ISSN 2074-9414 (Print)
ISSN 2313-1748 (Online)

https://doi.org/10.21603/2074-9414-2026-2-2634
https://elibrary.ru/YXUXCJ

Review article
Available online at https://fptt.ru/en

Targeted and Untargeted Methods for Fish Quality Assessment (*Oncorhynchus mykiss*)

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Received: 28.01.2026
Revised: 12.03.2026
Accepted: 07.04.2026

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Abstract.
Fish quality control demands efficient analytical solutions. While conventional (targeted) assessment methods are reliable, their labor-intensive and destructive nature drives the development of rapid, non-destructive (untargeted) technologies. This review features rainbow trout (*Oncorhynchus mykiss*) as a key global aquaculture species.
It compares, systematizes, and summarizes data on the capabilities, limitations, and prospects of applying targeted and untargeted assessment methods to fish raw materials in general and rainbow trout in particular. The review spans a decade of publications, comparing conventional quality assessment approaches (sensory, physicochemical, and microbiological methods) with advanced instrumental techniques (infrared spectroscopies, Raman spectroscopy, hyperspectral imaging, nuclear magnetic resonance spectroscopy).
Untargeted methods, e.g., spectroscopy and imaging, enable rapid, non-invasive assessment of such key freshness parameters as chemical composition, lipid oxidation degree, spoilage, and storage time. In contrast, targeted methods remain crucial for validation and precise quantification of specific indicators, especially at later stages of spoilage. Untargeted methods show strong suitability for integration into real-time monitoring systems at fish processing facilities. However, their implementation requires robust calibration, specialized mathematical models, and representative reference databases.
Rainbow trout (*Oncorhynchus mykiss*) demonstrates the effectiveness of spectroscopic fish quality control methods, combining speed, non-invasiveness and the potential for online monitoring.

Keywords. Fish freshness, rapid analysis, control methods, spectroscopy, chemometrics, *Oncorhynchus mykiss*

Funding. This research was supported by the Russian Science Foundation, Project no. 23-76-10038.

For citation: Kutuzov MN, Belova MA, Novichenko OV, Nikitin IA, Zaroual H, *et al.* Targeted and Untargeted Methods for Fish Quality Assessment (*Oncorhynchus mykiss*). Food Processing: Techniques and Technology. 2026;56(2):260–276. https://doi.org/10.21603/2074-9414-2026-2-2634

https://doi.org/10.21603/2074-9414-2026-2-2634
https://elibrary.ru/YXUXCJ

Обзорная статья
https://fptt.ru

Целевые и нецелевые методы оценки качества рыбного сырья: обзор на примере радужной форели

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Поступила в редакцию: 28.01.2026
Принята после рецензирования: 12.03.2026
Принята к публикации: 07.04.2026

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Аннотация.
Контроль качества рыбного сырья требует эффективных аналитических решений. Несмотря на надежность традиционных (целевых) методов оценки, их трудоемкость и деструктивный характер стимулируют развитие быстрых неразрушающих (нецелевых) технологий. Цель исследования – провести сравнительный анализ, систематизировать и обобщить данные о возможностях, ограничениях и перспективах применения целевых и нецелевых методов для оценки рыбного сырья на примере радужной форели.

Объектом сравнительного анализа является радужная форель (*Oncorhynchus mykiss*), представляющая собой один из ключевых видов мировой аквакультуры. На основе систематического обзора научной литературы за 2014–2024 гг. проведен анализ традиционных методов (сенсорный, физико-химический, микробиологический) и современных инструментальных подходов (ИК- и рамановская спектроскопия, гиперспектральная визуализация, ЯМР-спектроскопия).

Установлено, что нецелевые методы (спектроскопический, методы визуализации) обеспечивают быструю неинвазивную оценку ключевых параметров свежести: химического состава, степени окисления липидов, уровня микробной обсемененности и срока хранения. В свою очередь, целевые методы сохраняют значение для валидации и точного количественного определения специфических показателей, особенно на поздних стадиях порчи. Нецелевые методы демонстрируют высокий потенциал для внедрения в системы оперативного контроля рыбоперерабатывающих предприятий. Ключевыми условиями их успешного применения являются корректная калибровка, построение математических моделей и формирование репрезентативных эталонных баз данных.

Радужная форель (*Oncorhynchus mykiss*) демонстрирует эффективность спектроскопических методов контроля качества рыбы, сочетая быстроту, неинвазивность и потенциал для онлайн-мониторинга.

Ключевые слова. Свежесть рыбы, экспресс-анализ, методы контроля, спектроскопия, хемометрия, *Oncorhynchus mykiss*

Финансирование. Исследование выполнено при финансовой поддержке Российского научного фонда, проект № 23-76-10038.

Для цитирования: Кутузов М. Н., Белова М. А., Новиченко О. В., Никитин И. А., Заруал Х. и др. Целевые и нецелевые методы оценки качества рыбного сырья: обзор на примере радужной форели. Техника и технология пищевых производств. 2026. Т. 56. № 2. С. 260–276. (На англ.) https://doi.org/10.21603/2074-9414-2026-2-2634

Introduction

The recognized nutritional value of fish fuels a steady increase in global fish consumption. Fish is a reliable source of vitamins, minerals, and high-quality protein. It also provides the human body with long-chain omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid [1]. As the commercial fishery resources keep shrinking, the global food industry turns to aquaculture. According to the Food and Agriculture Organization of the United Nations (FAO), fish farms are responsible for 94.4 million tons, or 51% of the global fish production, which means that the share of aquaculture has surpassed that of industrial catch [2].

The salmonids (*Salmonidae*) are a valuable aquaculture family, with the rainbow trout (*Oncorhynchus mykiss*) being its most important commercial species. *O. mykiss* is the second most popular farmed trout in the world due to its adaptability, rapid growth, and high

feed consumption, as well as the superior sensory profile of its meat [2]. As a result of the growing demand, rainbow trout is now farmed on five continents, from the Arctic Circle to the south of Argentina [3]. Its production has nearly doubled over the past 20 years, as reported by the FAO. The high demand can also be explained by its high nutritional qualities: *O. mykiss* has a valuable dietary and delicacy fillet that contains iron, potassium, calcium, copper, magnesium, sodium, zinc, and phosphorus [4].

