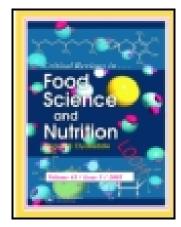
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Non-destructive Spectroscopic and Imaging Techniques for Quality Evaluation and

Assessment of Fish and Fish Products

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Abstract

Nowadays, people have increasingly realized the importance of acquiring high quality and

nutritional values of fish and fish products in their daily diet. Quality evaluation and assessment

are always expected and conducted by using rapid and non-destructive method in order to satisfy

both producers and consumers. During the past two decades, spectroscopic and imaging

techniques have been developed to non-destructively estimate and measure quality attributes of

fish and fish products. Among these non-invasive methods, visible/near-infrared (VIS/NIR)

spectroscopy, computer/machine vision and hyperspectral imaging have been regarded as

powerful and effective analytical tools for fish quality analysis and control. VIS/NIR

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spectroscopy has been widely applied to determine intrinsic quality characteristics of fish samples such as moisture, protein, fat, and salt. Computer/machine vision on the other hand mainly focuses on the estimation of external features like colour, weight, size and surface defects. Recently, by incorporating both spectroscopy and imaging techniques in one system, hyperspectral imaging can not only measure the contents of different quality attributes simultaneously, but also obtain the spatial distribution of such attributes when the quality of fish samples are evaluated and measured. This paper systematically reviews the research advances of these three non-destructive optical techniques in the application of fish quality evaluation and determination and discuss future trends in the developments of non-destructive technologies for further quality characterization in fish and fish products.

Keywords: Fish, spectroscopy, near-infrared (NIR), imaging, computer vision, machine vision, imaging spectroscopy, hyperspectral imaging, salmon, cod, trout, quality

1. Introduction

The current annual yield of fish produced by capture and aquaculture in the EU area is around 5.2 million tonnes and 1.3 million tonnes, respectively, the total of which accounts for about 4% of the globe fish production (Frid et al., 2012). The world fisheries production is projected at 164 million tonnes in 2020, with a growth of about 15% above the average level in 2008-2010 periods (OECD/FAO, 2011). As Shahidi (2009) and FAO (2009) investigated, the overwhelming majority of fish production is used for human consumption and the remaining is used to produce

other by-products. Report from OECD/FAO (2011) showed that in the period of 2008-2009, the average world *per capita* fish consumption kept at the level of about 17.1 kg/year and a slight growth occurred in 2010 because of increasing demand. By 2020, the world *per capita* fish consumption will come up to 17.9 kg/year, with Europe showing the highest growth rates. Because of such increasing fish production and consumption, trade in fish has become common to all societies and fish trade internationally has been increasing very rapidly, accounting for approximately 45% of the world fish production.

With increasing in globalization of fish trade, large amounts of fish and fish products have offered human beings with multiple choices for daily food purchasing and preparation. On the other hand, fish is an excellent source of many nutrients, including high quality protein contained the essential amino acids such as lysine and methionine, long chain omiga-3 fatty acids, and vitamin D (Nakagawa et al., 2007), which greatly induces consumers to spend in the fish business market. However, it is worth noting that quality assurance have increasingly gained attentions by producers and consumers when fish are processed and consumed. Processors, consumers, and regulatory officials have been seeking rapid and reliable methods for determining the quality, freshness as well as authenticity of fish products. Approaches developed to evaluate and measure fish quality typically involve sensory evaluation, chemical and physical determination, and microbiological examination. Sensory evaluation of fish quality is originally carried out through the sense of sight, smell, taste, touch and hearing (Hyldig and Nieisen, 2001), which are simple, cheap and rapid, but very subjective due to that the assessment by individuals are based on their preferences, although the bias is reduced apparently by means of proper sensory training for food description and record. Then instrumental method like electric nose was

developed and applied to simulate human sense of smell for analysing the odour profile of fish samples (Olafsdottir et al., 2004), which provides objective and reliable results, to a certain extent, but it is still not efficient when large samples are required to be measured. Chemical and physical constituents as main fish compositions are always associated with fish quality attributes and are measured initially by some chemical reagents or destructive tools (Sigurdsson, 1947; Sullivan et al., 1983), which are not only tedious and laborious but can also potentially damage the food itself and endanger the health of consumer. Microbiological examination is carried out for evaluating hygienic quality of fish such as the total aerobic bacteria (Debevere and Boskou, 1996), spoilage bacteria (Gram and Dalgaard, 2002), and various pathogenic bacteria (Boziari et al., 2011) and avoiding the possible pathogenic microorganisms occurred in the fish. These procedures generally conducted by using immunological reactions (ELISA, monoclonal antibodies) (Adams and Thompson, 1990) or genetic engineering (PCR, DNA-probes) (Bader et al., 2003) to keep the minimum spoilage, which however are time-consuming, costly and require special technical skills. Therefore, novel, rapid and non-destructive techniques need to be further developed for satisfying the producers and consumers.

With the development of optics and computer science, spectroscopic and imaging techniques emerged and have been viewed as promising and potential tools for quality evaluation and assessment in fish research. Among these non-destructive techniques, VIS/NIR spectroscopy, computer/machine vision and hyperspectral imaging have been regarded as powerful analytical tools in quality analysis and control. Studies showed that these advanced technologies are much more effective than traditional methods in providing useful information regarding fish quality and have obvious advantages in evaluating the quality attributes of fish, with less time and high

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efficiency (Bechmann and Jørgensen, 1998; Gümüş et al., 2011; He et al., 2012). Specifically, VIS/NIR spectroscopy provides detailed spectral information of tested products based on the interaction between light and specific physical and chemical constituents of fish compositions (Nortvedt et al., 1998). It has been widely reported in the application of evaluation and estimation of intrinsic quality properties in fish and fish products such as moisture, protein, fat, salt and others. Computer/machine vision has an ability to offer the image information at the pixel-level for explaining quality features of fish samples by means of high resolution digital camera (Sun, 2008). This technology has been effectively used for the estimation of external fish samples features such as colour, weight, size, shape and surface defects. Hyperspectral imaging technology into one system to characterise tested products with detailed spectral and spatial distribution information simultaneously (ElMasry and Sun, 2010). Using hyperspectral imaging, some chemical and physical attributes of fish samples can be visualized and mapped, which enables hyperspectral imaging to better explain the quality distribution in test products.

The main aim of this review thus focuses on application progresses of using these three non-destructive optical techniques, VIS/NIR spectroscopy, computer vision and hyperspectral imaging for assessment, measurement, and prediction of quality attributes of fish and fish products.

2. Quality attributes of fish and fish products

When it comes to quality attributes of fish and fish products, external features such as colour, size, shape and weight are always the visual impressions that directly affects the acceptance and satisfactory of fish products for consumers. Surface colour of products is generally considered as the first sensation by which the consumers perceive and use colour as a tool to accept or reject food products (Leon et al., 2006). Visual features like size, shape and weight are used as important quality parameters for commercial classification and grading of fish and fish products (White et al., 2006; Veliyulin et al., 2011). Other physical characteristic such as surface defects is also usually used as a basis for fish products sorting. Visual defects often appear in form of gaping, bruising, blood spots and even parasites, causing quality downgrading of fish products (Balaban et al., 2011a). Therefore, it is necessary to evaluate and measure external attributes to meet the different market demand for the fish processing industry.

Another aspect in relation to fish quality properties is intrinsic chemical attributes such as water, fat, protein, lipids, oils, and salt. These quality attributes vary greatly from one species or one individual fish to another (Sándor et al., 2011). In these parameters, water accounts for the most part of fish composition and is closely related to other attributes such as fat and lipid. In other words, the more moisture retains, the less fat content salmon fish has (Wold and Isaksson, 1997). In addition, moisture has a strong relationship with lipids that affect the eating quality of salmon (Katikou et al., 2001). Furthermore, moisture is a key factor that affects the microbial growth (Shimasaki et al., 1994). Fat is one of the important fish quality attributes, not only from a nutritional point of view, but also due to its sensory and functional properties (Wold et al., 1996). Protein of fish contains all essential amino-acids and has a high biological and nutritional value, which is good for human health (Nakagawa et al., 2007). High salt concentration in fish

inhibits microbial spoilage. The work of fish differentiation and classification is also demanded by producers and consumers due to different consumption groups. In addition, fish freshness makes a major contribution to the quality of fish or fish products. Many consumers prefer fresh fish despite its higher cost. For this reason, much more efforts are needed to identify the length of post-mortem of a fish and whether and how long a fish has been previously chilled or frozen. Traditional methods to evaluate and measure fish quality attributes are usually time-consuming, tedious and destructive. Therefore, it is imperative that rapid and non-destructive techniques are developed and applied for fish quality evaluation and assessment.

3. Principles of spectroscopic and imaging techniques

3.1 VIS/NIR spectroscopy

Since 1980s, spectroscopy, in particular VIS/NIR spectroscopy has been considered as a powerful tool and been increasingly used for food control and analysis (Jha, 2010). VIS/NIR spectroscopy typically utilizes the spectral range from 400 to 2500 nm (25,000–4,000 cm⁻¹), where the wavelength range from 400 to 700 nm is generally defined as VIS region and 700-2500 nm range is viewed as NIR region which can be divided into short-wave NIR (SW-NIR) (700-1300 nm) region and common NIR region (1300-2500 nm) (Cen and He, 2007). The principle of analysing physical and chemical properties in food and food products by using VIS/NIR spectroscopic methods bases on the interaction between electromagnetic radiation emitted from lights and physicochemical materials existed in foods, i.e., the responses of the

electromagnetic vibration of the molecular bonds C-H, O-H, N-H and C-O in chemical substances of food (Osborne, 2000; Cen and He, 2007). In fact, two vibration patterns involving stretch vibration and bent vibration are the major causes of vibrational energy changes when radiation happens. By absorbing light energy from vibration of these organic molecules using a VIS/NIR spectroscopic system, an absorption or reflectance spectrum is generated and information related chemical bonds can be extracted. An example is shown Fig. 1, illustrating the average spectra of beef meat samples in the VIS/NIR region which reveal the typical pigment, water and fat absorption. Over the last two decades, applications of using VIS/NIR spectroscopy has gained great popularity in the assessment and determination of quality parameters of food and food products, including meat (Horváth et al., 2008), edible oil (Wu et al., 2009), fruits (Magwaza et al., 2012), vegetables (Shiroma and Rodriguez-Saona, 2009), milk powder (Wu et al., 2012a), cheese (Woodcock et al., 2008), wine (Cozzolino et al., 2010), and juice (Wu et al., 2010). Especially for the quality evaluation of fish and fish products, VIS/NIR spectroscopy has been used for the measurement of chemical attributes such as moisture, fat and protein (Khodabux et al., 2007), freshness evaluation as well as product differentiation and classification (Nilsen and Esaiassen, 2005; Ottavian et al., 2012).

