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Marbling Analysis for Evaluating Meat Quality: Methods and Techniques

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Abstract: Marbling is one of the most important traits determining the quality of meat, and the richness of marbling is often considered by consumers as the primary factor when buying meat in view of its contribution to sensory characteristics of meat. In the market, there has been a constant demand for meat products with an assured degree of marbling. Conventionally, marbling of meat is assessed by visual appraisal or chemical analysis, which has the disadvantages of being subjective and time-consuming. In order to improve the detection accuracy and production efficiency, a variety of modern instrumental techniques, including spectroscopic techniques, imaging techniques, and hyperspectral imaging, have been developed for marbling analysis. This paper gives a comprehensive overview on the recent advances in marbling analysis. What's more, the advantages, limitations and some perspectives on the future trends of these techniques are also presented.

Keywords: bioelectrical impedance spectroscopy, computer image analysis, hyperspectral imaging, intramuscular fat, marbling, near-infrared reflectance spectroscopy, nuclear magnetic resonance spectroscopy, ultrasonic imaging, X-ray computed tomography

Introduction

Meat is a very important part of the human diet contributing valuable nutrients that are beneficial to health. However meat is highly perishable, therefore on one hand, techniques such as drying (Delgado and Sun 2002; Cui and others 2008) and refrigeration (Sun and Eames 1996; Wang and Sun 2002, 2004; Zheng and Sun 2004; Kiani and Sun 2011) should be employed to keep their qualities, on the other hand, effective and efficient methods should be developed to evaluate and classify their qualities. Among many quality attributes, marbling, which is defined as the amount and spatial distribution of visible white flecks of fat present within the lean in the *longissimus dorsi* (LD) muscle, is one of the most important traits determining the quality of meat (ElMasry and others 2012a). It has been generally accepted that an appropriate degree of marbling has a favorable effect on the juiciness, tenderness, palatability, and flavor of meat (McBee and Wiles 1967; Savell and others 1987; Wheeler and others 1994; Thompson 2004). Also, marbling is often considered as an important characteristic that directly affects a consumer's consumption decisions. In most developed countries, the degree of marbling is the main assessment index for grading the quality of meat and there is often a very high correlation between marbling score and price.

However, the marbling degree of meat is largely variable and is affected by many factors, such as breed, sex, diet, age, and weight at slaughter (Correa and others 2006; Olivares and others 2009; Bosch and others 2012), and the optimal marbling degree of meat is dependent on the country, the consumer, and the technological process (Ngapo and others 2007; Font-i-Furnols and others 2012). Therefore, in order to meet a consumer's various demands of marbling degree, many countries have developed systematic methods and standards for evaluating marbling degree of meat (Table 1).

So far, visual appraisal is still the most preferred method used by the meat industry for the evaluation of marbling degree. In addition, as another conventional method, chemical analysis has also been widely used as a standard method for the determination of marbling degree. However, these 2 methods have disadvantages of being subjective and time-consuming (Arneth 1998; Ferguson 2004). What's more, in order to overcome these disadvantages and realize rapid online grading of marbling degree, several instrumental techniques (mainly spectroscopic and imaging techniques) have been developed, such as near-infrared reflectance (NIR) spectroscopy (Barlocco and others 2006; Zamora-Rojas and others 2012; Su and others 2014), bioelectrical impedance (BI) spectroscopy (Marchello and others 1999; Altmann and Pliquett 2006), nuclear magnetic resonance (NMR) spectroscopy (Corrêa and others 2009; Pereira and others 2013), computer image analysis (CIA) (Du and others 2008; Jackman and others 2009; Pang and others 2014), ultrasonic imaging (UI) (Fukuda and others 2012; Lakshmanan and others 2012), X-ray computed tomography (CT) (Frisullo and others 2010; Font-i-Furnols and others 2013; Clelland and others 2014), and hyperspectral imaging (HSI) (Qiao and others 2007; Huang and others 2013, 2014a; Liu and Ngadi 2014). However, up to now, no comprehensive review is available on the methods and techniques for marbling analysis. Only Ferguson (2004) reviewed some online applications of

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Table 1–Methods and standards of some countries for marbling evaluation.

Meat species	Country	Method	Position	Total number of grades	Grades (from high to low)	Drafted institutions	Reference
Beef	U.S.A.	Visual appraisal	12th to 13th rib	10	very abundant, abundant, moderately abundant, slightly abundant, moderate, modest, small, slight, traces, and practically devoid	United States Dept. of Agriculture (USDA)	USDA (1997)
Beef	Japan	Visual appraisal	6th to 7th rib	12	BMS No. 12, BMS No. 11, BMS No. 10, BMS No. 9, BMS No. 8, BMS No. 7, BMS No. 6, BMS No. 5, BMS No. 4, BMS No. 3, BMS No. 2, BMS No. 1	Japan Meat Grading Assoc. (JMGA)	JMGA (1988)
Beef	Australia	Visual appraisal	5th to 13th rib	9	BMS No. 9, BMS No. 8, BMS No. 7, BMS No. 6, BMS No. 5, BMS No. 4, BMS No. 3, BMS No. 2, BMS No. 1	AUS-MEAT Limited	AUS-MEAT (2005)
Pork	U.S.A.	Visual appraisal	10th rib	7	Grade 10.0, Grade 6.0, Grade 5.0, Grade 4.0, Grade 3.0, Grade 2.0, Grade 1.0	Natl. Pork Producers Council (NPPC)	NPPC (1999)
Mutton	U.S.A.	Visual appraisal	Flank muscles	10	Abundant, moderately abundant, slightly abundant, moderate, modest, small, slight, traces, practically devoid, devoid	United States Dept. of Agriculture (USDA)	USDA (1992)

instrumental measurement techniques. In order to provide more information about recent advances of current methods and techniques, this review offers a detailed overview of the traditional and innovative methods and techniques for the analysis of meat marbling. The advances of current techniques are updated, and some new techniques for the analysis of meat marbling such as NMR spectroscopy, X-ray CT, and HSI are also presented.