Fish is usually consumed fresh: in 2022, fresh and chilled fish accounted for approximately 43% of total fish consumption [2]. Unfortunately, fish is a highly perishable product, which significantly complicates logistics and quality control procedures. Its rapid microbiological spoilage and oxidation remain a major challenge. The spoilage intensity directly depends on storage conditions. Therefore, the primary task of fish processing facilities is to ensure the quality and safety declared on the label. Compliance with this requirement is mandatory both for regulatory and oversight bodies, as well as for consumer transparency. This task is equally important for fish producers, seeking to maintain the brand reputation and consumer confidence.

Spoilage processes are complex and multi-stage as they involve physical, chemical, and microbiological changes that lead to the degradation of fats and proteins. Bacterial growth, enzymatic autolysis, and subsequent oxidation reduce the product quality. Robust fish quality control mitigates rapid spoilage, protects public health, reduces economic losses, and promotes sustainable use of natural resources.

Fish quality assessment methods are categorized into targeted and non-targeted. Targeted methods are also referred to as conventional, classical, or destructive. Non-targeted methods, on the contrary, are considered instrumental or non-destructive. Despite the differences in terminology, both approaches are analytical, and distinguishing between them is sometimes impossible.

Targeted methods measure a specific, predetermined parameter or property. For instance, physicochemical methods determine lipid oxidation, moisture, pH, and total volatile basic nitrogen. Sensory assessment evaluates such parameters as smell, color, taste, texture, etc. Microbiological methods reveal the total microbial count and the concentration of psychrophilic microorganisms. Although they are effective in assessing the quality of fish and fish products, most of these methods remain time-consuming and labor-intensive.

Non-invasive and non-destructive instrumental methods are attracting increasing attention from researchers. For example, infrared and Raman spectroscopy are fast, cheap, and operator-independent. Single spectral analysis provides a significant amount of information. Spectroscopic methods require minimal to no sample preparation. In addition to providing a comprehensive assessment of fish quality, these methods determine shelf life and identify the processing history, e.g., freezing or repeated freeze-thaw cycles.

This gap between the labor-intensive standard quality control and the industry's demand for rapid, real-time analysis needs a systematized comparative evaluation of analytical approaches to trout quality assessment. This review (Fig.) summarizes and compares data on targeted and non-targeted methods for rainbow trout quality assessment.

Study objects and methods

This systematic literature review followed the protocol developed by H. Snyder [5] and R. Torraco [6]. It covered articles published in 2015–2024 and registered in international (ScienceDirect, PubMed, Springer Link)

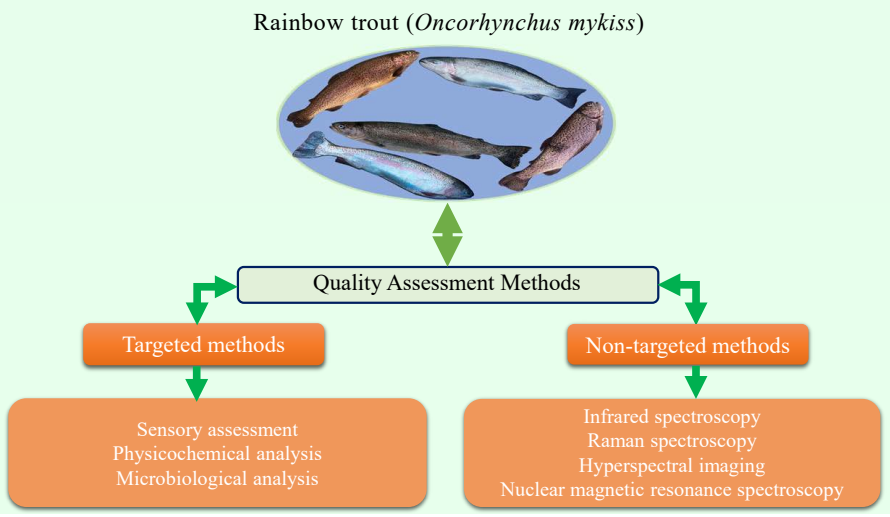

Figure. Methods for assessing the quality of *Oncorhynchus mykiss*: Schematic classification

Рисунок. Схематическая классификация методов оценки качества радужной форели

and Russian (eLIBRARY.RU, CyberLeninka) bibliographic databases. A number of older studies were included to describe some individual fundamental aspects.

The keyword search (English and Russian) covered rainbow trout, *Oncorhynchus mykiss*, fish quality, shelf-life, assessment, targeted methods, non-targeted methods, spectroscopy, rainbow trout, quality, shelf life, targeted methods, non-targeted methods, spectroscopy. The snowballing method made it possible to search the reference lists in the retrieved articles for potentially missed relevant sources. The final pool included peer-reviewed research on rainbow trout (*Oncorhynchus mykiss*) quality assessment by conventional (targeted) and advanced non-destructive (non-targeted) methods. The criteria disqualified duplicates, conference abstracts, and studies with flawed methodologies or unrepresentative samples.

The review categorized all reviewed methods into targeted and non-targeted ones for further comparative analysis. Targeted (destructive) methods included conventional approaches: sensory assessment (state-approved protocols); physicochemical parameters, such as K-index, water-holding capacity (WHC), total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and peroxide value (PV); microbiological tests, i.e., total viable count (TVC) and psychrotrophic total count (PTC). Non-targeted (non-destructive) methods included instrumental technologies: various spectroscopy, e.g., mid-infrared spectroscopy (MIR), near-infrared spectroscopy (NIR), Raman spectroscopy, and nuclear magnetic resonance (NMR), as well as hyperspectral imaging (HSI).

The methods were considered effective if they were validated and their results were statistically significant ($p < 0.05$). For non-targeted approaches, effectiveness depended on the availability of a reference targeted assay.

Results and discussion

Targeted methods. Conventional methods for monitoring fish quality deterioration are based on quantitative physicochemical, microbiological, and sensory techniques aimed at assessing various parameters that indicate postmortem changes in fish.

Sensory assessment. It determines the appearance, color, smell, texture, and taste. Its accessibility and simplicity make it the core method for product quality control. Russia and the CIS countries standardize sensory test methods by State Standard (GOST), International Organization for Standardization **(**ISO), Interstate Standard harmonized with ISO (GOST ISO), and Technical Regulation of the Customs Union (TR CU). The EU and the USA use the Codex Alimentarius (CAC/RCP 52-2003, Revision 4-2008) [7].

Sensory assessment remains the primary and critical method for determining fish freshness. It relies on recording and analyzing the responses of human senses (vision, olfaction, tactile sensations, etc.) to stimuli generated by the product. These stimuli directly correlate with the biochemical, microbiological, and physical changes in fish tissue.

