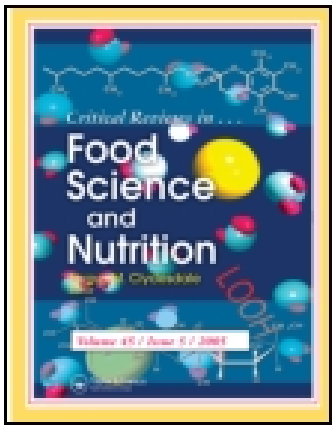

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Recent Advances in Methods and Techniques for Freshness Quality Determination and Evaluation of Fish and Fish Fillets: A Review

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Accepted author version posted online: 02 Jul 2013.Published online: 28 Jan 2015.

Critical Reviews in Food Science and Nutrition, 55:1012–1025 (2015)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2013.769934

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Recent Advances in Methods and Techniques for Freshness Quality Determination and Evaluation of Fish and Fish Fillets: A Review

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The freshness quality of fish plays an important role in human health and the acceptance of consumers as well as in international fishery trade. Recently, with food safety becoming a critical issue of great concern in the world, determination and evaluation of fish freshness is much more significant in research and development. This review renovates and concentrates recent advances of evaluating methods for fish freshness as affected by preharvest and postharvest factors and highlights the determination methods for fish freshness including sensory evaluation, microbial inspection, chemical measurements of moisture content, volatile compounds, protein changes, lipid oxidation, and adenosine triphosphate (ATP) decomposition (K value), physical measurements, and foreign material contamination detection. Moreover, the advantages and disadvantages of these methods and techniques are compared and discussed and some viewpoints about the current work and future trends are also presented.

Keywords Fish freshness, sensory evaluation, microbial inspection, volatile compounds, protein changes, lipid oxidation, ATP decomposition, *K* value, color measurement, texture measurement, parasites detection

INTRODUCTION

Fishery products are vital from a nutritional point of view, and the fishery industry is an important economic source and a fundamental industry related to international trade for many coastal states and districts. A report from the 30th Conference of Fisheries Commission of Food and Agriculture Organization held in Rome in 2012, pointed out that 128 million tons of fishery products are provided for human consumption every year around the world, and on average 18.4 kg is consumed per person per year, accounting for approximately 15% of the animal protein intake for 4.3 billion people. However, it is generally known that fish is one of the most vulnerable and perishable food items, and producers are paying special attentions to quality assurance. Therefore in the modern agricultural and food industry, the industry is continuously looking for new techniques and methods such as novel cooling (Sun, 1997; Sun and Brosnan, 1999; Sun and Zheng, 2006; Sun and Hu, 2003; Wang and Sun, 2001; HU and Sun, 2000), freezing (Delgado et al., 2009; Zheng and Sun, 2006), drying (Sun, 1999; Sun and Byrne, 1998; Sun and Woods, 1993, 1994a, 1994b, 1994c, 1997; Cui et al., 2004) and edible coating (Xu et al., 2001) to enhance product qualities. For fish and fishery products, freshness is one of the utmost principal attributes of fish quality, which makes a major contribution to the quality of fish and fishery products. It is well-known that the internal and intrinsic characteristics such as fragile muscle tissue and activity of endogenous protease and inappropriate handling methods and storage conditions can be prone to resulting in physical, chemical, biochemical, and microbial changes and contaminations in fish, thus affecting its freshness quality.

In order to provide fish and fish products with premium quality, reliable methods and techniques for determination and evaluation of the freshness quality of fish are required. These can be methods based on biochemical and instrumental measurements (Siripatrawan et al., 2009), methods using refractive index

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Table 1 Nondestructive techniques in determination and evaluation of fish freshness

Fish/fillet	Technique	Effectiveness/accuracy	Reference
Sardine	Refractive index	Cheap, useful	Gokoglu and Yerlikaya (2004)
Chub	Colorimetric sensor	87.5%	Huang et al. (2011)
Milkfish	Colorimetric sensor	Inexpensive visual indicator	Kuswandi et al. (2012)
Atlantic salmon, haddock, cod	Metal-oxide sensor array	The chemical analysis method results correlated well with sensor results	Hammond et al. (2002)
Argentinean hake	EN	An increase of the signals with an increase in storage period	O'Connell et al. (2001)
Moroccan sardines	EN	93.75%	Amari et al. (2006); ElBarbri et al. (2007, 2008)
Fresh and frozen-thawed cod fillets	VIS/NIR	100%	Sivertsen et al. (2011b)
Cod and salmon	VIS/NIR	The correlation coefficient of prediction was 0.97 and 0.98, respectively	Nilsen et al. (2002)
Sea bass fillets	NIR	90%	Trocino et al. (2012)
Fresh and frozen–thawed whiting fillets	MIR	100%	Karoui et al. (2007)
Carp, herring, sea bass	EIS	A simple, rapid and in situ measurement of the onset of fish spoilage	Niu & Lee (2000)
Cod, whiting, mackerel	Fluorescence spectroscopy	High-speed on-line measurements	Dufour et al. (2003); Karoui et al. (2007)

(Gokoglu and Yerlikaya, 2004) or spoilage and freshness indices (Uriarte-Montoya et al., 2010), and techniques based on sensor technology such as colorimetric sensor array (Huang et al., 2011; Kuswandi et al., 2012), semiconducting metal-oxide sensor array (Hammond et al., 2002), gas sensor (Amari et al., 2006; ElBarbri et al., 2007, 2008), or electronic nose (EN) (O'Connell et al., 2001; Natale et al., 2001; Olafsdottir et al., 2004). On the other hand, spectroscopy techniques are also widely used such as near infrared reflectance (NIR) spectroscopy (Sigernes et al., 1998; Nilsen et al., 2002; Sivertsen et al., 2011b; Trocino et al., 2012), mid-infrared reflectance (MIR) spectroscopy (Karoui et al., 2007), electrochemical impedance spectroscopy (EIS) (Niu and Lee, 2000), and front-face fluorescence spectroscopy (Dufour et al., 2003). Table 1 summaries relevant studies dealing with fish freshness determination and evaluation.