In VIS/NIR spectroscopy systems, two typical light interaction modes, transmission and reflection are widely applied for quality assessment of fish or fish products. The transmission analysis with long path length is suitable for SW-NIR region due to the absorption band of high overtones, while the reflection analysis is more efficient in common NIR region due to first or second overtone (Cen and He, 2007).

Fig. 1 Mean spectra corresponding to the different meat samples (Andrés et al., 2008)

3.2 Computer/Machine vision

Computer vision, or machine vision has been proved to be another useful and non-destructive tool in the application of extracting and quantifying many traits directly related to food quality evaluation and control (Brosnan and Sun, 2004; Du and Sun, 2004; Wu and Sun, 2012). This technique has an ability of analysing spatial information acquired from a specific object mainly based on the fact that computer vision can simulate human vision to see an object and perceive its optical features through the reflected light from the object being illuminated by artificial or natural light. A typical computer vision system consists of illumination device (light source), image-capturing system, frame grabber and computer (Fig. 2). An analysis task by computer vision generally involves three steps: image acquisition usually performed with a digital camera, image analysis includes pre-processing, segmentation and features extraction, and decision-making based on image understanding concerning image recognition and interpretation.

Fig. 2 Principal components of a computer vision system (Patel et al., 2012)

Computer vision technique started to be used in the food industry in early 1990s, and then it gained increasing acceptance and popularity in the fields of both scientific research and industrial application. Much evidences have showed that computer vision technique plays an important role in the food quality evaluation by maintaining objectivity and accuracy of

information and eliminating subjectivity of the manual inspections, which offers a reasonable substitute for making decision by human (Zheng and Sun, 2006; Sun, 2008). By far computer vision has been widely applied in the application of quality assessment of agricultural and food products such as beef (Zheng et al., 2006), pork (Mendoza et al., 2009), hazelnut (Pallottino et al., 2010), lettuce (Zhou et al., 2004), wheat (Konophka et al., 2004), and pizza (Sun, 2000). Also, it has been used to evaluate and control fish quality (Sun, 2008). As an essential part of human daily commodities, fish and fish products have been mainly studied by computer vision in the aspects of external features such as colour, shape, length, size, weight and surface defects (Gümüş et al., 2011), as well as some chemical characteristics like fat content (Stien et al., 2007).

3.3 Hyperspectral imaging

With the development of spectroscopy and imaging techniques, hyperspectral imaging or imaging spectroscopy emerged and it has been viewed as a novel, rapid and non-destructive technique for quality evaluation in food and food products (ElMasry et al., 2012a,b). It successfully integrates spectroscopy and imaging techniques into a new system and provides both spectral and spatial information from a tested object simultaneously. Hyperspectral image data, known as "hypercube", are three dimensional data blocks including two spatial and one wavelength dimension, providing hundreds of contiguous wavebands for each spatial position of a target. Each pixel of hyperspectral image contains the spectrum data in specific position, which can be used to characterise the composition in particular pixel (ElMasry and Sun, 2010). Fig. 3 illustrates one example of the hypercube extracted from a hyperspectral image acquired for a

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piece of meat. After the hyperspectral data are appropriately processed, a so-called *chemical image* can be generated for the visualization of different biochemical constituents presented in a sample based on their spectral signatures (Fig. 4), which enables hyperspectral imaging technique for quantitative analysis of each entity and labelling of different samples. Hyperspectral imaging has been reported for the evaluation of quality attributes of many agricultural and food products such as beef (Wu et al., 2012b), pork (Dissing et al., 2012), banana (Rajkumar et al., 2012), eggs (Liu and Ngadi, 2012), apples (Menesatti et al., 2009), and prawn (Wu et al., 2012c). In the work of fish quality assessment, hyperspectral imaging has been applied for determination of some chemical attributes like moisture estimation in different fish species (ElMasry and Wold, 2008, Menesatti and Costa, 2010), defect detection in cod fish (Sivertsen et al., 2011), and fat and colour distribution analysis in salmon fillets (Segtnan et al., 2009; Wu et al., 2012d).

Fig. 3 Hyperspectral image (*hypercube*) for a piece of meat showing the relationship between spectral and spatial dimensions (ElMasry and Sun, 2010).

Fig. 4 Application of PLS-R to obtain the distribution map of drip loss in pork meat samples by using hyperspectral imaging (Barbin et al., 2012).

4. Applications of non-destructive spectroscopic and imaging techniques for quality evaluation of fish and fish products

4.1 VIS/NIR Spectroscopy

4.1.1 Measurement of chemical composition by VIS/NIR spectroscopy

4.1.1.1 Moisture/water

Moisture is a major chemical component in fish composition and is an important quality indicator in fish and fish products. It is capable of using NIR spectroscopy for moisture evaluation due to the high absorbance of water in NIR spectral region. Since 1990s, NIR spectroscopy in reflectance mode has been widely applied to measure moisture content in many products of fish species. Rasco et al. (1991) reported NIR reflectance spectroscopy in the wavelength of 900-1800 nm to predict moisture content in fresh and frozen rainbow trout muscle. Calibration analysis including both partial least squares (PLS) and multiple linear regression (MLR) were applied to relate spectral data to moisture concentration, resulting in the standard error of prediction (SEP) of 1.5% and 1.1%, respectively. Later, Isaksson et al. (1995) investigated the NIR diffuse spectroscopy using three wavelength regions, 760-2500 nm, 760-2500 nm and 1100-2500 nm, for determining the moisture content in farmed Atlantic salmon fillets and compared the predictive abilities of three different calibration models. The results revealed that linear principal component regression (PCR) models using the 760-1100 nm range had lower errors in moisture prediction than that using 1100-2500 nm or 760-2500 nm ranges. In the following years, different wavelength ranges in NIR region were selected and applied to analyse moisture in salmon fish or salmon products, for example, 700-1000 nm (Downey, 1996), 800-1100 nm (Wold and Isaksson, 1997), 1100-2500 nm (Cozzolino et al., 2002) and 600-1100 nm (Huang et al., 2002, 2003). Table 1 shows the detailed information. In frozen cod fish, water

holding capacity (WHC) closely related to moisture was determined by NIR spectroscopy in 1000-2222 nm regions (Bechmann and Jørgensen, 1998). Xiccato et al. (2004) attempted to use NIR spectroscopy (400-1100 nm) for moisture concentration estimation in European sea bass and obtained a satisfactory result ($R^2 = 0.69$, SEP = 1.45%). In tuna fishes, skipjack and yellow fin, moisture was measured by Khodabux et al. (2007).

4.1.1.2 Protein

Protein is an important functional and nutritional component of fish and many studies on the application of NIR spectroscopy to predict this component have been reported in the past two decades. Mathias et al. (1987) analysed the protein of freeze-fried freshwater fish, rainbow trout and arctic charr, with 1000-2500 nm wavelength range applied in the test. By NIR spectroscopy, a considerably higher precision than conventional chemical methods in the measurement of protein in fish tissue was achieved. A comparable accuracy of prediction of crude protein in rainbow trout was also reported by Valdes et al. (1989) who further confirmed the potential use of NIR spectroscopy technique for fish carcass analysis. In early 1990s, Rasco et al. (1991) concluded that NIR spectroscopy (900-1800 nm) combined with PLS and MLR calibrations could be useful for making a rough estimate of protein content of rainbow trout muscle. By contrast, no satisfactory results were achieved by using NIR spectroscopy for protein prediction in whole salmon fillets (Isaksson et al., 1995), mainly because of protein concentration ranges considered. Due to the deeper and more representative ability of transmittance measurement into the fillet over the reflectance measurements in the fillet surface, Nortvedt et al. (1998) used NIR transmittance spectroscopy to predict protein of Atlantic halibut fillets. As a result, the accuracy

of the calibration models developed for protein was closed to that of chemical method, which was probably due to the narrow protein range in the examined fillets. In 2000s, endeavours on the exploration of potential use of NIR spectroscopy to estimate protein in fish or fish products continued. Xiccato et al. (2004) and Majolini et al. (2009) used NIR spectroscopy to predict protein in sea bass. However, calibration statistics for crude protein were considered of lower performance ($R^2 = 0.73$, SEPCV = 0.34%). In contrast, quantifying protein of tuna fishes by using NIR spectroscopy (600-1500 nm) coupled with PLS analysis yielded a desirable result, with a high correlation coefficient (R^2) of prediction being 0.99 (Khodabux et al., 2007).