The texture of fish muscle tissue is a key quality indicator for both raw and cooked salmon. It changes under a combination of various internal factors. These factors include biochemical degradation, e.g., lipid oxidation and proteolysis, and external factors, e.g., refrigeration storage (freezing and thawing) [8].

Colorimetric changes are another marker of fish quality. During refrigeration, muscle tissue loses its color intensity, resulting in pigment degradation, the degree of which depends on temperature and time conditions. Differences in color and appearance are visually noticeable in steaks and fillets.

With prolonged storage, fish loses its consumer properties, and the composition of its volatile compounds changes. As a result, the olfactory properties also change: the characteristic smell fades to be replaced by an unpleasant one, which gradually intensifies. Raw trout has a fresh seaweed or cucumber-like smell, which gradually becomes neutral. Eventually, it turns sour and then putrid due to the formation of short-chain fatty acids, alcohols, sulfur compounds, and amines [9, 10].

For objective recording and quantitative assessment of the described sensory changes in sensory analysis practice, standardized techniques are used. These are traditionally divided into two main approaches: analytical (expert) and consumer. Analytical methods include discriminative and descriptive procedures, as well as assessment using standardized scales and categories. They require specially selected, trained sensory assessors (panelists). In contrast, consumer methods record subjective preferences (likes or dislikes). As such, they involve regular consumers, who are well acquainted with the typical sensory profile of the product in question [11]. The reliability of analytical methods directly depends on the competence of the panelists, who are to be familiar with the system of sensory descriptors and able to work with scales [12].

Studies on rainbow trout (*Oncorhynchus mykiss*) clearly demonstrate the sensory changes in progress during refrigerated storage. For instance, M. Azizi *et al*. [13] showed that the quality of untreated fillets (control group) stored at +4°C rapidly deteriorated after ten days of refrigerated storage. Smell and color reached unacceptable levels by day 13, followed by texture on day 16. T. Mehdizadeh *et al*. [14] used a 10-point scale with an acceptability threshold of < 6 points to monitor fillets stored at +4°C. In this case, the overall sensory profile dropped below the acceptable level by day 6. The most drastic decrease belonged to smell and texture, which reached bottom values by day 9. The deterioration of sensory attributes correlated with proliferating psychrotrophic microflora.

While these studies focused on the progress of sensory changes, A. Anbi *et al*. provided a comparative quality assessment at fixed time intervals (15 days of storage).

They used a 10-point scale with an acceptability threshold of ≥ 5 points. The panelists assessed both raw and heat-treated fillets. After 15 days of storage at +4°C, the control samples (not treated with a 2% supernatant solution) lost all consumer properties: the scores for smell, taste, texture, and color were 3.0 ± 0.0 points, which was significantly below the acceptability threshold. The panelists recorded negative physiological reactions for both experimental and control samples, which indicated an advanced stage of spoilage and non-compliance with food safety requirements [15].

The method of sensory assessment relies on the human taster as a measuring instrument. Consequently, the subjectivity of individual perception remains a persistent limitation of this method. To minimize this factor and obtain quantitatively comparable data, sensory analysis results are expressed in points. Quality profile is a combination of key criteria (appearance, taste, smell, texture), each of which receives a number of points. The final score is the sum of all points for all criteria. It serves as an objective basis for comparative analysis of different samples [12].

Physicochemical analysis. Various biochemical processes occur in fish muscle tissue during storage under the impact of enzymes and bacteria. These processes form new substances that can serve as a criterion for fish quality. Physicochemical approach to fish quality assessment features total volatile base nitrogen (TVB-N) and other volatile amines, as well as biogenic amines [16, 17]. In addition, fish and seafood contain large amounts of polyunsaturated fatty acids (PUFAs), which makes them more susceptible to oxidation reactions. Peroxide value (PV) and lipid oxidation products, such as thiobarbituric acid-reactive substances (TBARS), are commonly used as freshness indicators [17, 18]. The concentrations of adenosine triphosphate (ATP) and its breakdown products are also reported as an indicator of fish freshness [19].

Free fatty acid content (FFAC) and thiobarbituric acid value (TBA) test. Rainbow trout is an oily fish [20]. Such fish deteriorates as a result of microbial activity and lipid oxidation [21]. Chemical reactions in muscle tissue oxidize and break lipids into simpler compounds, such as free fatty acids, peroxides, and thiobarbituric acid. These processes lead to oxidative rancidity manifested as unpleasant taste and odor. Fatty acid hydroperoxide is a primary product of lipid oxidation; it is measured as the peroxide value (PV). Peroxides are unstable compounds that break down into aldehydes, ketones, and alcohols, i.e., volatile substances that cause unpleasant taste in foods. Freshly caught fish has zero peroxide value. After death, postmortem changes in muscle tissue lead to hydrolysis and fat oxidation. An increase in the peroxide value indicates the formation of peroxides in partially hydrolyzed fat. A decrease in the peroxide value following its peak corresponds to the formation of secondary oxidation products, which marks the end of shelf life [19]. As reported by M. Rezaei & S. Hosseini, rainbow trout reached its maximum peroxide value on storage day 8 (6.25 mEq/kg), which dropped to 1.28 mEq/kg on day 16 due to the hydroperoxide decomposition [22].

Thiobarbituric acid-reactive substances (TBARS) reveal the degree of secondary lipid oxidation. The upper limit in frozen or chilled fish was reported as 5 mg MDA/kg tissue [8]. Given the increase in values during storage, thiobarbituric acid-reactive substances of trout remained below this limit throughout the storage period (≤ 2 mg MDA/kg fish) [23].

Similarly, the free fatty acid (FFA) content indicates hydrolytic activity caused by lipolytic enzymes. During lipid oxidation and hydrolysis, fish triglycerides break down, leading to an increase in the free fatty acid content. The presence of free fatty acids is undesirable they can be converted into volatile substances that cause unpleasant odor. In rainbow trout, the free fatty acid content was found to rise from 1.50 to 2.89 during 20 days of storage [22]. A Kolakowska *et al*. reported a free fatty acid content of 2.35 in whole trout after 14 days of storage [24]. Thus, a gradual increase in the free fatty acid content reliably indicates a decrease in fish quality.

Trimethylamine (TMA) and total volatile base nitrogen (TVB-N). Total volatile base nitrogen is a popular indicator for determining fish freshness as it reliably correlates with sensory changes during spoilage. In fish and seafood, total volatile base nitrogen is represented mainly by trimethylamine, dimethylamine, and ammonia, as well as other amines. These volatile compounds develop during the microbiological degradation of amino acids, especially in fish muscle tissue [19]. Trimethylamine nitrogen (TMA-N), a volatile amine, is a parameter used to determine the quality and shelf life of salmon fish. It is usually present in spoiled fish and causes an unpleasant odor. Trimethylamine is formed from trimethylamine oxide (TMAO) by the microbial enzyme TMAO reductase. The upper limit of acceptable fish spoilage is 10–15 mg TMA-N/100 g [25–27]. As fish spoils, trimethylamine produces dimethylamine and ammonia. The levels of total volatile base nitrogen and trimethylamine increase in parallel.