Despite the existing of extensive research activities as mentioned above, published reviews on the determination and evaluation of fish freshness are limited. An early review published in 1997 discussed a variety of approaches for determining and evaluating fish freshness (Olafsdottir et al., 1997), another review published in 2004 provided a concise overview about the fish quality determination and evaluation by a multi-sensor approach based on visible light spectroscopy (VIS), image analysis, EN, and texture (Olafsdottir et al., 2004). In 2007, Hyldig and Nielsen (2007) reviewed sensory methods for assessing the texture of fish muscle, which is indirectly related to the fish freshness. Thereafter, Abbas et al. (2008) discussed pH as one of the simple and reliable freshness indicators for cold-stored fish samples, and Mathiassen et al. (2011) presented the application of imaging technologies to inspection of fish and fish products. Most recently, Dowlati et al. (2012) reviewed the applications of machine vision for fish freshness quality assessment. However, no review is published on comparing different methods and techniques available for fish freshness determination and evaluation.

Therefore, in this paper, the recent advances of methods and techniques used to determine and evaluate fish freshness are reviewed. Details of sensory evaluation, microbial inspection, and chemical methods based on volatile compounds, protein changes, lipid oxidation, and adenosine triphosphate (ATP) decomposition and *K* value, and physical measurements and foreign material contamination will be presented, and finally outlook and future trends will also be discussed.

METHODS FOR EVALUATION OF FISH FRESHNESS

It is well known that freshness is one of the most critical qualities of fish. Choosing the effective and accurate methods for the freshness of fish quality evaluation is obviously significant in research and industry and some of these methods are discussed below.

Sensory Evaluation

Sensory evaluation is a scientific discipline used to analyze and interpret characteristics of food as perceived by the senses of sight, smell, and taste related to color, odor, and texture of food (Olafsdottir et al., 1997). Sensory evaluation as a conventional and useful tool has found a wide range of applications in fish freshness evaluation and interpretation perceived by experienced and trained personnel. Currently, the commonly used methods of sensory evaluation include European Union (EU) scheme and Quality Index Method (QIM), which can standardize sensory assessment for each fish species (Green et al., 2010).

EU scheme based on freshness category (E, A, B/unfit) is normally accepted in the EU countries. Some application examples are explained below. Simeonidou et al. (1997) used the EU freshness grading scheme to evaluate several Mediterranean fish

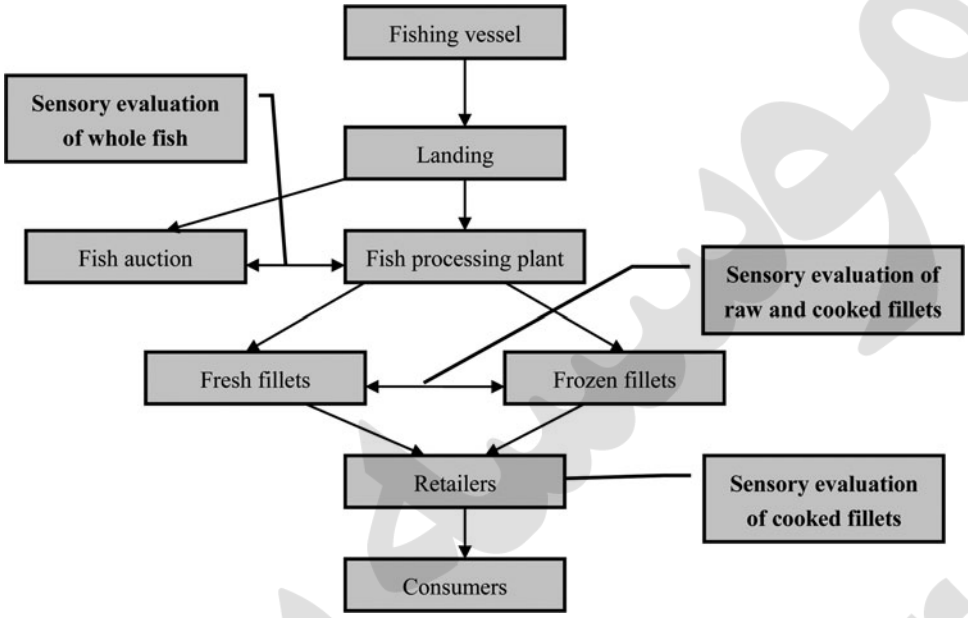

Figure 1 Sensory evaluation of fish in the fishery chain.

species during ice storage and found that better storage life was achieved for bogue, sardine, and striped mullet. In another study, un-gutted European sea bass stored in melting ice was offered an EU freshness grade E for up to three days, grade A for a further six days, and grade B for five more days after which it was graded as unfit (Kyrana and Lougovois, 2002). The EU freshness scheme was also applied for sensory assessment of raw fish and the sensory acceptability limit was —eight to nine days for filleted fish and 12–13 days for whole-ungutted fish (Taliadourou et al., 2003). However, the EU freshness grading scheme is only based on the whole fish, and there are some deficiencies about this method including different sensory attributes in one quality grade, and incompatibility of some sensory characteristics with other subdescriptions of the quality grade. Consequently, this method has been gradually replaced by QIM, which can surmount the demerits of the previous method and improve fish freshness evaluation. QIM as an essential tool plays an important role in keeping the quality of fish at a high level in each link of the whole complex fishery chain, as described in Fig. 1, and hence it has turned out to be the leading reference method for the quality assessment of fresh fish based upon objective evaluation of the relevant attributes of raw fish using a demerit points scoring system (from zero to three) that gives scores of 0 for very fresh fish and increasingly larger total result as the fish deteriorates.