4.1.1.3 Fat

Fat plays a very important role in eating quality of fish meat as it may affect flavour, juiciness, texture and appearance. The fat content in rainbow trout carcass was predicted by NIR spectroscopy, but the predictive ability was considered lower ($R^2 = 0.78$) due to the narrower range of fat values (Valdes et al., 1989). Then, researchers attempted to investigate the potential application of NIR spectroscopy for fat measurement in salmon fillets. Different wavelength regions were applied to develop calibration models, with different precision and accuracy found (Table 1). More specifically, Sollid and Solberg (1992) employed the 850-1050 nm region and showed a high correlation (R = 0.99, SEP = 0.49%) between measured and predicted fat values. With the same region, a similar prediction correlation of 0.97 with RMSECV of 0.75% was observed in the work of Wold (1996). The range of 760-1100 nm was used by Isaksson et al. (1995) and a very similar prediction error (RMSECV = 6.6 g/kg) for fat was yielded, compared with Solid and Solberg (1992). The fat content in the whole salmon fish was studied by Wold

and Isaksson (1997) and Solberg et al. (2003), in which almost the same wavelength range (800-1100 nm) in NIR regions was used and very similar results were found in these two studies, although the performance of using NIR spectroscopy for fat prediction was not good enough. In the recent work by Folkestad et al. (2008), NIR spectroscopy ranging from 760 to 1040 nm was applied to determine the fat concentration of live and slaughtered Atlantic salmon. A relative good result was achieved with R of 0.94 and RMSEP of 1.0 fat% unit. NIR spectroscopy was also used to analyse fat in other fish species such as halibut (Nortvedt et al., 1998), skipjack (Shimamoto et al., 2003) and tuna (Khodabux et al., 2007) besides salmon fish, which are shown in Table 1.

4.1.1.4 Lipid and oil

Mathias et al. (1987) applied NIR spectroscopy to estimate the lipid content of rainbow trout and arctic charr. In this research, NIR spectra were measured at the wavelength range of 1000-2500 nm and NIR spectroscopy showed a higher precision than standard methods in the measurement of lipids in fish tissue. After that, crude lipid content in rainbow trout muscle was studied by Rasco et al. (1991) and Lee et al. (1992), who suggested that NIR spectroscopy combined with MLR and PLR calibration could be useful for determination of crude lipids in fish. Then, in the work of Solberg (1995), a good prediction for lipid (R² = 0.98; SEP = 0.33%) in whole minced cod, capelin and salmon fillets was obtained. Xiccato et al. (2004) confirmed good prediction accuracy for lipid determination in European sea bass by using NIR spectroscopy (1100-2500 nm). According to consumer's perceptions, oil content in fish is greatly related to food taste and mouthfeel, fish high in oil will provide satisfactory flavour, therefore, oil concentration becomes

an important quality parameter in fish quality assessment. Downey (1996) developed NIR calibrations using 700-1100 nm range for analysing oil content in salmon flesh. Then, Cozzolino et al. (2002) measured oil content in raw mackerel, bluewhiting and other fish species. Results revealed that NIR spectroscopy in the 1100-2500 range could be potentially used to assess the oil content in minced raw fish with good precision ($R^2 = 0.96$).

4.1.1.5 Salt/sodium chloride

Salt or sodium chloride is not only related to food sensory characteristics but is also a key factor for inhibiting food spoilage occurrence. Theoretically, salt has no specific absorption band in the NIR region, but it is possible to detect sodium chloride mainly because salt has an effect on the shape and position of the water absorption band (Huang et al., 2001; Lin and Brown, 1992). Huang et al. (2002, 2003) used NIR reflectance spectroscopy in the short-wavelength range 600-1100 nm to determine salt content in different fish samples, cold-smoked and cured Atlantic salmon. ANN calibration exhibited a somewhat better capability (R² = 0.824, RMSEP = 0.55) for salt prediction than PLS (R² = 0.775, RMSEP = 0.63) in the cold-smoked salmon while in cured salmon ANN and PLS yielded similar results (ANN: RMSEP = 1.43% w/w, PLS: RMSEP = 1.37%). Using the same wavelength range coupled with PLS calibration, the prediction efficiency was improved by Lin et al. (2003), with R² of 0.83, 0.82 and SEP of 0.32% w/w, 0.25% w/w in king salmon (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*), respectively.

4.1.1.6 Other chemical components

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The total volatile basic nitrogen (TVB-N), dimethylamine (DMA-N) and formaldehyde (HCHO) in the whole thawed cod (*Gadus morhua*) were measured using NIR diffuse reflectance spectroscopy (4500-9996 cm⁻¹) by Bechmann and Jørgensen (1998). Unfortunately, no satisfactory calibration models were developed in this research for determining these chemical components. In contrast, high performance of calibration for total volatile nitrogen (TVN) prediction was obtained by Cozzolino et al. (2002) in minced raw mackerel, salmon, bluewhiting and other fish species. The R² is 0.96 with SECV of 3.51 mg/g, which indicated that NIR spectroscopy is a useful tool for the assessment of some chemical composition in raw fish. In addition, Nortvedt et al. (1998) analysed dry matter of wet homogenized Atlantic halibut (*Hippoglossus hippoglossus*) fillet by NIR in transmittance mode at the 850-1050 nm range, with a better accuracy (RMSECV = 4.2 g/kg) in PLS calibration model than that of chemical method obtained.

4.1.2 Quality inspection and differentiation by VIS/NIR spectroscopy

4.1.2.1 Defects detection

Bruising, in particular deep bruising generally caused by crushing or injuring during catching, transportation and storage, is one of major quality defect that results in reducing market value of fish and fish products, to a great extent. For this reason, NIR spectroscopy was used to detect the defect and ensure the fish with high quality. Based on characteristic absorbance bands of deoxyhemoglobin, a primary pigment in blood, at approximately 760 nm, Lin et al. (2003) attempted to apply visible and SW-NIR spectroscopy (600-1100 nm) to detect bruises in intact whole pacific pink salmon (*Oncorhynchus gorbuscha*). A PLS cross-validation model using six

latent variables yielded R of 0.83 with SEP of 0.05%, which suggested that visible and SW-NIR could be used to control the bruise defect of fish products and improve product consistency and quality. Also, good results were achieved by Hammers et al. (2007) who use the same wavelength range to non-destructively detect deep muscle bruising in whole pacific coho salmon (*Oncorhynchus kisutch*).

4.1.2.2 Freshness evaluation

Freshness is viewed as a very important quality indicator that makes a major contribution to the quality of fish or fish products and VIS/NIR spectroscopy has been successfully investigated for freshness evaluation. By defining freshness as storage time in ice, freshness of cod and salmon fillets was evaluated by Nilsen et al. (2002) who adopted VIS/NIR spectroscopy in the range of 400-1100 nm, with 400-700 nm in visible range for cod and 700-1100 nm in NIR range for salmon. The good correlations of prediction (cod: 0.97, salmon: 0.98) proved that VIS/NIR spectroscopy is useful for the assessment of fish freshness. Packed in three different atmospheres and frozen or chilled at different temperatures for different periods, thawed and chilled cod (Gadus morhua) fillets were assessed by Bøknæs et al. (2002), in which, NIR spectra at 4500-9996 cm⁻¹ was applied to develop a PLS calibration model, leading to a correlation coefficient of 0.90 with RMSECV of 3.4 d at 2 °C between measured and predicted duration of chill storage period (days at 2 °C). Another work by Nilsen and Esaiassen (2005) showed that visible spectroscopy (400-700 nm) could be used to predict the freshness of cod based on the quality index method (QIM) using QIM score (correlation of prediction > 0.9, RMSEP < 3.6). Furthermore, the result showed that a relatively narrow band in the mid-visible spectral range is

sufficient to use when measuring freshness. At the same time, further studies concerning the validity of this method were suggested.

4.1.2.3 Differentiation and classification/sorting

Product differentiation and classification is an emerging area of concern within the fish processing industry. In early 2000s, Isaksson et al. (2001) used VIS/NIR reflectance spectroscopy (400-1100 nm) combined with linear discriminant analysis to classify farmed Atlantic salmon (Salmo salar) into three categories: low Kramer shear force, medium Kramer shear force and high Kramer shear force. Up to 79% correct classification was achieved, which provided a possible way for fish sorting according to different Kramer shear forces before further processing or sale. Based on the report that NIR spectroscopy has the potential for addressing some discrimination issue in foods and is known to be a non-destructive rapid technique (Cozzolino et al., 2002; Downey et al., 2003), Uddin and Okazaki (2004) made an attempt to classify fresh and frozen-thawed fish by NIR spectroscopy due to fish muscle absorbs and reflects light in different ways during storage and thawing. In the work, live horse mackerel (Trachurus japonicas) was investigated and NIR reflectance spectra collected from 1100-2500 nm regions were analysed by MLR and PCA. A 100% correct separation was finally found between fresh and frozen-thawed fish. Then, Uddin et al. (2005) continued to use VIS/NIR spectroscopy in the 400-1100 nm range for further investigation of frozen-thawed red sea bream (Pagrus major). The developed linear discriminant analysis (LDA) model using original absorbance spectra also achieved 100% classification accuracy for the prediction in fish samples. In recent years, VIS/NIR spectroscopy was used to differentiate another fish species, European

sea bass. Costa et al. (2011a) employed VIS/NIR spectroscopy (400-970 nm) to determine the differences in meat quality between sea bass (*Dicentrarchus labrax*) cultured in cement land-based tanks (concrete tanks) (CT) or in sea cages (SC). Using spectral data, the PLS analysis was conducted on individual samples at 48 and 96 h post-mortem (p.m.). A high efficiency of discriminating SC from CT fishes (87%) at 48 h.p.m. and a low efficiency (66.7%) at 96 h.p.m. were observed. In the same year, Ottavian et al. (2011) discussed the possibility of using NIR spectroscopy for the authentication of wild European sea bass (*Dicentrarchus labrax*). The wavelength from 1100 to 2500 nm was used and three different chemometric techniques were developed to process the NIR spectra. Classification results showed that NIR spectroscopy is a very effective tool in assessing the authenticity of wild European sea bass.