The total volatile base nitrogen content in fish tissues depends on the species. Its concentration in freshly caught fish typically ranges from 5 to 20 mg TVB-N/100 g muscle tissue. According to M. Rezaei & S. Hosseini, the values of total volatile base nitrogen in rainbow trout gradually increased during refrigerated storage and reached a maximum value of 29.86 mg/100 g on day 4 [22]. Yet, the total volatile base nitrogen value remained below the upper acceptability limit, which for salmon fish is 35 mg TVB-N/100 g [28]. The method for determining the total volatile base nitrogen level in fish is fairly simple, but this indicator is not informative regarding the early stages of spoilage, since the concentration of volatile nitrogen compounds changes rather slowly during the first days of storage.

K-index. Adenosine triphosphate (ATP) breakdown products are a reliable indicator for assessing fish freshness. Their content remains relatively stable in the muscle tissue of live fish, depending on the species. After death, fish muscles undergo changes caused by the breakdown of adenosine triphosphate into a number of metabolites, including adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine, and hypoxanthine [18]. For quality control, inosine and hypoxanthine are the key products of adenosine triphosphate breakdown. Hypoxanthine is the end product of breakdown, and it does not break further in fish. However, such fish species as Pacific salmon demonstrate a different adenosine triphosphate breakdown pattern, when inosine, not hypoxanthine, becomes the main breakdown product. This phenomenon requires further study in freshwater rainbow trout. Most fish species exhibit a decrease in adenosine triphosphate content and an increase in its breakdown products during a certain storage period, which is characterized by the earliest signs of bacterial spoilage. They can be detected both by human senses and by chemical analysis. The adenosine triphosphate breakdown rate in fish depends on the species, the initial state of the raw material, and storage conditions. The nucleotide breakdown rate reliably correlates with the loss of freshness registered by sensory assessment [28]. T. Saito *et al*. [29] proposed a method for assessing the freshness of fish based on the adenosine triphosphate index. It calculates K-index as a percentage of inosine and hypoxanthine in the total amount of adenosine triphosphate and its breakdown products. The lower the K-index, the higher the degree of freshness, and conversely, high K-values indicate low freshness of the fish.

In fresh fish, the K-value remains below 20%. Fish that shows signs of spoilage but is suitable for processing may demonstrate a K-value of 40%. If the value exceeds 60%, the fish is unsuitable for processing [30]. As reported by Y.-T. Cheng [28], the K-value of rainbow trout increased significantly at the end of rigor mortis, regardless of the storage temperature. Moreover, the indicator increased to 61% at 35°C after 14 h of storage, reaching the spoilage level. It rose to 23 and 37% when stored for 4 days at 0 and 4°C, respectively. This coefficient reveals early signs of spoilage in fresh fish when the volatile base nitrogen and trimethylamine nitrogen levels do not yet differ from those of fresh fish. However, K-index does not always correlate with the nitrogen content of volatile bases and trimethylamine in frozen fish, although it objectively reflects its sensory properties [30]. Therefore, frozen fish should be subjected simultaneously to the trimethylamine test as an indicator of microbiological spoilage and the K-index test as an indicator of postmortem changes. In a broad sense, fish freshness depends on several indicators, and determining K-index alone is uninformative. Although, this indicator remains a reliable marker of fish freshness.

Water-holding capacity (WHC) and cook loss (CL). Water is the main component of fish muscle tissue. It occurs there in both free and bound states, forming stable structured systems with other components. The content and state of water determine the course of biochemical, microbiological, and physical processes that affect the sensory, nutritional, and functional properties of fish during processing and storage [31]. Water-holding capacity and cook loss are the crucial indicators for the state of the aqueous phase and its impact on the quality of the final product.

Water-holding capacity is the ability of structural components of meat proteins to retain their own moisture when exposed to external forces [32]. Due to its composition, fish muscle tissue has a high water-holding capacity, which makes it a pivotal technological parameter that depends on a number of factors. The intensity of tissue exudate (moisture) secretion allows for indirect assessment of structural changes in meat (histological changes, protein denaturation), as well as the condition of muscle fiber membranes, the degree of muscle contraction, and the intensity of mechanical stress.

The water-holding capacity of fresh fish varies depending on the postmortem stage, storage temperature, cutting method, and other conditions. The water-holding capacity of rainbow trout decreases during rigor mortis due to muscle contraction. It continues to decrease as the storage temperature goes up and after filleting [33]. High water-holding capacity correlates with low moisture loss and high protein functionality, which positively affects the appearance, juiciness, and texture. S. Shen *et al*. reported that moisture loss in rainbow trout fillets increased together with storage time at both 3 and –3°C, reaching 2.30 and 4.05%, respectively, by day 6. The higher loss rate at −3°C was associated with the partial freezing of water in muscle tissue. This phenomenon led to partial denaturation of myofibrillar proteins and damage to cell membranes, reducing the water-holding capacity and deteriorating the overall quality [34].

Cook loss is the degradation of taste qualities expressed as a percentage reduction in weight after heat treatment. This important quality indicator reflects not only sensory characteristics (juiciness/dryness, firmness/tenderness, appearance), but also the loss of soluble nutrients, i.e., nutritional value. Cook loss is caused by protein denaturation during heating: myofibrils and collagen fibers shrink while soluble proteins turn to gel. Its value depends on the temperature and time of heat treatment, as well as on the physicochemical properties of the raw material, e.g., the state of muscle proteins, pH, and histological integrity. O. Adebisi [35], who studied rainbow trout fillets, reported that cook loss increased together with storage time. Its values for samples stored for 0, 24, and 48 h after cutting were 11.09 ± 0.52, 12.40 ± 0.52, and 12.43 ± 0.52%, respectively. As a re-

sult, the moisture content of the finished product decreased from 69.41% (0 h) to 69.25% (48 h).