QIM is an accurate and objective method for the determination of fish freshness and is used in many studies in fish freshness quality evaluation. By using the demerit points of QIM, Wills et al. (2004) showed that the freshness degree of the asphyxiated fish was lower than that of the clubbed. The relationship between sensory evaluation scores of raw and cooked anchovy and enterobacteria counts was established and operating characteristic curves were obtained for corroborating the suitability of QIM (Pons-Sánchez-Cascado et al., 2006). In addition, European eel freshness assessment interrelated with sensory method under different storage situations was investigated with the results given in appearance score (poor/poorer), skin score (dry/drier), appearance of eyes score (cloudier/cloudy), limit for acceptability (12–14 days/5–7 days) in ice and in boxes without ice storage, respectively (Özogul et al., 2005b). Furthermore, QIM results were used to correlate with instrumental measurements. According to the QIM score, Nilsen and Esaiassen (2005) used VIS spectroscopy to predict cod freshness and the result showed that a relatively narrow band in the mid-visible spectral range was sufficient to measure fish freshness. In addition, QIM sensory attributes were used to associate with a new artificial quality index (AQI) system based on the signals of different instrumental techniques used. It was confirmed that this new system was more effective and was capable of predicting the freshness of fish (Macagnano et al., 2005; Alasalvar et al., 2010).

On the other hand, QIM has been further developed to determine and predict the shelf life of fish that can be used as an indication for fish freshness. Özyurt et al. (2009) described the shelf life of red mullet and gold-band goatfish during ice storage, and observed that the sensory acceptability and their shelf life limit was 8 days and 11 days, respectively. Kyrana and Lougovois (2002) reported the changes of gills odor of raw fish, and the fish was predicated unfit on gills odor at 15–16 days of iced storage, but the cooked flesh remained edible at 18–19 days. In another study, the storage time of the raw, farmed Atlantic salmon after cooking with quantitative descriptive analysis (QDA) based on QIM was observed and the prediction of the maximum storage time was 20–21 days with an accuracy of 1.5 days (Sveinsdottir et al., 2003). In addition, the shelf life of sea bass at different storage condition was explained in detail by Özogul et al. (2005a), and the best result was obtained at 16 days in ice, eight days in

aluminum foil, eight days in cling film, and four days in boxes without ice. According to Rodríguez et al. (2006), effects of storage in slurry ice about the sensory quality and the shelf life of farmed turbot were carried out and the sensory analysis was correlated well with the observed chemical and microbial changes. Most recently, Zhu et al. (2012) applied VIS/NIR hyperspectral imaging (HSI) technique combined with least squares-support vector machine classification models to differentiate between fresh and frozen-thawed fish fillets. Average correct classification rate of 97.22% for the prediction samples was achieved. This research finding also indicated that VIS/NIR-HSI had the potential to be used as an online technique for rapid and nondestructive differentiation of fresh fish from frozen-thawed fish and could have the opportunity to supplement and replace sensory evaluation method.

On the basis of these studies, it is evident that sensory evaluation is one of the most significant methods for assessing freshness in the fish sector and fish inspection services. This method performed in a proximal measurement of perceived attributes is a reliable tool capable of providing unique freshness information about fish and fish products. Accordingly, QIM should be part of seafood freshness quality control system and can be expected to be a popular and leading reference method for assessment of fresh fish in the future.

Microbial Inspection

The activity of microorganisms is one of the main factors causing fish spoilage. The total viable counts (TVC) as a traditional and helpful indicator are used to assess the freshness of different kinds of aquatic products. Meanwhile, most of countries have established standards, guidelines, and specifications of fish freshness evaluation based on TVC index with diverse storage conditions of temperature, time, and atmosphere. This indicator is useful for accurate detection of the degree of fish freshness and for predicting the remaining shelf life of fish. Generally speaking, there are many microorganisms in fish flesh just after capturing and the initial value of TVC is usually approximately 10^2–10^4 CFU/g (Gram and Dalgaard, 2002; Stringer, 2005; Liu et al., 2010).

Microbial deterioration process is a major contribution to the postmortem changes of fish and the remaining shelf life and therefore TVC values are used in various studies to predict the freshness of fish. Özogul et al. (2005b) studied the shelf life of eel based on the TVC acceptability limit of 10^6 CFU/g and showed that the shelf life of eel was about 13–14 days in ice and —six to seven days in boxes without ice, and the fish started to spoil after five days because of bacterial activity. Thereafter, Chantarachoti et al. (2006) compared the total plate counts on fish of Alaska pink salmon stored at 14°C and stored in slush ice, and the results showed that aerobic bacteria counts for fish stored at 14°C increased from 3.4 lgCFU/cm^2 (zero day) to 4.8 lgCFU/cm^2 (three days) and for fish stored in slush ice the counts ranged from 3.4 lgCFU/cm^2 (zero day) to 5.5 lgCFU/cm^2 (16 days). In a recent study, Song et al. (2011) declared that the TVC values were 5.74 and 4.66 log CFU/g on the day of sensory rejection under two different storage conditions linked to chilled and partial freezing storage. TVC values were also used to correlate with sensory assessment (Özyurt et al., 2009; Norton and Sun, 2008). On the other hand, studies on the special bacteria that can produce smelly odors were conducted. The common sulphide producer *Shewanella putrefaciens* was not a major spoiler of sea bass, but its counts would be an indicator to determine the time to sensory rejection (Kyrana and Lougovois, 2002; Lougovois et al., 2003, 2008). Another preliminary research was conducted by Taliadourou et al. (2003), and the behavior of H_2S-producing bacteria of whole-ungutted and filleted sea bass during the ice storage was studied and the TVC values for filleted sea bass samples were always higher than those for whole-ungutted sea bass.

Traditional TVC method is time-consuming and cumbersome. For rapid inspection of microorganisms, novel techniques have been established. Barbri et al. (2009) developed an EN technique to predict fish freshness and the results revealed that sardine samples could be classified in three freshness stages, and this was in good agreement with the results of a bacteriological analysis based on TVC values. Most recently, in order to provide noncontact and nondestructive measurement, hyperspectral imaging technique was investigated as a potential tool, and result indicated that HSI technique could be a rapid prediction tool for fish freshness quality (Peng et al., 2011).

Based upon the aforementioned studies, it is very necessary but challenging to establish universal and rapid microbial inspection platform for realizing simultaneous detections of multi-index and many samples, so that microbial inspection can be used for routine evaluation of fish freshness.