4.1.3 Microbial spoilage control by VIS/NIR spectroscopy

Microbial spoilage always gives rise to the short shelf-life of fish products. Therefore, controlling microbial spoilage is quite necessary and meaningful in practice. Applications of using VIS/NIR spectroscopy for detecting and quantifying microbial spoilage has been reported in meat and chicken (Ellis et al., 2002; Lin et al., 2004). Based on these works, Lin et al. (2006) used visible and short-wavelength near-infrared (600-1100 nm) spectroscopy to detect and monitor the onset of spoilage and quantify the microbial loads in intact and minced rainbow trout (*Oncorhynchus mykiss*) fillets stored at 4 °C and 21 °C. Quantitative PLS prediction models for microbial loads were established, with R of 0.97 and SEP of 0.38 log colony-forming units (CFU)/g (flesh side) and R of 0.94 and SEP of 0.53 log CFU/g (skin side) at 4 °C and R of 0.82 and SEP of 0.82 log CFU/g for minced fish held at 21 °C, respectively. The results indicated the

great potential of NIR spectroscopy in monitoring the spoilage process in fish with good accuracy. The author (Lin et al., 2006) suggested that this technique should be transferable to other food systems composed of intact or minced muscle tissue.

Table 1 Overview of VIS/NIR spectroscopy for quality evaluation and assessment of fish and fish products

4.2 Computer/machine vision

4.2.1 Measurement of physical attributes

4.2.1.1 Colour

Colour, one of important organoleptic features, is the first sensation that the consumer perceives and uses as a powerful indicator to accept or refuse food products (Hutchings, 1999; Leon et al., 2006). Colour evaluation of fish and fish products has been widely reported by using computer vision technique. Marty-Mahé et al. (2004) develop a colour image analysis method to measure colour (L*) and map the colour distribution in flesh of brown trout (*Salmo trutta*) that were fed with two different diets. Also, the colour of trout (*Oncorhynchus mykiss* W.) cutlet (Stien et al., 2006) and tuna (*Thunnus thynnus* L.1758) meat (Mateo et al., 2006) has been measured by automated image analysis system. Oliveira and Balaban (2006) compared the ability to measure changes of colour over 15 days of iced-storage of Gulf sturgeon (*Ancipenser oxyrinchus desotoi*) fillets between a machine vision system and Minolta CR-200 colorimeter. As a result, machine vision outperformed colorimeters when recording and estimating subtle colour changes in fish

fillets. Salmon as one of most popular fish species, was also investigated with respect to the colour measurement and identification by computer vision. With the technique, Erikson and Misimi (2008) determined the changes in skin and fillets colour of anesthetized and exhausted Atlantic salmon after killing, during rigor mortis, and after ice storage for 7 d. Yagiz et al. (2009) compared two different colour determination methods between Minolta colorimeter and computer vision system in measuring colour of irradiated Atlantic salmon, with a result of that using the Minolta colorimeter in reading the standard colour with given L*, a*, b* values was very closed to that of using computer vision method. A similar work was carried out between a computer vision system and a sensory panel by Quevedo et al. (2010) in salmon fillets, with a very strong correlation (R = 0.95) obtained and no differences occurred between these two methods. Recently, Menesatti et al. (2012) developed a novel "3D Thin-Plate Spline" warping approach to calibrate colours in RGB space for quantitative image analysis. The method reported a very high efficiency of calibration and was of great importance for colour evaluation when lighting conditions are not controlled. It is expected that the new calibration approach will be applied for quality evaluation in fish and fish products.

The refrigeration and preservation condition is a fundamental factor affecting final quality of fish and fish products, especially colours. Korel et al. (2001a) used a colour vision system to monitor the changes in the colour of tilapia (*Oreochromis niloticus*) fillets dipped in sodium lactate solutions. In another study (Korel et al., 2001b), raw and cooked catfish (*Ictalurus punctatus*) fillets were evaluated with computer vision and electronic nose throughout storage. Balaban et al. (2005) developed a computer vision for analyzing the colour changes (R, a* and hue) of fresh tuna that treated by 4% carbon monoxide + 20% carbon dioxide + 10% oxygen, or irradiated at 1

KGy or 2 KGy, or first gas treated then irradiated. The samples exposed to CO showed the higher redness (a*) and can be preserved up to 12 days in refrigerated storage. Irradiation at 2 KGy was effective in reducing microorganisms while 1 KGy was in effective. Recently, Erikson et al. (2011) used a computer vision system to assess the colour changes of salmon fillets (*Salmo salar*) under different chilling strategies. The samples stored at -3.6 °C indicated higher lightness (L*) than at -1.6 °C or under ice-stored conditions. No differences in redness (a*) and yellowness (b*) were found. Meanwhile, frozen storage of Atlantic salmon fillets at -20 °C resulted in increased L*, a* and b*.

4.2.1.2 Weight

Computer vision technique has also been used for weight estimation of fish. Odone et al. (1998) introduced a vision system with support vector machine to automatic estimation of trout weight, achieving good accuracy and reliability. In recent years, different relationship between fish weight and image analysis was reported. Liang and Chiou (2009) described a machine vision-based automatic system coupled with linear regression analysis for evaluating the weight of Taiwan Tilapia, with the high correlation (R² = 0.9303) yielded between weight and projected area. More interesting, a higher relationship (R² = 0.98) was achieved between weight and image features by Gümüş and Balaban (2010) in aquacultured rainbow trout (*Oncorhynchus mykiss*) using machine vision technique. Weight estimation of Alaskan Pollack (*Theragra chalcogramma*) has also been done based on the projected area (Balaban et al., 2010a) acquired from a digital camera, achieving an R² value of between 0.985 and 0.993, depending on the type of regression used. Balaban et al. (2010b) applied the weight prediction using computer vision

for accurately sorting salmon species, including pink (*Oncorhynchus gorbuscha*), red (*Oncorhynchus nerka*), silver (*Oncorhynchus kisutch*), and chum (*Oncorhynchus keta*), resulting in R² values between 0.928 and 0.983 in different models established between species and regression. In a recent work by Mathiassen et al. (2011), a 3D machine vision was developed to estimate weight of whole herring (*Clupea harengus*), with R² values between 0.919 and 0.938 found, varying with fish freshness and imaging conditions.

4.2.1.3 Length, size, shape and volume

A few applications of using computer vision in determination of external features of fish such as length, shape, size and volume, have been reported recently. White et al. (2006) used a computer vision system called CatchMeter to measure the length of seven species of fish (Table 2), resulting in a sorting reliability of 99.8% with a standard deviation of 1.2 mm for these fish samples. Using the same system, Svellingen et al. (2006) determined the length of other fish of seven species (Blue Whiting, Capelin, Cod, Haddock, Herring, Norway Pout and Saithe), besides three species (Long Rough Dab, Golden Redfish and Deepwater Redfish) mentioned in work of White et al. (2006). Based on image analysis and outline morphometry combined with multivariate techniques, Costa et al. (2012a) developed a methodological tool for length measurement (r = 0.9443) and size estimation (r = 0.9772) of farmed European seabass (Dicentrarchus labrax L.). With the developed discriminant analysis models, the discrimination efficiencies to select sex and malformed fish reached 82.05% and 88.21%, respectively. Recently, Hsieh et al. (2011) developed a digital imaging method based on developed computer software program to measure length of tuna fish, with estimation error of 4.5 ± 4.4% generated.

In addition, size and shape of Atlantic cod (Gadus morhua) and salmon (Salmo salar) fillets during rigor mortis and ice storage were evaluated by Misimi et al. (2008), who concluded that computer vision is useful for grading of fillets according to uniformity in size and shape, as well as fillet yield measured in thickness. Shape analysis also plays an important role in appearance features evaluation and is often necessary in research fields for a range of different purposes (Costa et al., 2011b). Geometric morphometrics combined with morpho-anatomy method were used to describe and quantify the shape features of sea bream (Sparus aurata, Sparidae) (Loy et al., 1999). After, by using geometric morphometrics and image analysis system, the shape of sea bass (Dicentrarchus labrax L., Moronidae) and shape differences between species were analysed (Loy et al., 2000a). The high incidences of anomalies associated with large scale shape changes in sea bream were proposed at the same time. Then, Loy et al. (2000b) proposed a remote system coupled with Elliptic Fourier analysis to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae) and compared with geometric morphometrics method. The results showed that the remote system technique had a very similar growth curves compared with geometric morphometrics method and it allowed for an appropriate visualisation of fish shape and shape changes. A computer coupled with digital camera was used to predict volume of whole Alaska pollock (Theragra chalcogramma) and two image analysis methods (dimensional measurement and the cubic splines) were developed and compared, leading to R² of 0.987 between the dimensions (length L, width W and depth D) versus measured volume and 0.99 between the cubic splines versus the measured volume, respectively (Balaban et al., 2011b).

4.2.1.4 Defects

It has been reported that about 40% of downgrading to lower value products during salmon (*Salmo salar* L.) fish processing is due to occurrence of defects like gaping typically appeared when the fish connective tissue failed to hold the muscle blocks together, which results in decreasing satisfaction and acceptability of consumer (Michie, 2001; Balaban et al., 2011a). For this reason, Ashton et al. (2010) analysed gaping in salmon (*Salmo salar* L.) fillets by digital photography combined with computer image technique. Among many parameters significantly correlated with gaping score, the tensile method parameter (maximum force) (R = 0.514, P < 0.001) was considered to be a strongest factor. Then, Balaban et al. (2011a) developed an image analysis method for analysing the defects including gaping, bruising and blood spots of red salmon (*Oncorhynchus nerka*) fillets with two different cameras, a dSLR camera and a video camera. After image analysis of samples by computer software, defects were well quantified, which is considered the first step for defects evaluation. The next step, differentiating between blood spots, gaping, and bruising, was suggested and expected at the same time.

Abnormalities of shape or body deformities were described and reviewed by Divanach et al. (1996). Scale abnormalities are frequent in many fish species and typically involve absence or loss of scales, much smaller or bigger scales than normal size and an abnormal scale distribution (Jawad et al., 2006/2007, Boglione and Costa, 2011). From the economic point of view, scale abnormalities may affect fish market acceptability and price. Monitoring the scale abnormalities seems necessary and important in fish capture and marketing. By scanned images of photographs or traces using image software on a computer, Corrales et al. (2000) characterized the features of scale disorientation and quantitatively measured the changes of scale disorientation in pinfish, Lagodon rhomboides, in Biscayne Bay, Florida (U.S.A.). Recently, Arechavala-Lopez et al.