Microbiological analysis. Microbial spoilage is responsible for 25–30% loss of fish products [36]. The surface and internal microflora of fresh fish includes mainly psychrophilic microorganisms with an optimal development temperature of about 20°C but capable of multiplying at 0°C [37]. The initial microbiological contamination of freshwater fish depends on the habitat conditions, primarily water temperature. According to literary data for rainbow trout and other fish species (tilapia, striped bass, silver perch), the initial microbial count remains within the range of 10^2–10^6 CFU/g [38, 39]. For chilled fish products, this indicator starts from 10^2 CFU/g and increases during storage. According to current regulations, the highest permissible count of mesophilic aerobic and facultative anaerobic microorganisms in chilled and frozen products cannot exceed 10^5 CFU/g [40]. Most of these are Gram-negative bacteria (*Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Cytophaga*), with Gram-positive bacteria represented by *Micrococcus* and *Corynebacterium*. As they proliferate, proteins and fats break down, leading to a gradual deterioration of fish quality. For some catch locations, spore-forming anaerobic microorganisms of the genus *Clostridium* and opportunistic bacteria (*Salmonella enteritidis*, *Escherichia coli*) may also be present [41]. Proteinases secreted by putrefactive bacteria decompose fish tissue proteins, releasing ammonia, hydrogen sulfide, amines, organic acids, and other compounds. As a result of bacterial protein decomposition, toxic breakdown products can accumulate in fish tissue, leading to nonspecific poisoning. Intoxication occurs due to biogenic amines. Most bacteria that produce biogenic amines belong to the *Enterobacteriaceae* family (*Escherichia coli*, *Enterobacter aerogenes*, *Morganella morganii*, *Proteus vulgaris*), as well as to members of the *Clostridium* and *Bacillus* genera [42].

Bacteria of the genus *Pseudomonas* are the major causative agents of fish spoilage. These microorganisms reproduce rapidly, outpacing other bacterial communities. During their life cycle, they produce volatile bases and volatile acids. After just 10 days, pseudomonads account for up to 50% of the total bacterial count and up to 96% after 18 days. Pseudomonas cause protein breakdown, developing various volatile compounds, e.g., trimethylamine and odorous gases H_2S and NH_3. Not only do pseudomonas grow rapidly, but they also exert strong enzymatic activity against proteins and lipids, which makes these bacteria the most responsible for fish spoilage [43].

Microbiological analysis includes the total viable count (TVC), which corresponds to the QMAFAnM indicator, and the psychrotrophic count (PTC). Studies demonstrate the rate of microflora growth on rainbow trout during storage. For fish stored in ice, the total viable count increased from 4.00 to 7.04 log CFU/g by day 20 [44]. For whole ungutted trout, the surface contamination reached 7.0 log CFU/cm^2 on day 18 [37]. Considering that the highest permissible total viable count is 10^6 CFU/g (6 log CFU/g), the shelf life of rainbow trout in ice is approximately 9–11 days. During storage of rainbow trout fillets, the initial psychrotrophic count was 2.47 log CFU/g, increasing to 5.98 log CFU/g by day 6 and reaching 7.02 log CFU/g by day 10 [45]. For vacuum-packed trout steaks stored at +3 and +8°C, the psychrotrophic count was 10^6 CFU/g on day 16 and 10^7 CFU/g on Day 18 [46].

Non-targeted methods. The increasing demand for high-quality food products drives the need for more advanced control methods. The list of instrumental methods able to address this challenge includes biochemical, microbiological, physical, and chemical approaches, e.g., chromatography, immunoassay, etc. [19]. However, these methods are, in fact, classified as targeted: they measure such specific parameters as chemical composition, color, lipid and protein oxidation, microbial contamination, etc. They are highly reliable, specific, and sensitive but time-consuming, destructive, expensive, and operator-dependent.

These limitations have redirected research efforts to-ward non-destructive technologies, such as optical spectroscopy, hyperspectral imaging, and X-ray diffraction analysis. These advanced techniques provide a reliable alternative to conventional methods of food quality assessment [47]. For instance, vibrational spectroscopy has already established a strong reputation in fish quality assessment. These include the methods of near-infrared (NIR), mid-infrared (MIR), Fourier-transform infrared (FT-IR), and Raman (RS) spectroscopy. In addition to being fast and accurate, these methods provide a large amount of information in a single test, which makes them effective for real-time monitoring and inline control.

Mid-infrared spectroscopy (MIR). Infrared spectroscopy analyzes the way substances interact with electromagnetic radiation in the infrared range. This method is rapidly expanding across food science research. Mid-infrared spectroscopy is highly efficient in assessing the quality of fish and fish products [48]. The mid-infrared spectrum is conventionally divided into four distinct regions: the single-bond stretching vibrations (4000–2500 cm^{-1}), the triple-bond region (2500–2000 cm^{-1}), the double-bond region (2000–1500 cm^{-1}), and the fingerprint region (1500–400 cm^{-1}). Absorption peaks in the mid-infrared spectrum are unique for each type of chemical bond, granting the method exceptional specificity. The most informative spectral ranges for assessing fish freshness are located around 3300–2700 cm^{-1} and 1800–900 cm^{-1}. These ranges represent absorption bands that correspond to vibrations of the functional groups of proteins and other macromolecules [49]. These bands are associated with the stretching of the C=O bond (1640 cm^{-1}, Amide I band) and the deformation of the N–H bond

(1520–1550 cm^{-1}, Amide II band). The peaks at 1238 cm^{-1} (N–H, C–H deformation vibrations) are connected with Amide III. The lipid-related bands peak at 1458 and 1160 cm^{-1} (CH_2 deformation vibrations, C–O stretching vibrations).

New approaches use mid-infrared spectroscopy to identify the structures of particular organic compounds, such as the secondary structure of proteins. For instance, the Amide I region between 1600 and 1700 cm^{-1} provides information on the α- and β-structure of proteins [50]. These new methods also provide rapid and accurate detection of microorganisms. L. Fengou *et al*. used Fourier transform infrared spectroscopy (FT-IR) to determine the microbiological composition of sea bream (*Sparus aurata*) fillets. In their case, the FT-IR spectra at 3100–2700 and 1800–900 cm^{-1} contained information on biochemical compounds formed as microbial metabolites [51]. Mid-infrared spectroscopy can be applied to identify the structural features of fish muscle proteins as indicators of its nutritional value and sensory properties. It provides an efficient chemical analysis, using characteristic absorption bands in the spectra to evaluate the content and conformation of peptide bonds. Amide I band (1600–1700 cm^{-1}) seems to be the most informative for this purpose as it yields data on the secondary structure of proteins [52].