Chemical Measurements

Chemical measurements mainly associated with the chemical composition changes of fish are a kind of important and indispensable method for determining and evaluating the freshness of fish. And chemical measurements normally refer to moisture measurement, volatile compounds measurements, protein changes, lipid oxidation, ATP decomposition, and *K* value measurement.

Moisture Measurement

Moisture content is very important for fish freshness quality, which can affect the texture and muscle of fish, and thus some nondestructive, fast determination techniques have been developed. For example, moisture was determined by oven-drying method and electronic moisture analyzer and by NIR spectroscopy in skipjack tuna and yellowfin tuna, and I was confirmed that there was a good comparison between the results from the traditional oven-drying method and the moisture analyzer and those quantified using the NIR spectroscopy method,

thus NIR spectroscopy could serve as an accurate and fast method for quantifying the moisture of fishes (Khodabux et al., 2007). Similar study was conducted in cured Atlantic salmon by short-wavelength near-infrared (SW-NIR) reflectance spectroscopy (600–1100 nm) using partial least square regression (PLS) and artificial neural networks (ANN) calibration methods for moisture determination. PLS and ANN models gave good and similar results for moisture analysis ($R^2 = 0.799$, $R^2 = 0.784$, respectively) (Huang et al., 2003). In addition, NIR multispectral imaging has been developed for on-line determination of moisture in 70 dried salted coalfish. The PLSR prediction models obtained had good correlation value of $R^2 = 0.92$ with RMSECV of 1.07% (Wold et al., 2006). NIR interactance spectral imaging technique with a spectral region of 760–1040 nm to determine water content and distribution in the fillets of six fish species in real time has also been implemented. It has been proved that this technique is suitable for high-speed assessment of quality parameters of water content and distribution in fish fillets (ElMasry and Wold, 2008).

Volatile Compounds Measurements

Volatile compounds are one of the vital parameters of fish freshness determination. The effects of microbial activity and endogenous enzyme decompositions can create some volatile compounds related to nitrogen, amine, ammonia, alcohols, sulfur-containing compounds, and others. Therefore, monitoring and determination of the freshness or spoilage stage of fish can be based on the valuable measurements of volatile compounds. Among them, odor is one of the most significant characteristics of volatile compounds, which can be used to evaluate fish freshness (Olafsdottir et al., 2004). The volatile compounds contributing to fish odor can generally be divided into three groups according to the origin of volatile compounds during the fish storage. The first group is fresh fish odor mainly related to C_6–C_9 alcohols and carbonyl compounds; the second group is microbial spoilage odor, which plays a fundamental role in evaluating fish freshness, and is mainly related to ammonia, trimethylamine (TMA), hydrogen sulphide, and methyl mercaptan; and the last group is lipid oxidation odor mainly related to hexaldehyde, and 2, 4, 7-decatrienal (Olafsdottir et al., 2004).

Extraction of the volatile components is still a great difficulty at present due to their diversities. Accurate experimental results cannot be obtained by a single measurement method. In the meantime, this difficulty encourages scientists to conduct further research, leading to the developments of some integrated methods and techniques, such as solid-phase micro-extraction headspace analysis (Triqui and Bouchriti, 2003), gas chromatography mass spectrometry (Duflos et al., 2010), Fourier transform infrared spectroscopy (FT-IR) (Armenta et al., 2006), and the rapid and nondestructive EN technique (Olafsdottir et al., 2004; Alimelli et al., 2007; Limbo et al., 2009; Dini et al., 2010).

On the other hand, many industries have practiced the total volatile basic nitrogen (TVB-N) and/or TMA as indicators for the determination of fish freshness. TMA is an important smelly odor from the second group, which can indicate the spoilage degree of fish. Béné et al. (2001) determined the volatile compounds in terms of TMA, dimethylamine, histamine, and dopamine and their results indicated that these volatile compounds had good correlation with the aging of fish on ice storage, which was intended to predict the freshness degree of fish. In another work, TMA content was determined in fish by FT-IR spectroscopy compared with the head space gas chromatography method (Armenta et al., 2006), however, the changes of TMA content cannot be used to comprehensively clarify the fish spoilage degree and was gradually substituted by TVB-N level. Thus, more studies on TVB-N level for fish freshness evaluation were conducted. Özogul et al. (2005b) estimated and suggested that the acceptability limit of TVB-N level could be about 10 mg TVB-N per 100 g flesh. Another study showed that the acceptability limit for the filleted fish of TVB-N level was 26.77 mg N per 100 g flesh, and for whole-ungutted sea bass it was 26.88 mg N per 100 g flesh, respectively (Taliadourou et al., 2003). Moreover, Castro et al. (2006) investigated the changes of TVB-N level in fish storage and indicated that there were no significant changes during the edible storage life before 21 days and an increasing changes after 21 days of storage. More importantly, for nondestructive detection, chemical sensor array can be employed to determine the volatile compounds as Alimelli et al. (2007) found that the chemical sensory array was sensitive and effective for evaluation of the spoilage of fish. In addition, an innovative method using ammonium ion-selective electrode (NH_4^+-ISE) to measure the signal changes of NH_4^+-ISE, due to the changes of the ammonia content of the fish were developed and the results were correlated well with the content of volatile amines (TVB-N) in the cod fillets (Heising et al., 2012).

Protein Changes in Postmortem Storage

Proteins are a basic, vital, and nutritional component of fish flesh, accounting for approximately 15–20% of fish. The proteins in fish flesh mainly comprise water soluble sarcoplasm protein, salt soluble myofibril protein, and insoluble matrix protein (Olafsdottir et al., 1997). However, proteins are susceptible to be decomposed by unsound handling and processing, and by microorganisms and enzyme activities. Therefore, fish after slaughter normally stores in low temperature or on ice in order to maintain the freshness. Although low temperature is able to slow down microbial growth and proliferation, to reduce the activity of enzyme, and also effectively to lengthen shelf life of fish products, the structures, physical, chemical, and biological properties of proteins are inevitable to go through some changes during storage, thus impacting the taste and flavor, and affecting the nutritional and commercial values as well as the freshness quality of fish.