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(2012) studied the differenced in external characteristics of scales using digital camera coupled with image analysis software and suggested that the use of scale characteristics as the easiest and quickest way to distinguish farmed or escaped fishes.

The body lateral line is a distinct external feature that can be used to judge the morphological quality and degree of conformity of reared fish to wild fish (Carrillo et al., 2001). The morphological malformation of the body lateral line is a problem in intensive aquaculture and has raised concerns (Divanach et al., 1996). Carrillo et al. (2001) observed the body lateral line abnormalities using image analysis techniques and provided a general description of the morphological modifications of the lateral line in gilthead sea bream (*Sparus aurata* L. 1758) reared under hatchery conditions. The lateral line anomalies in gilthead sea bream could be used as an alternative method for distinguishing wild from hatchery-reared fish.

Skeletal abnormalities are a fundamental problem in fish aquaculture, as they decrease the quality of the produced fish by affecting their external morphology, growth and survival (Koumoundouros et al., 1997a, 1997b). The development of saddleback syndrome and of caudal fin deformities in *Dentex dentex* (Linnaeus, 1758) was compared under two rearing methods, extensive and semi-extensive (Koumoundouros et al., 2001c). The results showed that these two rearing methodologies strongly affected the presence of morpho-anatomical abnormalities in *Dentex dentex*. With image analysis, gilthead sea bream (*Sparus aurata* L. 1758) was checked for sketal malformations and meristic counts (Boglione et al., 2001). Correspondence analysis was performed to rank groups of hatchery-reared sea bream according to their skeletal abnormalities. Then, Boglione et al. (2003) used the same analysis method and inspected the skeletal abnormalities of wild and reared juveniles of sharpsnout sea bream (*Diplodus puntazzo*)

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and pandora (*Pagellus erythrinus*). For the first time, Koumoundouros et al. (2002) described the ontogeny and the effects of vertebral kyphosis on the mortality rate of reared sea bass (*Dicentrarchus labrax*). It was concluded that a skeletal deformity had little effect on the final quality of the reared juveniles, but it can significantly affect the mortality rate and thus the productivity of the hatcheries. Sfakianakis et al. (2003) presented the development of a saddleback-like syndrome in reared white seabream *Diplodus sargus* (Linnaeus, 1758) and discussed the probable causative factors. Then, the research group studied the effect of temperature on the developmental plasticity and morpho-anatomical abnormalities in *Pagellus* erythrinus (L. 1758) (Sfakianakis et al., 2004). Through image observation, it was revealed that the role of temperature in developmental plasticity was enhanced by the induction of abnormalities mainly in the area of the caudal fin. After that, Sfakianakis et al. (2006a) tested the effect of water-temperature during the larval phase on European sea bass (Dicentrarchus labrax L. 1758) sensitivity to current-induced lordosis. The results revealed that fish that developed at 20 °C had significantly higher incidence of deformed vertebral centra and arches than those developed at 15 °C (p < 0.05). Meanwhile, the effect of lordosis on body shape of same sea bass fish was analysed by geometric morphometrics and computerized photographs (Sfakianakis et al., 2006b). Morphometric analysis revealed that lordosis angle was a useful measure of the degree of severity, since it significantly affected fish body shape (p < 0.05), which is valuable for quality assessment of reared sea bass. Recently, Bardon et al. (2009) estimated the genetic component of spine deformities in European sea bass. A positive linkage between spine deformities and growth rate was demonstrated. The effects of temperature on the development of

skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758) was examined (Georgakopoulou et al., 2010).

4.2.1.5 Rigor mortis

Rigor is a demanding indicator for quality evaluation in meat. The rigor process characterized by continuous muscle contraction occurs when oxygen delivery in the muscles stopped after slaughter, and then results in lacking of energy. Therefore, detecting and analysing rigor is quite necessary in meat industry. Imaging analysis method as a potential tool for identifying rigor in rainbow trout (*Oncorhyncus mykiss*) fillets has been reported by Stein et al. (2006). A clear inverse relationship (R = -0.43, p < 0.0001) was found between contraction and isometric tension.

4.2.2 Measurement of chemical attribute

Computer vision technique has also been used for fat measurement of fish. Borderías et al. (1999) determined fat of Atlantic salmon ($Salmo\ salar$) muscle using computer vision, but a low correlation coefficient (R = 0.41) was obtained between connective tissue and fat content. In the work of Rønsholdt et al. (2000), no significant (P > 0.05) correlation between meat percentage and fish size was found, and t-tests failed to expose any difference of fat in rainbow trout ($Oncorhynchus\ mykiss$). Differently, a good correlation (R = 0.76) was achieved by Marty-Mahé et al. (2004), who measured fat content from images of brown trout ($Salmo\ trutta\ L$) cutlets. Recently, a much better result (R = 0.84) was found in salmon ($Salmo\ trutta\ L$.) fillets by Stien et al. (2007).

4.2.3 Classification/sorting

Classification based on computer vision has also been investigated, with different classification rates reported in different fish or fish products. As shown in Table 2, 100%, 94% and 86% correct species classification in carp (Cyprinus carpio), St. Peter's fish (Oreochromis sp.) and grey mullet (Mugil cephalus) were achieved by Zion et al. (1999), according to fish images captured by a CCD camera coupled with an image processing algorithm. Then, Zion et al. (2000) discriminated the same three fish species in-vivo using computer vision technique, with the correct identification reached 100%, 91% and 91% for grey mullet, carp and St. Peter's fish, respectively. In another study (Storbeck and Daan, 2001) dealing with six different fish species (sole, plaice, whiting, dab, cod and lemon sole), over 95% correct species classification was obtained. By analysing colour images, only one sample misclassified in salted cod fillets (Kohler et al., 2002). For identification of flatfish from roundfish, 100% accuracy of differentiation was described by White et al. (2006). Based on different surface colour, Zion et al. (2007) sorted three underwater fish species with the correct classification of about 98.9%, 94.2% and 97.7%, respectively. Also in the work of Zion et al. (2008), 90% and 96% correct gender classification of guppy fish (*Poecilia reticulata*) based on shape and colour features were respectively illustrated. Using external geometrical information in the fish images, Misimi et al. (2008) classified Atlantic salmon (Salmo salar) with the correct classification of 90%. An instrumental method of colour calibration and discrimination based on the colorimetric imaging was proposed by Costa et al. (2012b) and was used to non-destructively classify whether gilthead seabream

(*Sparus aurata*) is fresh or not. The innovative and non-destructive approach was proved efficiently and it allowed the automatic assessment of fish freshness.

Table 2 Overview of computer/machine vision for quality evaluation and assessment of fish and fish products

4.3 Hyperspectral imaging

4.3.1 Prediction of chemical and physical attributes

4.3.1.1 Moisture/water

Hyperspectral imaging has been described as an emerging and promising method in the application of quality characteristics assessment (Sun, 2010). In recent applications, Wold et al. (2006) reported a multi-spectral imaging NIR transflectance system in the region of 760-1040 nm for on-line evaluation of moisture in dried salted coalfish (*Bacalao*). The best prediction model was achieved with correlation R² and RMSECV equalled to 0.92 and 0.70%, respectively. Similar results were also obtained by ElMasry and Wold (2008), who applied the same wavelength regions in other six fish species, Atlantic halibut (*Hippoglossus hippoglossua*), catfish (*Icatalurus punctatus*), cod (*Gadus morhua*), mackerel (*Scomber japonicus*), herring (*Clupea harengus*), and saithe (*Pollachius virens*) (Table 3). Moisture distribution was successfully mapped in these two studies, which can be used to differentiate the fish or fish products with different water content levels. Meanwhile, suggestion of using NIR spectral imaging to evaluate other important constitutes such as protein was proposed.

4.3.1.2 Fat

In addition to moisture, fat evaluation using hyperspectral imaging was also performed by ElMasry and Wold (2008) in the same six fish species mentioned above, with the result of correlation R^2 and RMSECV being 0.91 and 2.99%, respectively. Chemical images showed fat distribution in different parts of fish fillets, which enable the fish industry to sort fish according to fat content. Then, Segtnan et al. (2009) used online NIR interactance imaging (760-1040 nm) to analyse the fat distribution in raw and salted salmon (*Salmo salar*) fillets. It was observed that the correlation R and prediction error (RMSECV) for raw fillets were 0.947 and 1.96% and for salted fillets were 0.966 and 1.95%, respectively.

4.3.1.3 Salt, pigments and pH

In another work of Segtnan et al. (2009), results of R = 0.86 and RMSECV = 0.56% was found by NIR interactance imaging (760-1040 nm) alone to predict NaCl contents in salted salmon (Salmo salar) fillets. Moreover, adding fat predictions based on NIR interactance imaging further improved the NaCl prediction performance, yielding R = 0.95 and RMSECV = 0.34% NaCl. Due to the deposition of cartenoid pigments, astaxanthin, in the muscular tissue, a coloration of salmonid fishes happens to comply with the consumers preferences. Recently, Dissing et al. (2011) developed a multispectral imaging to evaluate and characterize the concentration of astaxanthin in rainbow trout (Oncorhynchus mykiss) fillets. The spectral range of 385-970 nm was applied and a PLS model was calibrated to predict astaxanthin content from novel images, giving a good accuracy of RMSEP = 0.27, higher than that of normal colour images analysis

(RMSEP = 0.45). pH is another parameter affecting the quality of fish and fish products. Imaging spectroscopy using 400-1000 nm region has been reported by He et al. (2012) to predict pH values in salmon (*Salmo salar*) fillets. A PLS prediction model was developed with result of R and RMSECV being 0.852 and of 0.050, respectively.