Mid-infrared spectroscopy was successfully applied to assess the freshness of rainbow trout steaks. D. Vilkova *et al*. [53] used it to grade trout samples by storage time. The 1700–1500 cm^{-1} range proved to be the most informative as it revealed a clear differentiation in the spectra of samples collected on the first and last days of storage. The absorption intensity in the regions corresponding to amide and amino groups increased progressively over the storage period.

When integrated with multivariate data analysis, mid-infrared spectroscopy can discriminate between chilled or thawed states of raw fish. Absorption band at ~1745 cm^{-1} characterizes the carbonyl groups of lipids, the ~1525 cm^{-1} region (Amide II) reflects the presence of the β-sheet structure of proteins, and ~1395 cm^{-1} is associated with vibrations of carboxyl groups. These spectral features suggest a shift in pH or hydrolytic processes [48].

Spectral data are highly dimensional as each sample is described by hundreds or thousands of spectral variables. These data require multivariate analysis methods for processing and interpretation, e.g., principal component analysis (PCA), hierarchical cluster analysis (HCA), discriminant analysis (DA), and regression methods. These methods simplify complex datasets by reducing dimensionality and separating objects into homogeneous groups [54].

Near-infrared spectroscopy (NIR). It can also be applied to assess fish quality and authenticate fish products. This research method measures the absorption of electromagnetic radiation in the wavelength range from 780 to 2526 nm. This range is conventionally divided into two regions: short-wavelength (SW-NIR; 780–1100 nm) and long-wavelength (LW-NIR; 1100–2526 nm). The near-infrared spectra record overtones and combination frequencies in vibrations of C–H, N–H, and O–H chemical bonds, which are unique to each sample and provide a reliable chemical analysis. For example, the spectral response of water in the near-infrared region is formed by overtones with maxima at approximately 760; 970; 1180; and 1450 nm, alongside a combination band at ~1940 nm. Characteristic absorption peaks of proteins are observed at 1510; 1980; 2050; and 2180 nm. Bands in the 1100–1390 nm region are associated primarily with stretching vibrations of C–H bonds and their first overtone [55, 56]. Lipids peak at 1722; 1760; 2310; and 2346 nm [57].

Near-infrared spectroscopy is increasingly used in the fishing industry to monitor microbiological spoilage, determine chemical composition, and assess freshness. According to M. Lin *et al*., shortwave near-infrared spectroscopy (SW-NIR) was able to differentiate between fresh and chilled ($\geq$ 4 days) rainbow trout mince and fillet. Applying principal component analysis (PCA) to the spectral data, the scientists were able to separate one-day-old refrigerated samples from older ones [58]. K. Xu *et al*. [59] used near-infrared spectroscopy to assess the freshness of rainbow trout fillet: the spectra of samples with various degrees of freshness differed significantly at 1500–1530 nm. Based on the near-infrared spectra, the team constructed a linear regression model using the partial least squares (PLS) method. The model demonstrated a high correlation between the experimentally determined and the modeled total volatile basic nitrogen values, confirming the high potential of near-infrared spectroscopy for fish fillet freshness assessment [59].

Near-infrared spectroscopy can be used to detect fraudulent practices in the sea food industry [60]. For instance, N. O'Brien *et al*. applied a portable NIR-spectrometer in combination with principal component analysis and soft independent modeling of class analogy (SIMCA) to discriminate between fish with higher economic value (salmon) and cheaper species (trout) [61].

Most studies on the application of near-infrared spectroscopy in the seafood industry focus on assessing product quality and authenticity while its use in freshness monitoring and early spoilage detection remains a relatively new research field [62]. Several recent studies featured near-infrared spectroscopy as a tool for identifying compounds that determine fish freshness and spoilage microorganisms. For instance, near-infrared spectroscopy was able to measure histamine [60,63], trimethylamine [64], and adenosine triphosphate breakdown products (K-index) [65]. It proved effective in assessing such lipid characteristics as free fatty acid and thiobarbituric acid-reactive substances [66]. Several studies, including those on rainbow trout, attempted to use near-infrared spectroscopy to quantify the microbial load expressed as total viable count [58, 67, 68].

Therefore, infrared spectroscopy is a robust analytical tool of food quality control. However, the near-infrared spectra might be challenging to interpret because they consist of combination bands and overtones of fundamental vibrations (C–H, N–H, O–H). This fact leads to significant band overlap and complicates direct identification of individual compounds. These data require pre-processing by chemometric methods to reduce noise, correct baselines, and separate overlapping signals.

Raman spectroscopy. It is another method of analytical vibrational spectroscopy used to analyze the quality and authenticity of food products. Surface-enhanced Raman scattering (SERS) addresses problems associated with weak Raman scattering. Raman spectroscopy and infrared spectroscopy have similar wavelength regions, which makes them complementary. Vibrations that are clearly visible in the infrared spectrum (strong dipoles) tend to demonstrate low intensity in the Raman spectrum. Furthermore, non-polar functional groups that produce very intense Raman bands typically produce weak infrared signals.

Raman spectroscopy provides information on the secondary and tertiary structure of proteins, as well as on lipid processes, e.g., oxidation and fatty acid hydrolysis [69, 70]. The peak centered at 1655 cm^{-1} is identified as the Amide I band, associated with proteins with a lot of α-helices. Another peak, observed between 1660 and 1680 cm^{-1}, also belongs to Amide I and may be associated with proteins rich in β-sheet or random coil structures. The region between 1200 and 1350 cm^{-1} represents the Amide III band. The bands at 1550 and 1608 cm^{-1} correspond to amino acids, i.e., tryptophan and phenylalanine, respectively [71]. Raman peaks at 1004, 1160, 1192, and 1520 cm^{-1} are associated with carotenoids. Bands observed at 968, 1065, 1078, 1266, 1300, 1440, and 1750 cm^{-1} are markers of lipids [72].

Raman spectroscopy can be used to monitor oxidative changes in fish. According to D. Vilkova *et al*., Raman spectroscopy is an efficient non-destructive tool for quality and shelf-life assessment of fish fillets. It was able to expose lipid oxidation-related differences between trout fillet samples with different storage periods [73]. Fresh samples (0–3 days) demonstrated the lowest intensity of spectral bands, which allowed for the clear differentiation of these samples from the older ones.

Combined with chemometric analysis, Raman spectroscopy can model the content of omega-3 and omega-6 fatty acids in seafood. E. Prado *et al*. reported that this approach provides high accuracy in predicting the content of polyunsaturated fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the visceral fat of rainbow trout [74]. A. Hassan *et al*. were able to detect different concentrations of heavy metal ions (mercury, cadmium, lead) in fish because the intensity and area of characteristic spectral peaks increased together with the concentration of metal ions [75].