Conventional Methods

As indicated before, marbling is a critical quality attribute of meat, and the meat industry demand effective and accurate evaluation methods. Two conventional industrial methods are discussed below.

Visual appraisal

As a conventional and useful tool, visual appraisal has been used by the meat industry worldwide for marbling evaluation for decades, and it continues to be the most widely used method. For example, the beef grading systems currently in use, including those used in the United States, Japan, and Australia, are based on human graders' visual appraisal. In the past decades, many countries have also formulated their own standard methods for visual appraisal of beef marbling, of which the methods formulated by the United States and Japan are representative ones. In the United States, beef marbling scores are assigned to the LD muscle between the 12th and 13th rib by highly trained graders from the United States Dept. of Agriculture (USDA) into 7 grades (Table 1) according to standard photos demonstrating marbling abundance and distribution of each grade (USDA 1997). Three higher degrees (moderately abundant, abundant, and very abundant) are also recognized for carcass evaluation programs and other purposes, since marbling degree is also closely related to the quality grade of a carcass. Figure 1 shows the relationship between beef marbling degree and carcass quality grade. In Japan, visual appraisal of beef quality is performed by the Japan Meat Grading Assoc. (JMGA), and beef marbling grades are classified into 12 categories (Table 1) according to the abundance degree of marbling in the LD muscle between the 6th and 7th rib, of which the distribution of marbling is more abundant than that of the 12th and 13th rib, based on beef marbling standard images (JMGA 1988).

In the pork and lamb industry, the determination of marbling degree also relies on a visual comparison between meat and meat marbling standard pictures by experienced graders. For example, in the United States, pork marbling is classified by professional inspectors into 7 grades (Table 1) according to the marbling standards of the Natl. Pork Producers Council (NPPC), which depict intramuscular fat (IMF) content and distribution from low to high with marbling scores from 1.0 (devoid) to 10.0 (abundant) (NPPC 1999), and the degrees of flank fat streakings are categorized into 10 grades (Table 1) from abundant to devoid in descending order of quantity (USDA 1992).

Although visual appraisal as a conventional method has been used by the industry for long, it has many major drawbacks. On one hand, since the structure of marbling is complicated and there are no clear boundaries between each grade, the processes of human observation, induction, and judgment are needed for every assignment which is laborious and tedious. On the other hand, due to the subjective nature of visual appraisal, although the inspectors are professionally trained, inconsistency between inspectors and variations often occurs (Cross and others 1983).

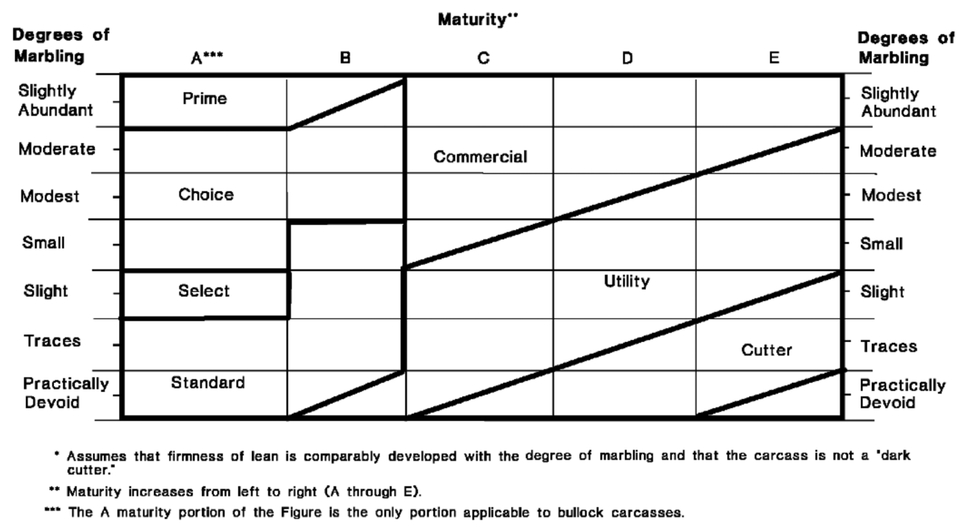

Figure 1–Relationship between marbling, maturity, and carcass quality grade (USDA 1997).

Chemical analysis

Another conventional method for the evaluation of marbling is chemical analysis, of which marbling degree is expressed in IMF content. IMF refers to the fat deposits within a muscle between muscle fibers and muscle fiber bundles. Although the chemically extracted fat content is not exactly the same as the marbling score determined by visual appraisal due to the fact that some invisible fat deposits that cannot be seen by visual appraisal can also be determined by chemical analysis, the relationship between them has been reported as linear (Savell and others 1986; Dow and others 2011). Therefore, sometimes the marbling degree of meat is evaluated by the IMF content.

The principle of chemical analysis is based on the extraction of fat by adding a pure or mixed solvent (hydrophilic or hydrophobic, and acidic, neutral or basic) to a dried meat sample. After extraction for a certain time under a specific condition, soluble fat is extracted into the solvent and insoluble substances can be separated by gravity or filtration. In chemistry, fats are defined as triglycerides that are made up of glycerol and fatty acids. Although pure fat can be dissolved by a wide range of organic solvents, the ideal pure or mixed solvent for extracting fat should be polar enough to remove fat from cell membranes and tissue constituents, but also not so polar that other nonpolar lipids cannot be readily dissolved by the solvent (Segura and Lopez-Bote 2014). The most commonly used solvents for IMF extraction is ether (Savell and others 1986; AOAC 1995) and a mixture of chloroform/methanol (Folch and others 1957).

Although chemical analysis as a standard method for marbling analysis has been used for many years, it has disadvantages in that chemical analysis is destructive, expensive, and time-consuming. Taking the ether extraction method, for instance, each meat sample must first be dried in an oven for at least 24 h and then extracted by petroleum ether in a Soxhlet extractor for another 8 h (Du and others 2008). What's more, this method also cannot reflect the visual appearance of samples, and could not correlate with what the consumers perceive which is valued for marbling evaluation.