Therefore, studies about the relationship between the freshness quality and protein actions have been performed, as affected by handling, processing, and storage time. Herrero (2008) used Raman spectroscopy technique to identify the structural

change of proteins during storage and found that this change was implicated in the loss of fish freshness quality, while Sivertsen et al. (2011b) studied the oxidation degree of fish proteins (hemoglobin and myoglobin) by means of VIS/NIR spectroscopy during freezing-thawing and cold storage on ice, and predicted that this change was correlated with most of the variations seen in the visible region of the spectrum. Protein changes can also be examined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique as indicated in studies conducted by Martinez et al. (2007) and Godiksen et al. (2009). Martinez et al. (2007) used the SDS–PAGE technique to reveal the protein patterns of wild and farmed cod and the results implied that wild cod muscle had a different protein expression and postmortem degradation pattern from farmed cod, while Godiksen et al. (2009) correlated protein band intensities and firmness of rainbow trout fillets by the SDS–PAGE technique and found that the textural change of trout could lead to the breakdown of the protein structure. Therefore, SDS-PAGE technique could be an innovative alternative method for indicating protein changes, thus the freshness quality of fish (Attouchi and Sadok, 2012).

Lipid Oxidation Monitoring

It is known that fish is rich in unsaturated fatty acids and low in saturated fatty acids. The unsaturated fatty acids include tetradecene acid, palmitoleic acid, eicosapentaenoic acid, and docosahexaenoic acid (Rodriguez-Casado et al., 2007). As these fatty acids in fish can be easily oxidized by light and high temperature, and gradually break down into the low molecular weight substances such as aldehydes, ketones, and carboxylic acid groups, resulting in alterations of smell, texture, color, and nutritional values of fish (Trocino et al., 2012), lipid oxidation can be used to indicate the freshness of fish. Lipid oxidation is the main oxidation reaction in relation to lipase activity, fish species, and storage conditions and the thiobarbituric acid (TBA) value is considered as a helpful indicator for predicting the degree of lipid oxidation and assessing fish freshness. TBA content is usually expressed as mg malonaldehyde (MDA) kg^{-1} muscle, and studies on lipid oxidation based on TBA value have been performed to evaluate fish freshness. In 2003, Taliadourou et al. (2003) measured the increase in the TBA values of whole-ungutted and filleted sea bass during storage and the TBA values increased from the initial values of 1.55 and 10.21 mg MDA kg^{-1} muscle to 8.15 and 26.27 mg MDA kg^{-1} muscle after 16 days of storage, respectively. Furthermore, a few papers found that peroxide value (PV) was another useful indicator for determining the lipid oxidation. For example, Herrero (2008) and Özogul et al. (2005a) determined the peroxide value and fatty acid composition of fish using the traditional quality methods and Raman spectroscopy technique, and Guillén and Ruiz (2004) studied the ^{1}H Nuclear Magnetic Resonance spectra of the salmon lipids, as well as their usefulness for determining the proportions of different acyl groups. Furthermore, the rate of degradation of different acyl groups and the rate of generation of oxidation compounds were evaluated simultaneously throughout the oxidation process, which showed that this technique was useful to study the oxidative stability of fish lipids samples and their oxidation degree that could indicate fish freshness. Besides, the fat content of fish also plays a significant role in the oxidation courses and application of NIR spectroscopy proved to be successful to predict the lipid oxidation degree for Atlantic halibut fillet (Nortvedt et al., 1998; Folkestad et al., 2008) and Tuna fish and yellow fin (Khodabux et al., 2007). Similar to the success of NIR spectroscopy, spectral imaging technique has also been proved to be helpful for quantitative measurement of fat distribution as indicated by ElMasry and Wold (2008), who measured six species of fish fillets using the spectral imaging technique and their results presented a good correlation value of 0.91 with traditional measurements.

ATP Decomposition and K Value Indicator

Generally, after death, fish flesh undergoes postmortem changes due to a certain number of biochemical and physiochemical reactions occurred in the complicated structure and composition of fish muscles. Therefore, it is of great importance to comprehend the changes of fish muscles after death so as to maintain the fish freshness. Fig. 2a details the elaborate postmortem changes of fish. In this process, the decomposition of ATP is predominant and then a number of metabolites are generated as shown in Fig. 2b.With respect to the correlation between the changes of fish muscle and ATP content, some preliminary studies have been conducted and the fluctuating metabolite contents of ATP decomposition were reported (Itoh et al., 2012). Based on the ATP decomposition, Ehira and Uchiyama (1987) introduced a K value as a fish freshness quality indicator that was defined as the ratio (%) of the total amount of inosine and hypoxanthine to that of ATP-related compounds, and is given below.

$$K-\text{value}(\%)=\frac{\text{HxR}+\text{Hx}}{\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{HxR}+\text{Hx}}\times 100, \quad (1)$$

where ATP means adenosine triphosphate; ADP means adenosine diphosphate; AMP means adenosine monophosphate; IMP means inosine monophosphate; HxR means inosine; and Hx means hypoxanthine.

On one hand, K value as an useful indicator has been widely used to evaluate fish freshness, and a higher K value indicates a higher rate of ATP decomposition (Wills et al., 2004). To determine the K value, ion mobility spectrometry technique can be adopted that has been proved to be an effective and useful approach (Raatikainen et al., 2005). Tejada (2009) and Alasalvar et al. (2010) presented details on ATP-derived products and K value determination and showed that K value could be used for fish freshness and quality assessment. Moreover, Ocaño-Higuera et al. (2011) showed that K value expressed an