J. Landry *et al*. [72] used surface-enhanced Raman spectroscopy for rapid quality assessment and authentication of fish samples, and this method provided reliable data for the qualitative and comparative analysis of lipid, protein, and carotenoid composition in fish tissues. Its capacity to identify biochemical markers confirms the significant potential of Raman spectroscopy for aquaculture.

Z. Chen *et al*. [76] reinforced Raman spectroscopy with machine learning algorithms to speed up the detection of rainbow trout imitations of Atlantic salmon: the species had significantly different intensities of characteristic spectral peaks. In particular, the peaks associated with rainbow trout were more intensive than the corresponding bands in salmon.

Surface-enhanced Raman spectroscopy demonstrated strong prospects for entering standard control protocols as a quality assessment method. For instance, E. Witkowska *et al*. [77] demonstrated the feasibility of Raman microspectroscopy for the detection of *Listeria monocytogenes* and *Salmonella* spp. in smoked salmon and other food products. It reduced the pathogen detection time from 6 to 2 days, but the procedure was found too labor-intensive and time-consuming [77, 78].

Raman spectroscopy and surface-enhanced Raman scattering (SERS) are promising tools for fish quality control. However, their practical implementation is hampered by a number of limitations, including low penetration depth, interference of fluorescent background, high equipment costs, and the complexity of spectral data interpretation.

In general, spectroscopy requires a single measurement to provide sufficient information about various classes of compounds that determine product quality. A simultaneous analysis of proteins, lipids, and moisture efficiently traces the processes that have occurred in muscle tissue during storage. This integrated assessment both confirms product spoilage and measures its remaining shelf life.

Hyperspectral imaging (HSI) is a non-invasive technique that combines spectroscopy and imaging into a single system [79]. It provides images with both spatial and spectral data. This combination makes the technique popular in the food industry, where it is used to monitor complex, heterogeneous products. Spectral imaging can be either hyperspectral (HSI) or multispectral (MSI), depending on spectral resolution. Hyperspectral imaging records a signal over a continuous range of wavelengths with a small step, e.g., 400–1000 nm with a step of 5 nm. Multispectral imaging operates on a discrete set of pre-selected wavelengths, e.g., 400–1000 nm with a step of 100 nm. As a result, hyperspectral systems generate a significantly larger volume of data per image pixel. Most modern spectral imaging systems operate in the visible and near-infrared range (400–2500 nm) [80].

Hyperspectral and multispectral imaging techniques are popular in the food industry. Hyperspectral imaging has been applied for the assessment of various seafood

quality attributes, including sensory profile [81], lipid-oxidation secondary products (thiobarbituric acid reactive substances) [82, 83], total volatile basic nitrogen concentration [84], K-index [85], and moisture content [86]. S. Khoshnoudi-Nia & M. Moosavi-Nasab [87] combined hyperspectral imaging at 430–1010 nm with linear and nonlinear models for a more rapid non-destructive measurement of thiobarbituric acid reactive substances in rainbow trout fillets during 12 days of storage at 4 ± 2°C. Shan *et al*. used it to discriminate between fresh and thawed fish [88]. K. Washburn *et al*. used hyperspectral imaging to differentiate between once- and twice-thawed cod: the technique demonstrated significant potential when used as an online method for assessing freeze–thaw cycles of fish [89]. J. Xu *et al*. reported the spectral range of 400–1000 nm as the most informative for classifying salmon samples by freshness [90].

Hyperspectral imaging is a non-invasive measurement for the chemical analysis of seafood, e.g., moisture or fat content. H. Zhang *et al*. accurately determined the fat and moisture content in salmon fillets using near-infrared hyperspectral imaging in the 950–1650 nm range [91]. Blood analysis is another important indicator of fish quality, e.g., M. Skjelvareid *et al*. [92] used hyperspectral imaging to perform a qualitative and quantitative blood analysis of fish fillets.

Hyperspectral imaging can be used to determine the s helf life as a freshness indicator in salmon and other species [93, 94]. S. Khoshnoudi-Nia & M. Moosavi-Nasab integrated hyperspectral imaging with nonlinear chemometric models and obtained accurate modelling of the volatile base nitrogen content, psychrotrophic microbial count, and sensory analysis of rainbow trout fillets over 12 days of storage [81].

Some research teams used hyperspectral imaging to estimate the volatile base nitrogen content of rainbow trout fillets [84] and other fish species [95–97]. All these studies presented accurate models for predicting volatile base nitrogen values using the visible and near-infrared spectral range. For example, M. Moosavi-Nasab *et al*. [84] combined hyperspectral imaging with deep neural networks to obtain highly accurate models of volatile base nitrogen content in rainbow trout fillets across 12 days of storage. These experiments prove the strong potential of hyperspectral imaging for the fish industry.

Both hyperspectral and multispectral imaging methods are highly effective tools for fish analysis. They record images at specific wavelengths in the visible, near-infrared, and mid-infrared ranges, identifying individual features that correlate with various product quality characteristics. Standard methods provide mean values for a homogenized sample, but hyperspectral technology visualizes the spatial distribution of key components (moisture, fat, pigments) directly within muscle tissue. This approach makes it possible to identify localized texture defects, uneven lipid distribution, hemorrhages, and individual foci of microbiological spoilage, which affect product appearance and quality but often go undetected by standard methods. Yet, the practical implementation of these technologies in the food industry is hampered by the poor availability of commercial and reliable equipment. Moreover, large volumes of generated data remain a serious challenge as they need specialized interpretation algorithms.

Nuclear magnetic resonance (NMR) spectroscopy. It is a non-destructive analytical technique. It yields comprehensive data on the chemical composition and molecular structure, facilitating reliable and comprehensive qualitative and quantitative profiling. This method is based on the phenomenon of resonant absorption of radiofrequency radiation by atomic nuclei in a magnetic field [98]. Depending on the magnetic field strength, a distinction is made between high-field (≥ 1 T), mid-field (0.5–1 T), and low-field (≤ 0.5 T) nuclear magnetic resonance spectroscopy [98]. The food industry utilizes two complementary approaches. High-resolution nuclear magnetic resonance spectroscopy analyzes the chemical composition and metabolite profile while low-field nuclear magnetic resonance relaxometry defines the state of water and structural changes in tissues [98, 99].

High-resolution nuclear magnetic resonance spectroscopy. It identifies and quantifies individual chemical compounds in complex mixes. In the food industry, it usually targets ^{1}H, ^{13}C, and ^{31}P nuclei [100]. The fishing industry uses this method to monitor postmortem biochemical changes, determine the metabolic profile, and assess the freshness of raw materials [101]. It can calculate the K-index of freshness based on the concentrations of adenosine triphosphate and its breakdown products.