Instrumental Techniques

As discussed above, conventional methods for the evaluation of meat marbling have various disadvantages and, in recent years, there has been a constant demand for more objective and efficient marbling grading techniques, resulting from the demand from producers intending to improve production efficiency, as well as from consumers for products of assured quality. Therefore, numerous studies on the nondestructive and objective evaluation of marbling by instrumental methods have emerged and some of them are discussed below.

Spectroscopic techniques

With the development of chemometrics, many spectroscopic techniques including NIR spectroscopy, BI spectroscopy, and NMR spectroscopy have been exploited for marbling analysis. Table 2 summarizes relevant representative studies in marbling analysis by spectroscopic techniques.

Near-infrared spectroscopy. NIR spectroscopy is a nondestructive and rapid technique on the basis of the principle that different chemical bonds absorb or emit different wavelengths of light when the sample is irradiated by continuous changing frequency of near-infrared light, and the absorption intensity is related to the content of the chemical substances (Prevolnik and others 2004; Cen and He 2007). With the development of chemometrics and computer technique in the 1970s, NIR spectroscopy has been extensively studied as a nondestructive tool for the evaluation of food quality (Liu and others 2011; Wu and others 2012; Bao and others 2014; Ignat and others 2014; Ye and others 2014). The suitability of NIR to predict IMF content is owing to the absorption of light by the C–H bonds of fatty acids in the wavelengths of 1100 to 1400, 1700, and 2200 to 2400 nm (Williams and Norris 1987). Since fat is the main component of marbling, it is promising to apply NIR spectroscopy to evaluate marbling degree. By constructing calibration models between NIR spectra and IMF content, NIR spectroscopy can offer a prediction of IMF content (Prieto and others 2006; Prieto and others 2009).

In the past several years, studies have been done for predicting IMF content by NIR spectroscopy (Chan and others 2002; Cozzolino and Murray 2002; Prevolnik and others 2005; Barlocco and others 2006; Viljoen and others 2007; Su and others 2014), and some encouraging results were obtained. However, among these studies, the majority of good results were obtained for minced and homogenized meat samples. For instance, NIR spectroscopy was

Table 2–Spectroscopic techniques in evaluation of marbling degree.

Meat species	Technique	Method	Accuracy	Reference
Minced pork	NIR spectroscopy	Modified PLSR	R^2_{CV} (0.93 to 0.99), RMSECV (0.14% to 0.39%)	Maja Prevolnik and others (2005)
Minced beef	NIR spectroscopy	Modified PLSR	R^2_{CV} (0.93 to 0.98), RMSECV (0.26% to 0.44%)	Maja Prevolnik and others (2005)
Homogenized pork	NIR spectroscopy	PLSR	R^2_{C} (0.87), RMSECV (1.8 g/kg)	Barlocco and others (2006)
Homogenized beef	NIR spectroscopy	MSC + PLSR	R^2_{CV} (0.924), RMSECV (16.22 g/kg)	Prieto and others (2006)
Minced mutton	NIR spectroscopy	PLSR	r (1.0), RMSEP (0.43)	Viljoen and others (2007)
Minced beef	NIR spectroscopy	MC + 1D + PLSR	R^2_{P} (0.998), RMSEP (0.986)	Su and others (2014)
Ground beef	BI spectroscopy	MLR	R^2_{P} (0.84 to 0.95), RMSEP (2.96 to 5.60)	Marchello and others (1999)
Ground pork	BI spectroscopy	MLR	R^2_{P} (0.87 to 0.96), RMSEP (2.75 to 4.95)	Marchello and others (1999)
Cylindrical beef slice	NMR spectroscopy	Linear regression	r (0.90)	Corrêa and others (2009)
Cylindrical beef slice	NMR spectroscopy	PLSR	r (0.99)	Pereira and others (2013)

applied to predict the IMF content of minced pork and beef, as well as intact pork, by Prevolnik and others (2005) who developed a model 6500 NIR system for the nondestructive determination of IMF content. Modified partial least-square regression (PLSR) prediction models, established with both NIR spectrum (1100 to 2500 nm) and whole spectrum (400 to 2500 nm) showed remarkable results for predicting IMF content in minced pork and beef with coefficient of determination in cross-validation (R^2_{CV}) between 0.93 and 0.99, and root mean square error in cross-validation (RMSECV) between 0.14% and 0.44%. However, the calibration models were less accurate for intact samples than for minced ones. Similar results were found by Barlocco and others (2006) who also employed a NIR 6500 system to predict the IMF content of intact and homogenized pork meat. In this study (Barlocco and others 2006), PLSR calibration models established with homogenized samples showed a fine result for the prediction of IMF content with coefficient of determination in calibration (R^2_{C}) of 0.87 and RMSECV of 1.8 g/kg. However, a poor calibration model was developed for the prediction of IMF content in intact pork with R^2_{C} of 0.30 and RMSECV of 4.0 g/kg. In addition to beef and pork, the study by Viljoen and others (2007) also showed better prediction ability of fat content in minced freeze-dried mutton than in raw meat, resulting from the more homogenous features of the former sample. The poor ability of NIR spectroscopy for predicting IMF content in intact meat was suggested to be due to the fact that the NIR spectral data were from only a 50-mm diameter view of the sample in each measurement. With fat more uniformly distributed in minced or homogenized samples, it was more probably that a single scan of minced or homogenized samples would detect the actual IMF content compared to intact samples (Prevolnik and others 2005; Prieto and others 2009). Although studies have been conducted to solve this problem by increasing the number of scans (Prevolnik and others 2005; Ripoll and others 2008) or using a larger sampling area (Hoving-Bolink and others 2005), the results were not satisfactory.

In addition to the sample preparation methods, the variability of samples scanned also have a significant effect on the prediction accuracy. For example, Su and others (2014) recently improved the ability of NIR spectroscopy in predicting the fat content of minced beef by expanding the variability of samples scanned. As a result, the PLSR model in predicting IMF content established with spectral data collected from 4 different breeds achieved a coefficient of determination in prediction (R^2_{P}) of 0.998 and root mean square error in prediction (RMSEP) of 0.986. Moreover, recently, handheld NIR spectroscopy instruments have also been developed for the objective grading of marbling. In the study by Zamora-Rojas and others (2012), a new miniaturized spectrometer that combined NIR spectroscopy technology with microelectromechanical platforms was developed. This instrument enabled the transfer of sample spectra from large databases to new devices, which allowed for the fast and low-cost analysis of meat products.