4.3.1.4 Colour

As a freshness index, the characteristic colour is one of the main factors that make the salmon with elite image and high market value (Sigurgisladottira et al., 1997). An attempt of colour determination and visualization was made by Wu et al. (2012d), who applied hyperspectral imaging in long-wave near infrared spectral range (LW-NIR) (897-1753 nm) for rapid and non-invasive measurement of colour distribution in salmon fillet. Successive projections algorithm (SPA) was used to select effective wavelengths. Instead of selecting different sets of effective wavelengths for each colour component respectively, instrumental effective wavelengths (IEW) were identified for the prediction of all three colour components, leading to reducing the number of band-pass filters for designing the multispectral imaging system. Meanwhile, predictive effective wavelengths (PEW) were further chosen from IEW to optimize calibration models. Correlation coefficients of prediction models for L*, a*, and b* (0.876, 0.744, and 0.803) as well as obtained colour distribution map revealed that hyperspectral imaging is a potential technique to quantitative colour analysis of salmon fillet.

4.3.1.5 Texture

Texture is an important parameter that determines the quality perception and acceptance of salmon products. Soft fish meat always leads to the consumption reduction and unacceptability by consumers. Wu et al. (2012e) investigated the potential of hyperspectral imaging (400-1000 nm) coupled with PLS analysis to non-destructively evaluate the texture of salmon fillets. In this research, three texture profile analysis (TPA) parameters of hardness, cohesiveness, and adhesiveness were measured, with correlation coefficients (R) of 0.665, 0.555 and 0.606 and RMSECV of 4.09, 0.067 and 0.504 for these three parameters, respectively, which proved the potential of hyperspectral imaging for texture evaluation in fish fillets.

4.3.2 Parasites detection

Parasites, known as nematodes or roundworms survived in whitefish such as cod (*Gadus morhua*), have been viewed as one of high priority defects and reported in several studies. Nematodes infection occurred in fish and fish products will result in quality degrading and consumer rejection, thus reducing fish consumption (Bublitz and Choudhury, 1992; Fishcler, 2002). Hyperspectral imaging offered a great potential for detection of nematodes in fish meat based on several previous studies concerning the optical properties of fish muscle and parasites (Petursson, 1991; Dixon et al., 1993; Stormo et al., 2004). In early 2000s, endeavours on the application of hyperspectral imaging were reported by a few authors. Wold et al. (2001) described how multispectral imaging (400-1100 nm) in combination with soft independent modeling of class analogies (SIMCA) classification was used for automatic detection of parasites in cod fillets. With this technique, parasites at depths down to about 6 mm into the fish muscle can be detected. In another work by Heia et al. (2007), the performance of detecting nematodes

in cod (*Gadus morhua*) fillets by imaging spectroscopy (from 350 to 950 nm) was further confirmed and proved, with parasites embedded into as deep as 8 mm being effectively detected. Then, a transillumination hyperspectral imaging system (400-1000 nm) was developed by Sivertsen et al. (2011) for automatic nematodes inspection of cod (*Gadus morhua*) fillets under industrial conditions. Results revealed that the overall rate of nematodes detection was 58%, with 71% for dark nematodes and 46% for pale ones, respectively. Meanwhile, the over 60% of false alarm rate occurred, which needs further development.

4.3.3 Differentiation and classification

The feasibility of VIS/NIR hyperspectral imaging (380-1030 nm) combined with least squares-support vector machine (LS-SVM) classifiers was investigated to determine whether fish has been frozen—thawed or not (Zhu et al., 2012). In the work, 48 fresh and 60 frozen—thawed (F-T) halibut (*Psetta maxima*) fillets were studied. High average correct classification rate (CCR) of 97.22 % was obtained based on combined spectral and textural variables, which was better than the average CCR of 91.67 % based on spectral variables or textural variables. Sone et al. (2012) tried to use hyperspectral imaging (400-1100 nm) to study spectral changes in fresh salmon (*Salmo salar L.*) fillets stored under different atmospheres (air, 60% $CO_2/40\%$ N_2 and 90% vacuum) and to determine whether hyperspectral imaging can be applied to classify fillets by the type of packaging. Five wavelengths including 606, 636, 665, 705, and 764 nm were considered to be useful and effective for classifying fresh salmon fillets according to the type of packaging used during storage. The conclusion showed that good classification efficiency of (88.3 \pm 4.5%) largely depends on spectral characteristics at the wavelength of 606 and 636 nm.

Table 3 Overview of imaging spectroscopy for quality evaluation and assessment of fish and fish products

5. Discussion and future trends

The results of previous work described in this review confirmed that VIS/NIR spectroscopy, computer vision and hyperspectral imaging are well suited for fish quality evaluation and control. As rapid and non-destructive techniques, VIS/NIR spectroscopy has successfully been used for chemical quality attributes assessment and computer vision makes a great contribution to physical features estimation. However, these two optical techniques still have some limitations for further industrial applications in fish quality evaluation. In specific, no spatial distribution information can be obtained by using VIS/NIR spectroscopy that only provides detailed fingerprints of the intrinsic attributes. On the contrary, computer vision only provides spatial image and is unable to provide spectral information of quality features. In modern society, it is not enough to meet the increasing demands for characterizing samples with detailed spectral information and spatial distribution. With the further development of optical technology, hyperspectral imaging emerged as a novel and non-destructive technique, assembles the spectroscopy and imaging together and provides both spectral and spatial information simultaneously, making up for the existed deficiency of VIS/NIR spectroscopy and computer vision techniques. Hyperspectral images can provide much more detailed information about a sample than a spectrometer or a digital camera, which reflect the advantages of hyperspectral

imaging in quality evaluation of fish and fish products. However, despite the great value of hyperspectral imaging, it is currently suffering from some constraints. Hyperspectral image with substantial amount of redundant information is the main problem that challenges the rapid data processing and screening. With such enormous raw image data produced, hyperspectral systems are currently used off-line in laboratory to select some effective wavelengths for developing multispectral imaging systems. It is expected that future improvements in solutions for more powerful data acquisition and image processing will enable hyperspectral imaging for on-line and real-time fish quality evaluation and characterization.

6. Conclusions

This review summarised applications of spectroscopic and imaging techniques in quality evaluation of fish and its products. As rapid and non-invasive techniques, VIS/NIR spectroscopy, computer vision and hyperspectral imaging have been used for texture analysis, physical and chemical attributes determination as well as microbial examination of fish and fish products. Compared with traditional sensory evaluation and destructive instrument measurements, these three optical techniques have obvious advantages in improving work efficiency and reducing intensity of manual inspection, enabling fish quality evaluation in a rapid and simple way. Especially, spectroscopy is suitable for quality inspection of homogenous samples while computer vision performs well in external features estimation with no need for providing spectral information. Hyperspectral imaging combining spectroscopy and imaging techniques, provides spectral information and spatial distribution of tested samples, leading to detailed analysis of

quality distribution of fish and fish products at pixel-level. In practice, it is critical to apply these non-invasive techniques specifically in their appropriate situations, thereby improving efficiency and accuracy and reducing inspection cost. As important optical inspection and analysis techniques, it is anticipated that spectroscopy, computer vision and hyperspectral imaging techniques may progressively become a routine method for fish process monitoring and for fish safety and quality control.

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Figure captions

- Fig. 1 Mean spectra corresponding to the different meat samples (Andrés et al., 2008).
- Fig. 2 Principle components of a computer vision system (Patel et al., 2012).
- Fig. 3 Hyperspectral image (*hypercube*) for a piece of meat showing the relationship between spectral and spatial dimensions (ElMasry and Sun, 2010).
- Fig. 4 Application of PLS-R to obtain the distribution map of drip loss in pork meat samples by using hyperspectral imaging (Barbin et al., 2012).

Table 1

Overview of VIS/NIR spectroscopy for quality evaluation and assessment of fish and fish products

Property	Fish species	Mode	Wavelength (nm)	Calibration	Performance	Authors
Lipid, protein	Rainbow trout (Salmo gairdneri Richardson), Arctic charr (Salvelinus alpinus L.)	Reflectance	1000-2500		Lipid: R = 0.94, Protein: R = 0.97	Mathias et al., 1987
Fat, protein	Rainbow trout (not specified)	Reflectance	1445-2348	PLS, MLR	Fat: R ² = 0.78, SEP = 1.4; Protein: R ² = 0.76, SEP = 1.8	Valdes et al., 1989
Moisture, lipid, protein	Rainbow trout(Oncorhynchus mykiss)	Reflectance	900-1800	PLS, MLR	PLS: Moisture: R = 0.908, RMSEP = 1.1%; Lipid: R = 0.691, RMSEP = 3.1%; Protein: R = 0.861, RMSEP = 5.4% MLR: Moisture: R = 0.893, RMSEP = 1.5%; Lipid: R = 0.657, RMSEP = 3.5%; Protein: R = 0.921,	Rasco et al., 1991

					RMSEP = 9.3%	
Lipid	Rainbow trout (Oncorhynchus mykiss)	Reflectance	700-1050	PLS, MLR	PLS: R = 0.81, SEPCV = 2.27%; MLR: R = 0.76, SEPCV = 2.48%	Lee et al., 1992
Fat	Atlantic salmon (Salmo salar)	Transmission	850-1050	PLS	R = 0.99, REP = 0.49%	Sollid and Solberg, 1992
Fat, moisture, protein	Atlantic salmon (Salmo salar)	Reflectance	760-1100, 1100-2500, and 760- 2500	PCR	760-1100 nm: Fat: RMSEP = 6.6g/kg; Moisture: RMSEP = 3.8g/kg; Protein: RMSEP = 2.0g/kg 1100-2500 nm: Fat: RMSEP = 7.4g/kg; Moisture: RMSEP = 6.6g/kg; Protein: RMSEP = 3.6g/kg 760-2500 nm: Fat: RMSEP = 3.6g/kg 760-2500 nm: Fat: RMSEP = 6.2g/kg; Protein: RMSEP = 7.3g/kg; Moisture: RMSEP = 3.7g/kg	Isaksson et al., 1995