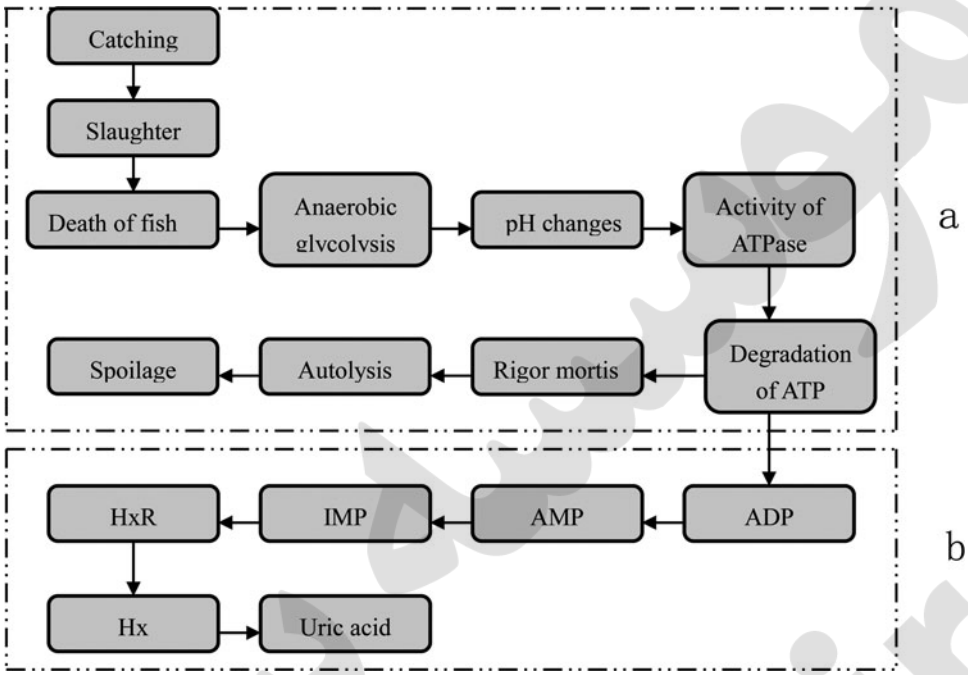

Figure 2 The postmortem changes in fish flesh. (a) The course of fish postmortem changes; (b) The course of ATP decomposition.

exponential increase with an initial value of 4.7% and a final value of 47.5% for indicating the signs of freshness and deterioration. In another work, Liu et al. (2010) confirmed that *K* value was a good indicator in monitoring the loss of fish freshness when they studied the postmortem changes of tilapia muscle. In addition, oxidation–reduction potential (ORP) method was used to evaluate fish freshness and the relation between ORP and *K* value was established, showing that ORP increased proportionally to the *K* value when it was in the range of 0.30–0.38 V, and then decreased with further increase in *K* value depending on storage temperatures (Agustini et al., 2001). On the other hand, it was discovered that ATP, ADP, and AMP contents decreased rapidly and nearly disappeared at 0°C, and HxR and Hx contents increased when IMP content decreased (Li et al., 2011). In this case, the contents of ADP and AMP were negligible. Therefore, the *K* value was simplified as K_1 value shown below (Hamada-Sato et al., 2005).

$$K_1 - \text{value}\ (\%) = \frac{\text{HxR} + \text{Hx}}{\text{IMP} + \text{HxR} + \text{Hx}} \times 100. \quad (2)$$

Hamada-Sato et al. (2005) illustrated that K_1 value was more useful and effective than *K* value for evaluating fish freshness. Based on the K_1 value, Barat et al. (2008) employed an amperometric flow-injection system with a 16-way switching valve and immobilized enzyme reactors to determine the freshness of sea bream stored at 4°C, and K_1 value was obtained with a correlation coefficient of 0.992 between K_1 value and the storage time. In a separate study, a nondestructive quality evaluation sensor was proposed to determine *sashimi* freshness quality and the result showed a good relationship between K_1 value and the remaining days of shelf life (Watanabe et al., 2005). However, according to Nanjyo and Yao (2002), Kyrana and Lougovois (2002) and Lougovois et al. (2003), K_1 value was only useful for monitoring early stage of stored fish, but could not be used to determine loss of acceptability or end of storage life.

Physical Properties Measurements

Effects of variations in fish physical properties on freshness quality are obvious and direct. These physical properties include color (Balaban et al., 2005), texture (Coppes-Petricorena, 2010; Careche and Barroso, 2009), shape, size (Poli et al., 2001), weight (Balaban et al., 2010; Gümüş and Balaban, 2010), and volume (Balaban et al., 2011). Among them, color and texture have direct impact on freshness quality of fish.

Color Measurement

Color is a major attribute of fish freshness quality and can also be used as an indirect estimate of chemical components and sensory attributes of food (Francis, 1995). In fish research, the color space used is the L^* a^* b^* or CIE Lab. Normally, fish color feature is measured by a colorimeter, showing L^*, a^*, b^* values (León et al., 2006; Hoang et al., 2005), however, novel methods are also investigated for measuring color of fish. Several studies have focused on the development of noncontact color measurement techniques. A nondestructive method based on the colorimetric imaging of the whole external body of sea breams to evaluate through multivariate partial least squares the

differences in the freshness under four refrigeration modalities has been developed. The results quantified significant colorimetric differences between fresh and nonfresh fish and the proposed imaging method merging different image analysis techniques had the ability to obtain the automatic assessment of fish freshness (Costa et al., 2012; Karoui and Blecker, 2011). In other studies, a color computer vision or machine vision system was used to analyze the fish skin color development in live goldfish (Wallat et al., 2002) and a long-wave near infrared hyperspectral imaging system was studied for measuring color distribution in salmon fillet (Wu et al., 2012a). Results showed that both computer vision and hyperspectral imaging had potentials in fish color measurement in a rapid and noninvasive way. Measurements of color of salmon fillets using computer vision and sensory panel based on the Roche color card industrial standard also confirmed no differences in the measurements about the two methods mentioned above (Quevedo et al., 2010). Other studies also proved that computer vision or hyperspectral imaging was a powerful tool to replace manual vision in fish processing (Misimi et al., 2007; Erikson and Misimi, 2008; Quevedo et al., 2008). Comparing with Minolta colorimeter, Yagiz et al. (2009) also illustrated that results of a machine vision system in measuring color of irradiated Atlantic salmon were very close to colorimeter readings.

Texture Measurement

Texture measurement is also a direct method to evaluate fish freshness. Texture measurements for fish and fish products are important in fish quality control and product development in the seafood industry. Numerous mechanical methods have been used to measure texture. However, there is little consensus on which is the most suitable method (Shankar et al., 2010).