Bioelectrical impedance spectroscopy. The inspection of IMF content by BI spectroscopy is based on the difference of electronic conductivity between fat and lean muscle. Lean muscle is a far better conductor of current than fat. By introducing a small alternating electrical current into the meat products of different IMF contents, the electrical impedance spectroscopy obtained is expected to be different from each other (Shirsat and others 2004).

Marchello and others (1999) first applied BI spectroscopy to determine the fat content in both ground and trimmed beef and pork. In this study (Marchello and others 1999), prediction

Table 3–Imaging techniques in evaluation of marbling degree.

Meat species	Technique	Method	Accuracy/efficiency	Reference
Beef	CIA	ANN	Agree well with grades assigned by graders	Shiranita and others (2000)
Beef	CIA	DTSM	High-quality grading of marbling (100%)	Yoshikawa and others (2000)
Beef	CIA	FCM + downsampling	Segmentation error (1.97%), average error pixel distance (4.4 pixels), similar to imprecision of reference method	Subbiah and others (2004)
Beef	CIA	FCM + thresholding	r (0.96)	Jackman and others (2009)
Live pigs	UI	Ultrasound echo signals, MLR	R^2_P (0.76), RMSEP (0.34%)	Lakshmanan and others (2012)
Live cattle	Dynamic UI	ANN + texture analysis	r (0.75)	Fukuda and others (2012)
Beef	X-ray μCT	Fat structure parameters, regression	r (0.92 to 0.99)	Frisullo and others (2010)
Pig loins	X-ray CT	Relative volumes, OLR	R^2_P (0.83), RMSECV (0.46%)	Font-i-Furnols and others (2013)
Live lamb	X-ray CT	Fat and muscle density values, linear regression	R^2_P (>0.65)	Clelland and others (2014)

equations established with bioelectrical resistance, temperature, and weight of product showed better performance for the determination of ground beef and pork (ground through a 0.95-cm plate) with R^2_P of 0.84 and 0.87, respectively, than those for the trimmed ones. In addition, when beef and pork were ground through a smaller plate (0.32 cm), the prediction ability of the equations was even better with R^2_P of 0.95 and 0.96, respectively. On the other hand, in order to improve the measurement speed and accuracy of intact beef and pork, Altmann and Pliquett (2006) developed a computer-controlled device, which could measure the impedance when the probe passed through muscle. In this study (Altmann and Pliquett 2006), regression parameters with high correlations to the variability of the impedance were selected for predicting IMF contents in beef and pork. However, the results obtained by the regression model agreed poorly with the reference method with the correlation coefficient (r) only ranging from 0.28 to 0.69.

The above studies indicate that BI spectroscopy is suitable for the prediction of fat content in ground meat products. However, for inspecting IMF in intact meat, the prediction accuracy needs to be further improved.

Nuclear magnetic resonance spectroscopy. NMR spectroscopy is an important analytical technique that can be used in food science (Erikson and others 2012; Cheng and others 2013; Zhang and others 2013; Wu and others 2014; Rondeau-Mouro and others 2015). The suitability of NMR spectroscopy in determining IMF content is based on the fat–water chemical shift in the NMR spectrum. However, traditional NMR spectrum taken with fast inversion-recovery (FIR) or Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence is complex and costly, and thus it is not suitable for industrial applications. Recently, NMR spectra taken by the continuous wave-free precession (CWFP) method, which is a simpler pulse sequence than FIR and CPMG, were introduced for the nondestructive and fast determination of IMF content in meat (Corrêa and others 2009; Pereira and others 2013). For instance, a simple high-throughput low-resolution NMR (LR-NMR) CWFP method was introduced by Corrêa and others (2009) to measure IMF content in beef, and a higher correlation coefficient (r) of 0.9 was found. A similar result was found by Pereira and others (2013) who determined the fat content in beef samples by time-domain NMR (TD-NMR) relaxometry using both CPMG and CWFP sequences. A higher correlation coefficient of 0.99 was obtained by the PLSR model established between CWFP data and reference results.

As discussed above, NMR spectroscopy is a promising technique for the determination of IMF contents, however, before being applied at an industrial scale, more studies are needed for confirming the robustness of the system, and the cost of the instrument must be reduced.

Imaging techniques

With the development of image processing technology, many imaging techniques, including CIA, UI, and X-ray computed tomography (CT), have been developed for marbling analysis. Table 3 summarizes relevant representative studies in marbling analysis by imaging techniques.

Computer image analysis. Computer vision is a science about how to make computers "see" by installing eyes (camera) and brain (algorithm) to it, and it is able to provide superior spatial information of detected samples (Wang and Sun 2002; Brosnan and Sun 2004; Sun 2004; Jackman and others 2009; Quevedo and others 2010; Costa and others 2011; Vélez-Rivera and others 2014). Marbling assessment by computer vision began with the work by Chen and others (1989) who quantified the area ratio of marbling in the LD surface by image processing. Since then, computer vision has been extensively studied and considered as the most potential technique for the objective grading of beef marbling due to its best match to human eyes and a combination of nondestructiveness, speediness, and simplicity in sample preparation (Yang and others 2006; Jackman and others 2009). When employing computer vision technology to automatically evaluate marbling scores, the image processing process usually involves the operations of segmentation of the LD muscle from the steak image, segmentation of marbling from lean LD muscle, and extraction of marbling features (Figure 2). Among these operations, segmentation of the LD muscle from the steak image is necessary because graders assign marbling grades mainly based on visual appraisal of the LD muscle, and therefore it is the primary step in developing a successful marbling evaluation system by computer vision, which can be realized by thresholding, region growth, and morphological operations (Subbiah and others 2004; Chen and others 2010; Pang and others 2014). Accurate segmentation of marbling from lean LD muscle and effective marbling feature extraction has been the research hotspot in the past 20 y.

