified by: "We have taught it this way, so why change?" [113] However, the research studies discussed in this review show clearly that reconciling novelty and tradition is absolutely possible.

In summary, the continuous monitoring of various processing processes and quality changes occurring in meat, fish, and their derivative products can be achieved by using spectroscopic techniques. These techniques, once the aforementioned challenges are successfully overcome, will be used as a valuable analytical tool not only for the traditional processing processes but also for the food industry in general.

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