Fat	Farmed Atlantic salmon (not specified)	Transmittance	850-1048	PLS	R = 0.97, RMSECV = 0.75%	Wold et al., 1996
Moisture, oil	Farmed salmon (not specified)	Reflectance	700-1100	PLS	Calibration for dorsal site: Oil: R^2 = 0.70, SEP = 2.04%; Moisture: R^2 = 0.69, SEP = 1.45% Calibration ventral sites: Oil: R^2 = 0.74, SEP = 2.41%; Moisture: R^2 = 0.77, SEP = 1.90%	Downey, 1996
Moisture, fat	Atlantic salmon (not specified)	Reflectance	800-1100	PLS	Fat: R = 0.87, RMSEP = 1.12%; Moisture: R= 0.86, RMSEP = 0.98%	Wold and Isaksson, 1997
WHC, TVB- N, DMA-N, HCHO	Cod(Gadus morhua)	Reflectance	1000-2222	PLS	WHC: R = 0.84, RMSEP = 5.82%	Bechmann and Jørgensen, 1998
Fat, protein, dry matter	Atlantic salmon (Hippoglossus hippoglossus)	Transmittance	850-1048	PLS	Fat: RMSECV = 2.7g/kg; Protein: RMSECV = 5.2g/kg; Dry matter: RMSECV = 4.2g/kg	Nortvedt et al., 1998
Classification	Atlantic salmon	Reflectance	400-1100	LDA	79% correct	Isaksson

	(Salmo salar)				classification	et al., 2001
Moisture, oil, TVN	Mackerel (Scomber scombrus), Herring (Clupea harengus), Salmon (Salmo salar), Bluewhiting (Micromesistius poutassau), other species (not specified)	Reflectance	1100-2500	Modified partial least squares (MPLS)	Moisture: R ² = 0.99, SEP = 3.86%; Oil: R ² = 0.96, SEP = 8.01%; TVN: R ² = 0.96, SEP = 3.51%	Cozzolino et al., 2002
Moisture, salt	Atlantic Salmon (Salmo salar)	Reflectance	600-1100	BPNN, PLS	BPNN: Salt: R ² = 0.824, RMSEP = 0.55%; Moisture: R ² = 0.946, RMSEP = 2.44% PLS: Salt: R ² = 0.775, RMSEP = 0.63%; Moisture: R ² = 0.936, RMSEP = 2.65%	Huang et al., 2002
Freshness	Cod (Gadus morhua), Salmon (Salmo salar)	Transflection	Cod: 400- 700; Salmon: 700-1100	PLS	Cod: R = 0.97, RMSEP = 1.04d; Salmon: R = 0.98, RMSEP = 1.20d	Nilsen et al., 2002
Freshness	Cod (Gadus morhua)	Reflectance	4500-9996 cm ⁻¹	PLS	R = 0.90, RMSECV = 3.4d	Bøknæs et al., 2002
Fat	Farmed Atlantic salmon (not specified)	Reflectance	800-1100	PLS	R = 0.90, RMSECV = 14g/kg	Solberg et al., 2003

Bruise	Pacific Pink Salmon (Oncorhynchus gorbuscha)	Reflectance	600-1100	PLS	R = 0.83, SEP = 0.05%	Lin et al., 2003
Moisture, salt	Atlantic Salmon (Salmo salar)	Reflectance	600-1100	BPNN, PLS	BPNN: Salt: $R^2 = 0.701$, RMSEP = 1.43%; Moisture: R^2 = 0.784, RMSEP = 2.08% PLS: Salt: R^2 = 0.726, RMSEP = 1.37%; Moisture: R^2 = 0.799, RMSEP = 2.04%	Huang et al., 2003
Salt	King (Oncorhynchus tshawytscha), Chum (O. keta)	Reflectance	600-1100	PLS	King: $R^2 =$ 0.83, SEP = 0.32%; Chum: $R^2 =$ 0.82, SEP = 0.25%	Lin et al., 2003
Water, lipid, protein	Sea bass (Dicentrarchus labrax L.)	Reflectance	1100-2500	PLS	$R^2 = 0.69,$ SEP = 1.45%	Xiccato et al., 2004
Differentiation	Horse mackerel (<i>Trachurus japonicus</i>)	Reflection	1100-2500	PCA and MLR	Fresh and frozen-thawed fish could be separated 100% correctly	Uddin and Okazaki, 2004
Differentiation	Red sea bream (Pagrus major)	Interactance	400-1100	SIMCA and LDA	100% correct classification	Uddin et al., 2005
Freshness	Cod (Gadus morhua)	Transflection	400-700	PLS	R > 0.9, RMSEP < 3.6d	Nilsen and Esaiassen,

						2005
Spoilage	Rainbow trout (Oncorhynchus mykiss)	Reflectance	600-1100	PLS	Fillets (4 °C): R = 0.97, SEP = 0.38 log (CFU)/g (flesh side); R = 0.94, SEP = 0.53 log CFU/g (s kin side); Minced (21 °C):R = 0.82, SEP = 0.82 log CFU/g	Lin et al, 2006
Moisture, protein, fat	Skipjack (Katsuwonus pelamis), Yellow fin (Thunnus albacares)	Reflectance	350-2500	PLS	Moisture: R^2 = 0.98; Protein: R^2 = 0.99; Total fat: R^2 = 0.95 Free fat: R^2 = 0.96	Khodabux et al., 2007
Bruise	Pacific colo salmon (<i>Oncorhynchus kisutch</i>)	Reflectance	600-1100	PCA and SIMCA	Correct rate = 90%	Hammers et al., 2007
Fat, pigment	Farmed Atlantic salmon (Salmo salar L.)	Transmittance	760-1040	PLS	Fat: r = 0.94, RMSEP = 1.0%; Pigment: r = 0.85, RMSEP = 0.9mg/kg	Folkestad et al., 2008
Water, protein	European sea bass (Dicentrarchus labrax)	Reflectance	1100-2500	PLS	Water: $R^2 = 0.964$, SEPCV = 0.67%; Protein: $R^2 = 0.734$, SEPCV = 0.34%	Majolini et al., 2009
Differentiation	European Sea Bass	Reflectance	400-970	PLS	87%	Costa et

	(Dicentrarchus labrax)				correction at 48 h post- mortem; 66.7% correction at 96 h post- mortem	al., 2011
Differentiation	European Sea Bass (Dicentrarchus labrax)	Reflectance	1100-2500	PLS-DA and WPTER	Wild and farmed sea bass can be reliably discriminated	Ottavian et al., 2011

Table 2

Overview of computer/machine vision for quality evaluation and assessment of fish and fish products

Prop erty	Fish species	Data analysis	Performance	Authors
Colo ur	Brown trout (Salmo trutta)	CIELAB space	L* and L* flesh had the best correlation coefficients with lipid rates (R = 0.76, R = 0.75)	Marty-Mahé et al., 2004
	Tuna (not specified)		a* was higher when samples exposed to CO	Balaban et al., 2005
	Trout (Oncorhynchus mykiss W.)	PCA	The correlation of mean L* value with fat percentage was only 0.52	Stien et al., 2006
	Tuna (Thunnus thynnus L.1758)	Just noticeable difference (JND) and Gray level co-occurrence matrix (GLCM)		Mateo et al., 2006
	Gulf sturgeon (Ancipenser oxyrinchus desotoi)	Measuring L*, a* and b* values	Machine vision outperformed colorimeters in estimating subtle colour changes	Oliveira and Balaban, 2006

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	Atlantic salmon (not specified)	Unpaired Student's <i>t</i> -tests	Computer vision is better in assessing the colour scales	Erikson and Misimi, 2008
	Brown trouts (Salmo trutta)	t-test	Correlation coefficients: $L^* = 0.99$, $a^* = 0.98$, $b^* = 0.92$; Errors: $L^* = 2.7\%$, $a^* = 1\%$, $b^* = 1.7\%$	Quevedo et al., 2010
	Atlantic salmon (Salmo salar)		L* was higher when samples stored at -3.6 °C	Erikson et al., 2011
Weig ht	Trout (not specified)	Support vector machine (SVM) regression	Good accuracy and reliability	Odone et al., 1998
	Taiwan tilapia (not specified)	Regression analysis	$R^2 = 0.9303$	Liang and Chiou, 2009
	Rainbow trout (Oncorhynchus mykiss)	Regression analysis	$R^2 = 0.98$	Gümüş et al., 2010
	Alaskan Pollack (Theragra chalcogramma)	Regression analysis	R ² was between 0.985 and 0.993, depending on the regression	Balaban et al., 2010a
	Pink (Oncorhynchus gorbuscha)	Regression analysis	R ² was between 0.928 and 0.983 in different models	Balaban et al., 2010b
	Red (Oncorhynchus nerka)			
	Silver (Oncorhynchus kisutch)			
	Chum (Oncorhynchus keta)			
	Herring (Clupea harengus)	Linear regression	R ² was between 0.919 and 0.938 in different conditions	Mathiassen et al., 2011

Leng	Long Rough Dab (Hippoglossoide s platessoides), Sole (Solea vulgaris), Lemon Sole (Microstomus kitt), Plaice (Pleuronectes platessa), Golden Redfish (Sebastes marinus), Deepwater Redfish (Sebastes mentella), Flounder (Platichthys	CatchMeter system	A standard deviation was 1.2mm	White et al., 2006
	flesus)			