Measurements of fish textural changes are usually achieved by texture analyzer with texture profile analysis, and many studies are available in this aspect. For example, the hardness, toughness, and stiffness of fish skin were studied by texture analyzer with a positive relationship between fish flesh stiffness and pH (Jain et al., 2007). Postmortem changes of muscle texture of Atlantic salmon were evaluated depending on shear force and rigor mortis (Roth et al., 2006). Textural properties of different locations of raw Atlantic salmon fillets were elucidated and the results showed that the hardness and shear force increased from head to tail of fish (Sigurgisladottir et al., 2006).

Fish texture is affected by fish structure behavior. Taylor et al. (2006) determined Atlantic salmon fillet texture by myofiber–myofiber and myofiber–myocommata attachment and noticed that the textural changes were correlated with structural alterations as the texture decreased significantly within 24 hours and synchronized with loss of attachment of muscle fibers. In another study, Badii and Howell (2002) discussed the changes in the texture and structure of cod and haddock fillets during frozen storage, and the results indicated that the textural changes were accompanied by a decrease in protein solubility, and an increase in hydrophobicity.

Besides the above traditional instrumental methods, innovative techniques were also developed for measuring fish texture. Steen and Lambelet (1997) measured the texture changes in frozen cod by using low-field nuclear magnetic resonance spectroscopy, and found that this technique could be an effective alternative tool to investigate textural modifications of cod during frozen storage. In another work, VIS/NIR spectroscopy and dynamometric analysis were used to discriminate concrete tank-cultured sea bass from sea cage-cultured sea bass based on texture changes. It was shown that differences in texture were observed between the two groups of fishes, but the texture changed with time. The texture in sea cage-cultured fish showed higher values of measured force (N) with respect to fish reared in concrete tanks at 48-hour postmortem (Costa et al., 2011). Instead, opposite results were observed at 96-hour postmortem (Costa et al., 2011). Meanwhile, VIS/NIR spectroscopy and hyperspectral imaging techniques were applied in texture analysis of farmed Atlantic salmon and results confirmed that VIS/NIR spectroscopy measurements offered fair predictions of Kramer shear force (Coppes et al., 2002; Coppes-Petricorena, 2010), while HSI also provided satisfactory measurements (Wu et al., 2012b). However, measuring texture in whole fish is still challenging due to inhomogeneous structure (density of muscle fiber, contents of fat and collagen) and difficulty in preparation of standard size of fish samples. Therefore, further research is needed in this area.

Besides, Menesatti et al. (2010) developed the HSI combined with geometric morphometric techniques to evaluate fish freshness. This novel approach was based on a priori determination of which wavelengths were more discriminating in relation to fish freshness, with considering fish samples of nonhomogeneous spectral quality. The results proved that HSI was a technique of high technological and methodological complexity, but with great application potential. It has been also demonstrated that the quality of fish from both fishery and aquaculture can be evaluated using the hyperspectral video-imaging morphometric-based analysis (Menesatti et al., 2010; Böhme et al., 2011).

Foreign Contaminant Detection

Foreign contaminants have a significant impact on the freshness and quality of fish. In particular, parasites, nematodes, and other hazardous substances pose significant risk to consumers (Zhu, et al., 2011). In wild-caught marine fish, *Anisakis simplex* is considered to be the most common parasites (Werner et al., 2010) and currently, no effective methods are available to detect parasites except manual vision inspection on candling tables. However, manual vision inspection is not accurate, and is time-consuming and laborious. To overcome this difficulty, multispectral imaging in the VIS/NIR region was used for automatic detection of parasites in cod fillets. The result indicated that the spectral characteristics of nematodes differed from those of fish flesh, thus allowing one to obtain fairly good classifications (Wold et al., 2001). Meanwhile, Park et al. (2005, 2007) developed a HSI technique as a potential tool for identifying the

Table 2 Evaluation methods for fish freshness based on different kinds of destructive and nondestructive inspection techniques

Evaluation method	Fish/fish fillets	Parameter	Technique	Reference
Sensory evaluation	Ungutted European sea bass, European eel, red mullet, gold-band goatfish	Skin, eyes, gills, abdomen	EU scheme, QIM	Kyrana and Lougovois (2002); Özogul et al. (2005b)
Microbial inspection	Tilapia, eel, sea bass	TVC	EN, HSI	Song et al. (2011); Peng et al. (2011)
Volatile compounds	Cod	TMA, TVB-N	NH_4^+-ISE	Heising et al. (2012)
Protein changes	Rainbow trout fillets	—	VIS/NIR, SDS-PAGE	Sivertsen et al. (2011b); Godiksen et al. (2009)
Lipid oxidation	Tuna fish and yellow fin	PV, TBA	Raman spectroscopy, ^{1}H-NMR	Khodabux et al. (2007)
ATP decomposition	Sea bass, sashimi	K-value, K_1-value	ORP	Watanabe et al. (2005)
Color measurement	Atlantic salmon, salmon fillets	Color	Colorimeter, HSI	Yagiz et al. (2009); Wu et al. (2012a)
Texture measurement	Atlantic salmon	Texture	VIS/NIR spectroscopy	Coppes-Petricorena (2010); Wu et al. (2012b)
Contaminants detection	Marine fish, cod fillets	Parasites	HSI	Sivertsen et al. (2011a); Sivertsen et al. (2012)

type and source of fecal contaminants. Also, results by Sivertsen et al. (2011a) indicated that HSI technique had a successful detection rate of 71% for pale nematodes of cod fillets, which in fact was better than the detection rate of 58% based on manual inspection method under industrial conditions. Further study was conducted by the authors (Sivertsen et al., 2012) to install the HSI system to meet the requirement of industrial speed of assessing one fish fillet per second in a production line moving at 400 mm/s. These studies illustrate that HSI technique can be effective for industrial on-line detection of parasites in fish.