Segmentation of marbling from lean LD muscle. Segmentation of marbling from lean LD muscle is the most important step for marbling evaluation by computer vision. It serves as the basis for the following feature extraction operations, and small errors in segmentation can have a big influence on the marbling score (Subbiah and others 2004; Chen and others 2008; Pang and others 2014). In the past decades, numerous segmentation methods for segmenting marbling from lean LD muscle have been reported. For instance, McDonald and Chen (1990, 1991, 1992) were the

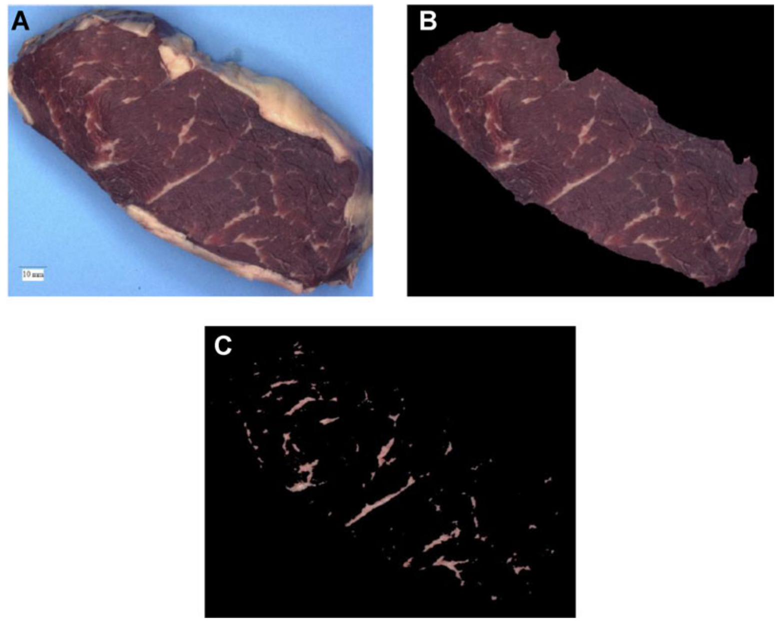

Figure 2–Steps of computer image analysis for evaluating beef marbling: (A) original image; (B) LD muscle segmented from the steak image; (C) marbling features extracted from the lean LD muscle (Du and others 2008).

first who segmented marbling from lean LD muscle. Based on reflectance characteristics, they separated fat from lean muscle and generated binary muscle images. Shiranita and others (2000) applied artificial neural network (ANN) techniques to discriminate between fat and lean muscle based on a grey-level histogram of fat and lean. Shiranita and others (1998) considered the marbling of meat as a texture pattern and proposed a method for the prediction of beef marbling using the grey-level cooccurrence matrix (GLCM). In this study (Shiranita and others 1998), standard texture-feature vectors of each grade were first collected, and then the grade of the unevaluated image was judged by comparing the texture-feature vector with the standard texture-feature vectors. In order to improve the robustness of the online beef grading support system, Yoshikawa and others (2000) employed the method of nonparametric discriminant threshold selection method (DTSM) proposed by Otsu (1975) for region segmentation. This method could dynamically determine the optimal threshold of a beef LD image from the gray-level histogram independent of the distribution.

Most of the above studies were performed based on thresholding, which is a fast and simple method in image segmentation. However, this segmentation method can be significantly affected by the shape of the grey-level histogram. When the histogram of the image is a single peak or peak-to-valley, characteristics are unclear and the optimal threshold cannot be converged, which could lead to low segmentation accuracy or even segmentation failure (Pang and others 2014). Relatively good segmentation results have been obtained by the clustering method, which can realize the segmentation of fat flecks from lean muscle regardless of the image histogram (Subbiah and others 2004; Du and others 2008; Pang and others 2014).

However, clustering analysis is an iteration process that has the disadvantages of being time-consuming and of low efficiency compared to the method based on thresholding (Subbiah and others 2004). In order to reduce computation time, some efforts have been made to reduce the dimensionality of the original image. For instance, Subbiah and others (2004) reduced the image size by downsampling and Pang and others (2014) reduced the dimensionality of the original image by resampling. In another study, Jackman and others (2009) proposed a new segmentation method, which incorporated the benefits of both clustering and thresholding, in which the first estimate of the LD muscle was obtained by clustering and then a universal threshold enhanced by a customized grayscale was found. This segmentation method showed good ability to evaluate marbling degree.

Marbling feature extraction. Marbling feature extraction is another important step for evaluating marbling degree by computer vision. In the earlier studies, for the selection of features representing the richness of marbling, McDonald and others (1991) attempted to evaluate the marbling score of beef in the LD muscle based on the visible total fat area alone. However, an $R^2{}_P$ of only 0.47 was found between total fat area and sensory scores, indicating that predicting marbling score based on total fat area alone is unreliable. This is because marbling score is not only affected by the total fat area of fat flecks, but also by the distribution of fat.

In order to improve the prediction accuracy, the effects of fleck size and distribution were taken into consideration by later studies (Gerrard and others 1996; Tan 2004; Du and others 2008). Among these studies, various factors affecting the evaluation of marbling scores were investigated, and area densities and count densities of marbling have been generally proved important for the evaluation of marbling scores. For instance, an earlier classic study was performed by Gerrard and others (1996) who introduced color images for marbling analysis in which marbling particles were classified into 3 categories according to their area. Both count and area marbling features were then used to measure the richness degree of marbling. After regression analyses of sensory scores and image features, 5 image features including mean red color components (μ_R), count densities of marbling for size category A_1 (D_{c1}), area densities of marbling for size category A_1 and A_3 (D_{a1}, D_{a3}), and global area densities of marbling (D_a) were proved to be significant for the evaluation of marbling scores. Also, a relatively good result with R^2_P of 0.84 was obtained for marbling score prediction (Gerrard and others 1996). In another study, 5 features (the number of marblings, percentage of marblings in the binarized region, the number of large marblings of fat, the number of small marblings of fat, the amount of scatter of the distribution of marblings in the binarized region) presented to graders were evaluated by Shiranita and others (2000) using multiple regression analysis to identify which features were most correlated to the marbling score given by the graders. And then a multiple regression equation based on 3 features identified (percentage of marblings in the binarized region, the number of large marblings of fat, and the amount of scatter of the distribution of marblings in the binarized region) was formulated for the determination of the marbling grade. It was shown that results obtained using the proposed method were well consistent with the judgment of the graders. Similar results were also confirmed by Du and others (2008). They proved that 5 IMF characteristics including area densities of middle and large fat particles, count densities of middle and large fat particles, and total fat area per unit LD area were significantly correlated with reference IMF contents obtained by chemical analysis, with the highest coefficient of 0.852 being obtained for large fat particles.