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Blue whiting (Micromesistius poutassou), Capelin (Mallotus villosus), Cod (Arctogadus glacialis), Deepwater redfish (Sebastes mentella), Golden redfish (Sebastes marinus), Haddock (Melanogrammu s aeglefinus), Herring (Clupea Harengus), Long rough dab (Hippoglossoide s platessoides), Norway pout (Trisopterus esmarkii), Saithe (Pollachius virens)	CatchMeter system	Standard deviation of the length measurement was 3 mm	Svellingen et al., 2006
Tuna (not specified)	Proportional relationship between the fish body pixel length and the image reference scale	Estimation error was 4.5 ± 4.4%	Hsieh et al., 2011
European sea bass (<i>Dicentrarchus</i> labrax L.)	Morphometry combined with multivariate techniques	r = 0.9443	Costa et al., 2012a
Atlantic cod (Gadus morhua) Salmon (Salmo salar)	A 2-way analysis of variance (ANOVA)	Automatic monitoring the changes of size and shape effectively	Misimi et al., 2008
Alaska pollock	Dimensions	R ² of 0.987 between the	Balaban et al.,

me	(Theragra chalcogramma)	Method and Cubic Spline Method	dimensional versus measured volume and 0.99 between the cubic splines versus the measured volume	2011
Defe cts	Salmon (Salmo salar L.)	Hierarchical cluster analyses	R = 0.514, P < 0.001	Ashton et al., 2010
Rigor morti s	Rainbow trout (Oncorhyncus mykiss)	Regression analysis	Gaping: R = -0.43, p < 0.0001	Stein et al., 2006
Fat	Atlantic salmon (Salmo salar)	Particle analysis	R = 0.41	Borderías et al., 1999
	Rainbow trout (Oncorhynchus mykiss)	Student's <i>t</i> -test		Rønsholdt et al., 2000
	Brown trout (Salmo trutta L.)	Linear regressions	R = 0.76	Marty-Mahé et al., 2004
	Salmon (Salmo trutta L.)	Linear regressions	R = 0.84	Stien et al., 2007
Class ificat ion/s ortin g	Carp(Cyprinus carpio), St. Peter's fish (Oreochromis sp.), Grey mullet (Mugil cephalus)	Moment- invariants (MI) coupled with geometrical considerations	100%, 94% and 86% correct classification, respectively	Zion et al., 1999
	Carp (Cyprinus carpio), St. Peter's fish (Oreochromis sp.), Grey mullet (Mugil cephalus)	Moment- invariants (MI) coupled with geometrical considerations	100%, 91% and 91% correct classification, respectively	Zion et al., 2000

Sole (Solea solea), Plaice (not specified), Whiting (Merlangius merlangus), Dab (Limanda limanda), Cod (Gadus morhua), Lemon Sole (Microstomus kitt)	Neural network	95% correct classification	Storbeck and Daan, 2001
Cod (not specified)	Soft independent modelling of class analogies (SIMCA)	Only one sample misclassified	Kohler et al., 2002
Long Rough Dab (Hippoglossoide s platessoides), Sole (Solea vulgaris), Lemon Sole (Microstomus kitt), Plaice (Pleuronectes platessa), Golden Redfish (Sebastes marinus), Deepwater Redfish (Sebastes mentella), Flounder (Platichthys flesus)	Canonical discriminant analysis	100% correct differentiation	White et al., 2006

Carp (Cyprinus carpio), St. Peter's fish (Oreochromis sp.), Grey mullet (Mugil cephalus)	Bayes classifier	98.9%, 94.2% and 97.7%, correct classification, respectively	Zion et al., 2007
Atlantic salmon (Salmo salar)	Linear discriminant analysis (LDA)	90% correct classification	Misimi et al., 2008
Guppy (Poecilia reticulata)	Bayes classifier	90% and 96% correct classification using shape and colour features, respectively	Zion et al., 2008

Table 3

Overview of imaging spectroscopy for quality evaluation and assessment of fish and fish products

Property	Fish species	Mode	Wavelength (nm)	Calibration	Performance	Authors
Moisture	Coalfish (Bacalao)	Transflectance	760-1040	PLS	$R^2 = 0.92,$ RMSECV = 0.70%	Wold et al., 2006
Moisture, fat	Halibut (Hippoglossus) hippoglossua), Catfish (Icatalurus) punctatus), Cod (Gadus morhua), Mackerel (Scomber japonicus), Herring (Clupea harengus), Saithe	Interactance	760-1040	PLS	Moisture: R = 0.94, RMSECV = 2.73% Fat: R = 0.91, RMSECV = 2.99%	ElMasry and Wold, 2008

	(Pollachius virens)					
Fat	Salmon (Salmo salar)	Interactance	760-1040	PLS	Raw fillets: R = 0.947, RMSECV = 1.96% Salted fillets:	Segtnan et al., 2009
					R = 0.966, RMSECV = 1.95%	
Salt	Salmon (Salmo salar)	Interactance	760-1040	PLS	R = 0.86, RMSECV = 0.56%	Segtnan et al., 2009
Pigments(astaxanthin)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Reflectance	385-970	PLS	$R^2 = 0.86,$ RMSEP = 0.27	Dissing et al., 2011
рН	Salmon (Salmo salar)	Reflectance	400-1000	PLS	R = 0.852, RMSECV = 0.50%	He et al., 2012
Colour	Salmon (Salmo salar)	Reflectance	897-1753	MLR	R = 0.876, 0.744, and 0.803 for L*, a*, and b*, respectively	Wu et al., 2012
Texture	Salmon (Salmo salar)	Reflectance	400-1000	PLS	Hardness: R = 0.665, RMSECV = 4.09%	Wu et al., 2012
					Cohesiveness: R = 0.555, RMSECV = 0.067%	
					Adhesiveness: R = 0.606, RMSECV = 0.504%	
Parasites	Cod(not specified)	Transmission	400-1100		Parasites embedded into 6 mm can be detected	Wold et al., 2001

Nematodes	Cod (Gadus morhua)	Transmission	350-950	Discriminant partial least square (DPLS)	Parasites embedded into 8 mm can be detected	Heia et al., 2007
Nematodes	Cod (Gadus morhua)	Transillumination	400-1000	Fisher Discriminant Ratio (FDR)	Overall detection rate = 58%; Rate for dark nematodes = 71%; Rate for pale nematodes = 46%	Sivertsen et al., 2011
Differentiation	Halibut (Psetta maxima)	Reflectance	380-1030	Least squares- support vector machine (LS-SVM) classifier	Correct classification rate (CCR) = 97.22%	Zhu et al., 2012
Classification	Salmon (Salmo salar L.)	Interactance	400-1100	PLS	Classification efficiency = (88.3 ± 4.5%)	Sone et al., 2012

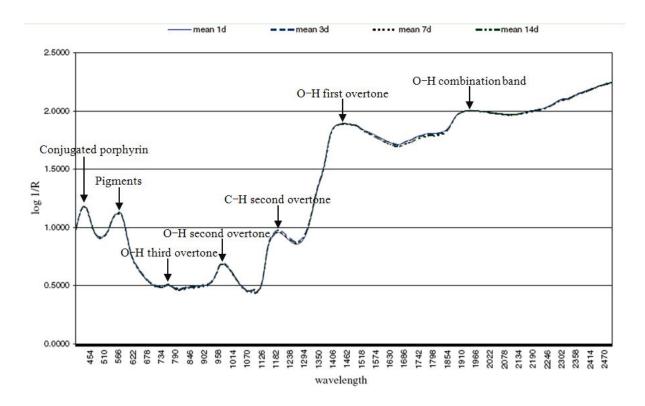
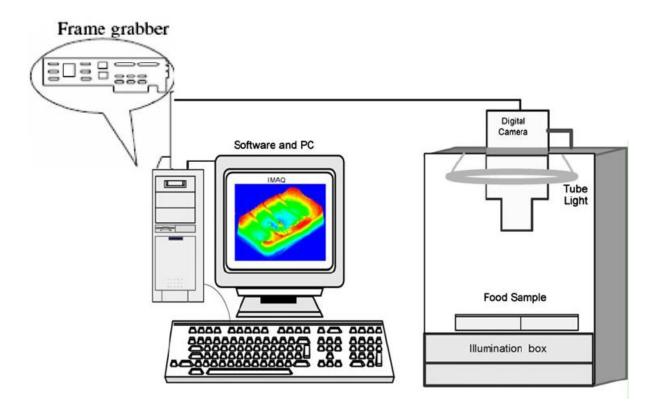


Fig. 1



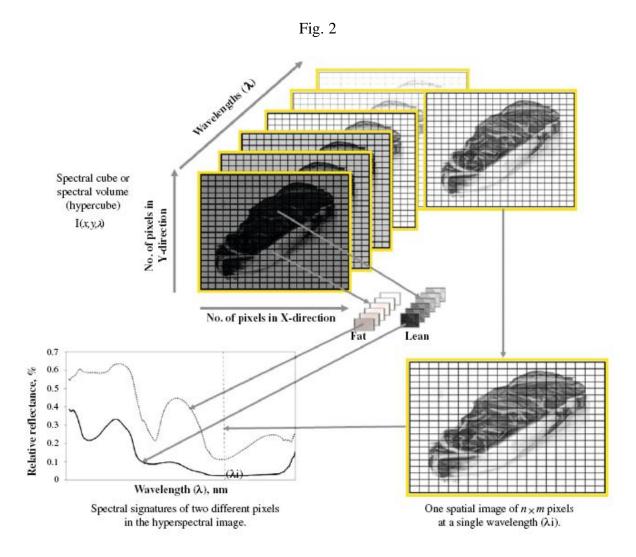


Fig. 3

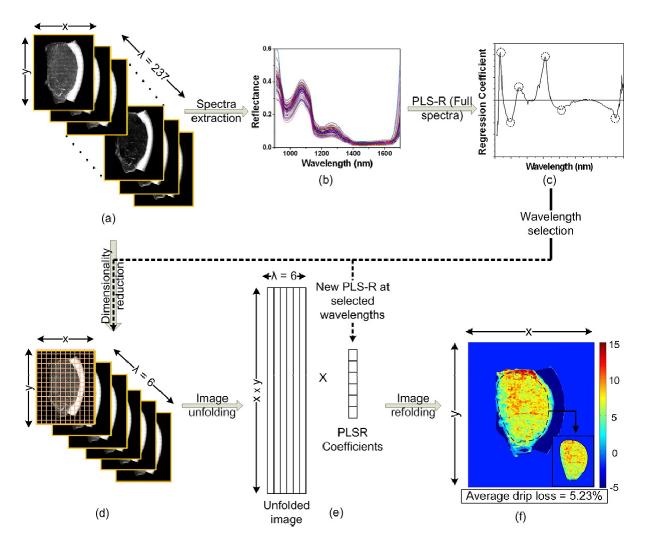


Fig. 4