ADVANTAGES AND DISADVANTAGES OF CURRENT EVALUATION METHODS

The state of fish freshness can be determined and evaluated by the above-mentioned methods based on different kinds of destructive and nondestructive inspection techniques as illustrated in Table 2. However, none of them could be recommended as a general method for assessing fish freshness (Barat et al., 2008). These methods and techniques possess their advantages and disadvantages as discussed below.

- Sensory evaluation is an important, traditional, and easily accepted method to determine and evaluate freshness of fish based on QIM, because it is most approximate to people's perceptions and can acquire relatively objective and accurate assessments. However, the disadvantages and limitations in using this method are time-consuming, costly, cumbersome, lack of reliability for in situ or on site monitoring, and the need of experienced, specially trained and professional panels' participation. For this reason, to a certain extent, the extensive application of this method is restricted in the industry.
- Microbial inspection is a vital and sensitive method to evaluate fish freshness due to the main role of microorganisms in fish decay. Some microbial criteria are established and developed in sea-foods and aquatic products based on culture and colony counting method, but this method cannot be used to effectively predict the remaining shelf life and its measurement is destructive and needs overelaborative experimental steps and lengthy testing time.
- Proteins are the main component of fish, but there are limited reports available about using protein as an indicator of fish freshness, because it is difficult to assess and control the complexities of protein changes during fish postmortem storage. While SDS-PAGE technique could be an innovative alternative method for indicating protein changes, its measurement process is very tedious and the pH of the solution is difficult to control.
- Lipid oxidation is a complicated process occurred in fish body based on the effect of enzymes and external condition changes. The value of PV and/or TBA is usually used to assess the degree of lipid oxidation and fish freshness. However, it is hard to monitor the progression of lipid oxidation due to its instability and limitation in determination of different types of fish products.
- The process of ATP decomposition can generate different types of metabolites. One single metabolite content index cannot be persuasive to evaluate fish freshness, and thus K value is developed to weigh the freshness but it is necessary to measure the concentrations of almost every metabolite in order to determine K value, which is time-consuming and expensive.
- The measurements of physical and foreign contaminants involve some instruments and techniques such as colorimeter, spectroscopy, and hyperspectral imaging. These techniques have great potential to be widely used for fresh fish quality inspection and safety assessment owing to their fast and nondestructive inspection and possibility for online monitoring. However, their drawbacks cannot be ignored. The established model of NIR spectroscopy technique must take a large number of chemical measurements and the accuracy of the analysis results is susceptible to the influence of external factors. Concerning the HSI technique, it is necessary to conduct further

in-depth research for improving the precision of the prediction model, reducing hyperspectral data redundancy, accelerating the detection speed and choosing optimized wavelengths for multispectral imaging systems.

CONCLUSIONS AND FUTURE TRENDS

The recent advances of freshness quality evaluation of fish in research and industry have been reviewed that covers a variety of methods including sensory evaluation, microbial inspection, physicochemical measurements, and some destructive and nondestructive techniques such as QIM, EN, SDS-PAGE, computer vision, spectroscopy, and hyperspectral imaging. Sensory evaluation as a traditional and helpful method for evaluating fish freshness is mainly employed in laboratory conditions. Microbial inspection based on the TVC value is capable of predicting the shelf life and the freshness or spoilage degree of fish. Chemical measurements as an indirect and valuable method can also be used to determine the fish freshness and K_1 value as an influential and powerful indicator has been widely used for evaluating the freshness of fish. Physical measurements mainly related to color and texture measurements are the most direct and critical method for fish freshness assessment.

On the other hand, despite so many research efforts on fish freshness determination and evaluation by means of the abovementioned methods and techniques, there are still many challenges that remain to improve fish freshness evaluation level. Further research is required to strengthen the objectivity and practical applicability of sensory evaluation. Moreover, it is observed that microbial inspection and chemical measurements have become valuable and sensitive methods for assessing fish freshness. However, some more difficult tasks need to overcome due to their destructive inspection, which also offer further research opportunities to explore new ideas for improving the level of microbial inspection and chemical measurements, thus promoting the speed and accuracy of fish freshness evaluation. In addition, implementations of spectroscopy and computer vision as relatively mature technologies for fish physical properties measurements have the chances to overcome the variability and to enhance the repeatability and authenticity in determining and predicting fish freshness. Besides, hyperspectral imaging as a nondestructive and emerging technique, which integrates both spectroscopy and computer vision is also available to predict the freshness of fish. However, as an emerging and promising technique, there are plenty of room for further research including selection of optimized wavelengths for multispectral imaging systems and establishment of robust prediction models that indicate fish freshness and realization of industrial online detection. In general, further research and developments should focus on establishing some feasible and achievable standardized methods for determination and evaluation of the freshness quality of fish.

ACKNOWLEDGMENT

Special thanks to Dr. Lingzhu Deng from North Dakota State University and Dr. Hongbin Pu from South China University of Technology for their kind suggestions.

FUNDING

The authors are grateful to the Guangdong Province Government (China) for its support through the program "Leading Talent of Guangdong Province (Da-Wen Sun)".

ABBREVIATIONS

ADP	= adenosine diphosphate
AMP	= adenosine monophosphate
CFU	= colony forming unit
EIS	= electrochemical impedance spectroscopy
EU	= European Union
Hx	= hypoxanthine
HSI	= hyperspectral imaging
MDA	= malondialdehyde
NIR	= near infrared reflectance spectroscopy
ORP	= oxidation–reduction potential
PV	= peroxide values
QIM	= quality index method
SDS–PAGE	= sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBA	= thiobarbituric acid
TVB-N	= total volatile basic nitrogen
U	= uric acid
NH_4^+-ISE	= ammonium ion-selective electrode
AQI	= artificial quality index
CIE	= Commission Internationale de L'Eclairage
EN	= electronic nose
FT-IR	= Fourier transforms infrared reflectance spectroscopy
HxR	= inosine
IMP	= inosine monophosphate
MIR	= mid-infrared reflectance spectroscopy
NMR	= nuclear magnetic resonance
PLSR	= partial least-squares regression
QDA	= quantitative descriptive analysis
TVC	= total viable counts
TMA	= trimethylamine
VIS	= visible light spectroscopy

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