In summary, computer vision has been extensively studied in the past decades for the evaluation of marbling in beef muscles and some good results have been obtained. However, compared to beef, marbling in pork is much more variable and provides a low color contrast against the lean muscle, which make its assessment more difficult (Faucitano and others 2005). Therefore, the employment of computer vision to assess marbling characteristics of pork has been unsatisfactory and was only limited to a couple of preliminary studies (Scholz and others 1995; Faucitano and others 2005). Nevertheless, computer vision is a useful technique for the nondestructive and objective evaluation of beef marbling. Although some computer vision systems have been developed for the commercial evaluation of beef marbling, there are still some problems to be settled before such systems can be widely used, including the improvement of the robustness of the system and development of adaptable real-time inspection equipment. The core of these problems is image segmentation. Currently, there is still no general image segmentation method available for evaluating beef marbling by computer vision, regardless of the biological variations in the meat products, and improvement on the current algorithm and combination of 2 or more traditional segmentation methods are future development directions.

Ultrasonic imaging. Another technique that can be used for evaluating marbling quality attributes is UI. However, since the prediction accuracy of this technique is relatively lower than other imaging techniques, such as computer vision, most of the studies were for live animals which is of significant benefit for genetic studies of breeding stock and for sorting of feedlot cattle (Mörlein and others 2005; MacNeil and others 2010). The basic principle of ultrasound is based on the measurement of the echo reflected from the fat/muscle interface (Houghton and Turlington 1992). When the ultrasonic wave propagates in the tissue of the animal body, energy is reflected back to the transducer from the interface of tissues with different densities. According to the intensity and distribution of the echo from the image that displays each echo returns to the transducer, appropriate measurements of the marbling can be made (Houghton and Turlington 1992). With the development of image processing technology, ultrasound images of live animals have been analyzed in the past few years (Brethour 1994; Kim and others 1998; Newcom and others 2002; MacNeil and others 2010; Nade and others 2014). For example, Brethour (1994) pioneered the analysis of ultrasonic images, combined with ANN procedures, and showed that the error of this method was not higher than the human error in judging marbling scores, indicating the feasibility of ANN procedures to make unsupervised assessments of marbling in live cattle. In another study, Kim and others (1998) applied wavelet-transform (WT) and GLCM-based texture analysis techniques for evaluating intramuscular fat percentage (IMFAT) of beef cattle from ultrasound images, and a RMSEP of 1.44 for predicting IMFAT was obtained by the regression model established using WT features.

On the other hand, the spectra of the backscattered ultrasound signals proved to contain richer information about tissue composition in comparison with conventional images (Mörlein and others 2005; Lakshmanan and others 2012). For instance, Mörlein and others (2005) investigated the use of spectral analysis of ultrasound echo signals for predicting IMF content in porcine LD muscle, and IMF content was predicted by a PLSR model with an R^2_P of 0.58 and a RMSEP of 0.36% IMF. Lakshmanan and others (2012) used a modified commercial hand-held ultrasound device to obtain the images, and based on preprocessing of the ultrasound data, better prediction result was obtained with an R^2_P of 0.76 and RMSEP of 0.34%. Recently, a new method of dynamic ultrasound imaging was proposed by Fukuda and others (2012) for estimating the beef marbling standard (BMS) number of live beef cattle. In this study (Fukuda and others 2012), a neural network model was established using dynamic image features, and an estimation of the BMS number was obtained with $r <$ of 0.75 (P 0.01) by the leave-one-out method.

Results of the above studies indicated that the UI technique is suitable for the assessment of marbling in live animals. This technique is a good supplement to other techniques. With the development of better ultrasonic equipment and data processing techniques, more accurate prediction of marbling by UI can be expected. In addition, the development of integrated systems that combine ultrasound with new or now available techniques may further improve the prediction accuracy.

X-ray computed tomography. X-ray CT is a nondestructive detecting technique that is based on the difference of the attenuation of an X-ray when it passes through tissues of different densities (Guelpa and others 2015). The degree of X-ray attenuation is characterized as CT values, which are measured in Hounsfield units (HU) (Kalender 2011). As a sample is rotated under the X-ray scanner, a series of 2D X-ray images can be obtained. The series of images covering the entire sample, can not only provide information on carcass tissues, but can provide muscle density as well, which has also been proved to be a good predictor of IMF (Clelland and others 2014).

Recently, X-ray CT was investigated for the analysis of IMF content in beef, pig and lamb loins (Lambe and others 2008; Frisullo and others 2010; Prieto and others 2010; Font-i-Furnols and others 2013; Clelland and others 2014). For instance, Prieto and others (2010) applied X-ray CT to predict the IMF content of beef, in which PLSR prediction models were established with tissue density values, which offered an R^2_P between 0.71 and 0.76 and a RMSECV between 0.54% and 0.57%, respectively. Better results were obtained by Frisullo and others (2010) who used X-ray microcomputed tomography (μCT) to quantify the IMF content of beef, and they investigated the correlations between all μCT parameters and IMF content analyzed by official method. High correlation coefficients (r) of 0.92to 0.99 were found between percentage object volume (POV) and IMF content (Frisullo and others 2010). In addition to beef, Font-i-Furnols and others (2013) evaluated the suitability of 2 statistical methods, ordinary linear regression (OLR) and PLSR, to estimate IMF content in pig loins using data from 1 tomogram or 2 different ones. Alongside the use of relative volumes associated with ranges of HU values, the best prediction model was obtained by OLR with an R^2_P of 0.83 and RMSECV of 0.46%, respectively.