For instance, Shumilina *et al*. demonstrated the high efficiency of ^{1}H-NMR spectroscopy in the quality and quantity analysis of metabolites in the muscle tissue of Atlantic salmon (*Salmo salar*) stored at 0 and 4°C [102]. The method made it possible to calculate the K-index of freshness based on the concentrations of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, inosine monophosphate, inosine, and hypoxanthine without additional chromatographic separations.

L. Abramova *et al*. also used nuclear magnetic resonance spectroscopy for a quantitative analysis of compounds formed during autolytic and bacterial spoilage during storage. They developed several variants of quality index formulas, depending on the type of raw material [103]. The ^{1}H-NMR method was able to determine a wide range of metabolites, including alanine, acetate, creatine, dimethylamine (DMA), trimethylamine (TMA), trimethylamine-N-oxide (TMAO), glucose, and lactate [104, 105]. The changes of their content mark the key biochemical processes in fish muscle tissue during storage, i.e., autolysis, proteolysis, lipid oxidation, microbiological spoilage, etc. [99].

High-resolution nuclear magnetic resonance spectroscopy also demonstrates a strong potential for assessing

the sensory profile of fish products [103]. The authors applied it to assess the taste profile of Atlantic salmon during heat treatment and storage [103]. Based on the quantitative analysis of free amino acids, nucleotides, and organic acids, the authors calculated the taste index (TI) for the major compounds responsible for sweet, bitter, sour, and umami tastes.

High-resolution nuclear magnetic resonance spectroscopy can be used to discriminate between fresh and thawed fish. K. Kaltenbach *et al*. combined this method with multivariate data analysis (principal component analysis followed by linear discriminant analysis, PCA-LDA) to achieve a classification accuracy of 90.0% in the lipid analysis of trout samples [106].

Some publications report the high prospects of high-resolution nuclear magnetic resonance spectroscopy for the metabolomic analysis of rainbow trout muscle tissue. Roques *et al*. used this method to study metabolic changes that occurred when they replaced fishmeal in feed with alternative sources. The result was a wide range of water-soluble metabolites in muscle tissue [107].

P. Gunnarsson *et al*. combined it with sensory analysis to study the effect of various protein feeds (insects, micromycetes, mollusks) on *β*-alanine, creatine, and amino acids in rainbow trout [108]. The changes, however, did not affect the sensory properties of the fish.

All these studies demonstrate the efficacy of high-resolution nuclear magnetic resonance spectroscopy for a comprehensive assessment of trout quality as it captures the impact of dietary and environmental factors on both fish metabolism and end-product consumer properties.

Nuclear magnetic resonance relaxometry. Unlike spectroscopy, it focuses on the physical state of a substance, primarily the mobility of water and lipids in biological tissues [99]. The method measures the relaxation times (T_1 and T_2) of protons, which characterize the distribution of water across different fractions, i.e., bound water (strongly associated with macromolecules), immobilized (structural) water, and free water. The method provides real-time monitoring of water migration and the changes in its mobility during storage, freezing, thawing, and heat treatment.

A significant correlation exists between the relaxation parameters and such technological indicators as water-holding capacity and cook losses [98]. Nuclear magnetic resonance relaxometry can be used to develop express methods for monitoring the thermal state of raw fish. It was successfully applied to assess the effect of heat treatment on fish quality [109].

The method is officially recognized by regulatory documentation for food quality and safety: Methodological Guidelines MUK 4.3.3551-19 determine the quality of chilled fish products by nuclear magnetic resonance relaxometry.

M. Zhao *et al*. [110] used nuclear magnetic resonance relaxometry to assess the quality of rainbow trout during storage. The authors focused on the effect of transportinduced heat stress on the proton relaxation times in trout fillets during refrigerated storage (4°C). The proportion of free water increased throughout storage, especially in the samples subjected to heat stress, while the proportion of immobilized water decreased. These changes correlated with the accumulation of total volatile basic nitrogen and malondialdehyde, as well as a progressive rise in the K-index. This method made it possible to differentiate the fish samples based on their shelf life. In addition, the authors evaluated the efficacy of ascorbic acid against adverse changes in water fractions and spoilage indicators [110].

The synergy of nuclear magnetic resonance spectroscopy and relaxometry provides a comprehensive quality assessment of fish products. Spectroscopy yields high-resolution data on chemical changes (metabolomic approach, K-index, flavor compounds) while relaxometry reveals the structural and mechanical changes, as well as water mobility and distribution. These methods provide highly reproducible results and require minimal sample preparation. Combined with chemometric methods, they possess an enormous potential for real-time quality control of fish products.

Conclusion

Conventional quality control of fish and fish raw materials combines sensory, physicochemical, and microbiological methods. Despite their high reliability, these targeted approaches are time-consuming, destructive, and operator-dependent, which significantly curbs their use for operational monitoring. These shortcomings stimulate the development of non-targeted (instrumental) analytical methods.

Using the case of rainbow trout (*Oncorhynchus mykiss*) and some other fish species, this review proved the effectiveness of mid-infrared and near-infrared spectroscopy, Raman spectroscopy and surface-enhanced Raman scattering, spectral imaging, and nuclear magnetic resonance spectroscopy in the fish industry. They provide rapid assessment of freshness and shelf life while being able to differentiate raw materials based on pre-treatment methods, e.g., chilled or thawed fish. They offer effective authentication tools able to detect species substitution. In addition, they can determine indirect biochemical spoilage markers, e.g., total volatile basic nitrogen, lipid oxidation products, and K-index. Non-invasive by nature, these methods can be automated and integrated into online monitoring systems.

However, the implementation of spectroscopic approach in the fish industry is hampered by a number of methodological challenges. For instance, these methods are highly sensitive to sample heterogeneity and require robust calibration using representative datasets for each species. Moreover, they need advanced statistical data processing methods to identify hidden patterns and construct species-specific predictive models.

As a highly valuable aquaculture species, rainbow trout represents a relevant model for demonstrating the capabilities and limitations of modern instrumental methods. Further progress in operational quality control is associated with new analytical protocols that combine spectroscopic platforms with optimized chemometric algorithms targeting specifically this raw material.

Contribution

All the authors participated equally in the study design, manuscript preparation, and final editing.

Conflict of interest

The authors declared no conflict of interest regarding the publication of this article.

Критерии авторства

Авторы в равной степени принимали участие в исследовании, написании и оформлении рукописи.

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

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