Another advantage of X-ray CT is its potential application to live animals. In an attempt by Kongsro and Gjerlaug-Enger (2013), X-ray CT was investigated for predicting IMF content of pigs *in vivo*. However, the results were not satisfactory with a maximum prediction accuracy of $R^2_P = 0.53$. Most recently, Clelland and others (2014) optimized the use of CT parameters correlated with tissue densities for predicting IMF in the loins of Texel lambs *in vivo*, and they investigated a range of CT parameters correlated with fat and muscle density values for predicting IMF content and obtained a better prediction accuracy of $R^2_P > 0.65$ was obtained.

As discussed above, it was demonstrated that CT is a promising technique for the evaluation of IMF content of both meat carcass and live animals. With more information provided, its prediction accuracy of live animals is expected to be better than UI. However, before being used for commercial applications, more investigations are still needed to enhance the prediction accuracy and to reduce the cost of the present detecting instrument.

Hyperspectral imaging technique

Considering the specialties of spectroscopic and imaging techniques, integration of spectroscopic and imaging techniques has been expected to overcome their disadvantages in marbling analysis. In recent years, as an emerging spectral imaging technique, HSI has been extensively studied for (Brosnan and Sun 2004) food especially meat quality analysis (Barbin and others 2012a,b; ElMasry and others 2012a,b; Kamruzzaman and others 2012; Wu and others 2012; Wu and Sun 2013).

HSI is a technique which integrates conventional imaging and spectroscopic techniques (Pu and others 2014; Cheng and Sun 2015). A typical HSI system usually consists of an illumination, a translation stage controlled by a stepper motor, a spectrograph, a high definition camera, and a computer system installed with image acquisition software (ElMasry and others 2012b; Xiong and others 2014). When an object is scanned by a HSI system, a 3-dimensional hyperspectral image called "hypercube" can be obtained, of which the first 2 dimensions represent the spatial information and the third represents the spectral information (ElMasry and others 2012b; Wu and Sun, 2013). In recent years, the HSI technique has been intensively studied by researchers for marbling analysis of intact pork, which can be hardly achieved by spectroscopic or imaging techniques alone, and good results were obtained (Qiao and others 2007; Liu and others 2012, 2014; Huang and others 2013, 2014a, 2014b). Figure 3 depicts the main steps for marbling degree evaluation by HSI.

Qiao and others (2007) were the first to develop a HSI system for automatic assessment of the marbling level of pork. In this study, Qiao and others (2007) exploited the possibility of using the angular second moment (ASM) value, which is an indicator reflecting the uniformity degree and size of textures, as the texture index of marbling. They first modeled pork marbling standards by calculating the ASM value of digitalized marbling standards (from 1.0 to 10.0) by GLCM. Then the image of the samples at the wavelength of 661 nm, where the color contrast is more obvious (Figure 4) was selected to assess the marbling scores by computing the ASM value of the selected region of interest (ROI). Experimental results showed that ASM can successfully predict the marbling scores, except the score at 10.0 of which the ASM value was the same as the score of 5.0. In another study, marblings were regarded by Liu and others (2012) as a kind of line patterns, and were extracted by the wide line detector (WLD) which is efficient in extracting lines. The proportion of marblings (PM) obtained using WLD on digital color images of Natl. Pork Producers Council (NPPC) standards was used to determine marbling scores by multiple linear regression models. Their results (Liu and others 2012) showed that the established multiple linear regression models could successfully distinguish the marbling scores of the NPPC standards, and the prediction ability of the simple linear model based on only the blue channel was almost equivalent to the multiple linear model developed at all 3 channels, which showed the efficiency of the WLD. A similar result was reported by Huang and others (2013) who compared the ability of 2 pattern analysis techniques, WLD and the texture extraction technique based on an improved GLCM, in assessing pork marbling, and their results showed that the WLD-based models were more effective than the GLCM based models. They also found that both the WLD-based models and the GLCM-based models developed at the green channel showed the best prediction ability for pork marbling, suggesting that the simple linear model developed at the green channel could substitute the multiple linear model developed at all 3 channels. Most recently, in a study conducted by Liu and others (2014), 5 wavelengths (1076, 1129, 1191, 1210, and 1258 nm) were selected as critical wavelengths by correlation analysis between reference IMF content and each of the spectral features, 1st derivative of spectral features and 2nd derivative of spectral features. IMF features were extracted by WLD, and the proportion of IMF fleck areas (PFA) at critical wavelengths was used for model development to predict IMF content. Both stepwise procedures and PLS analysis were used to establish prediction models, and the best result was obtained by a 3-component PLS model with an adjusted coefficient of determination in validation (R^2_V) of 0.93 and root mean square error in validation (RMSEV) of 0.17.

Results up to now show that HSI is powerful for the accurate and fast assessment of marbling levels due to the abundant spatial and spectral information it provides. However, the high dimensionality of the hyperspectral data needs to be reduced to the most meaningful dimension in order to improve the computational speed and meet the demands of real-time inspection. How to reduce the hyperspectral data to a meaningful dimension retaining the predictive power of the original data and the exploitation of powerful algorithms for establishing robust prediction models would be the direction of future investigations.

Advantages and Limitations of Current Evaluation Methods and Techniques

The richness of marbling can be evaluated by the above-mentioned various conventional and instrumental techniques. However, none of them could be recommended as a general method for marbling evaluation, these methods and techniques possess their advantages and limitations as discussed below.

- Visual appraisal is an important, traditional and easily accepted method to determine the marbling degree of meat and has been widely used in the meat industry for many years. However, with the requirements of trained professional graders, the disadvantages and limitations of this method are tedious, time-consuming, and have lack of inconsistency. For this

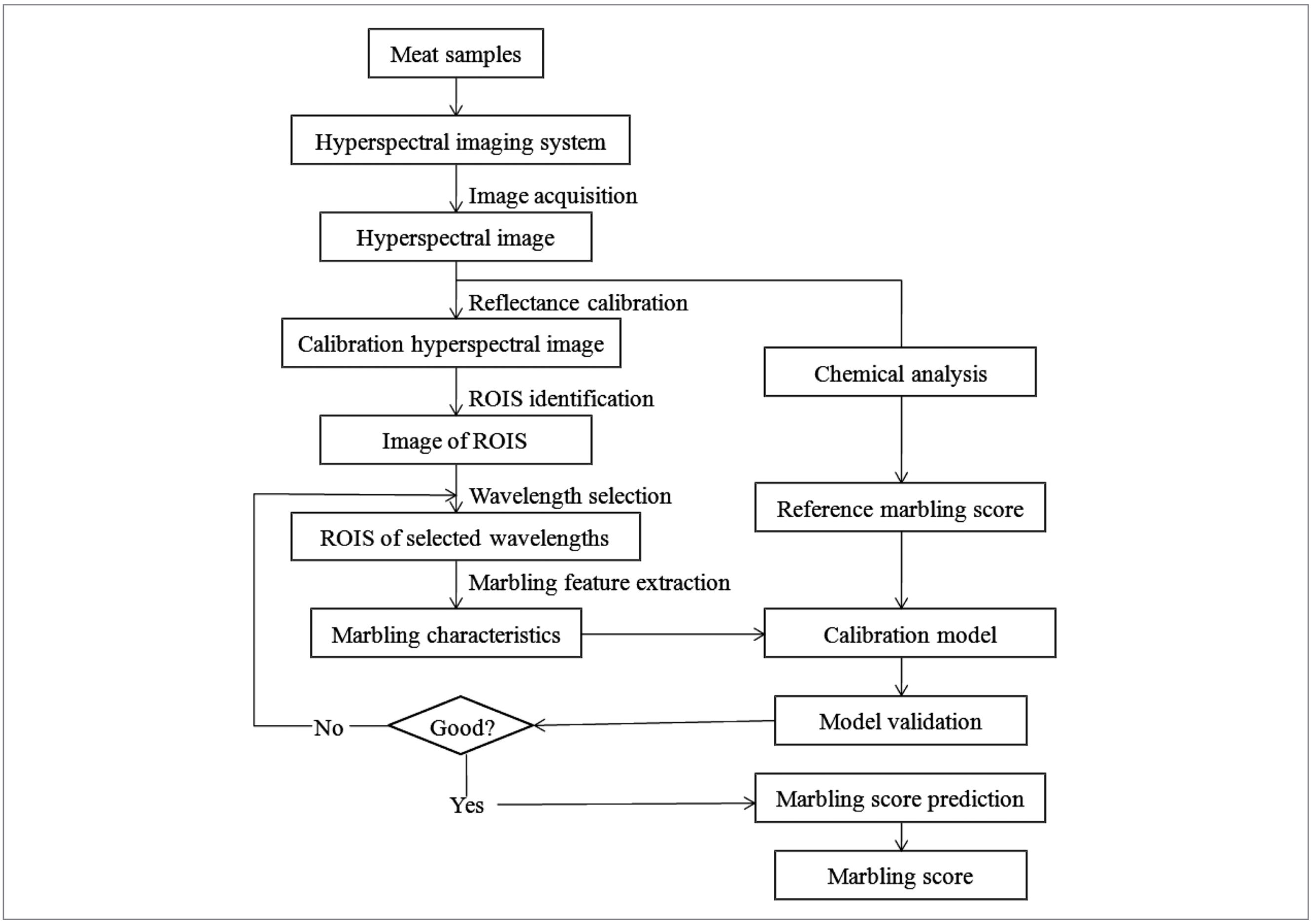

Figure 3–Main steps for marbling degree evaluation by HSI.

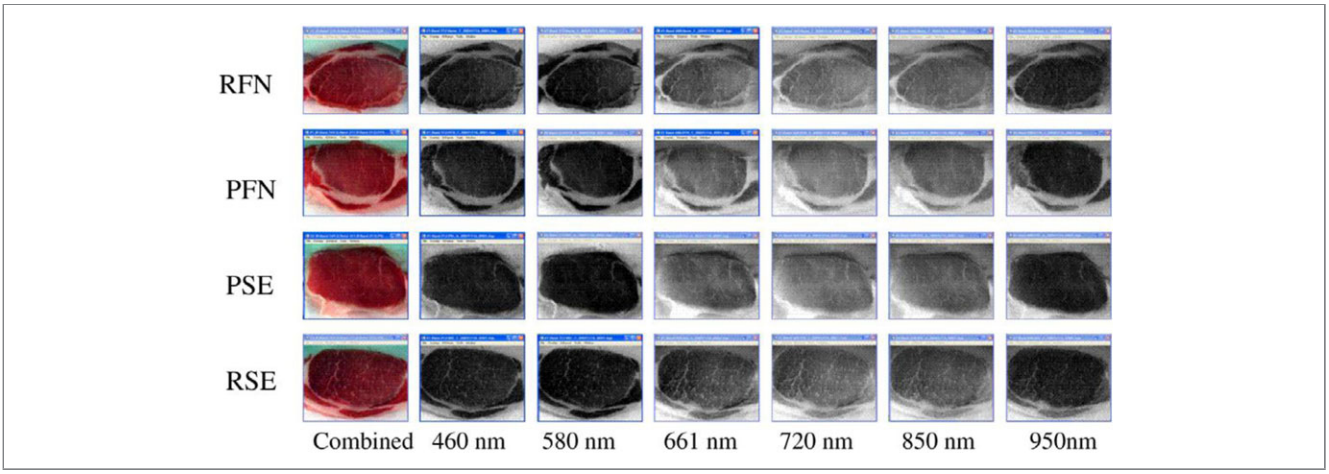

Figure 4–Hyperspectral images (RFN, reddish pink, firm and nonexudative; PFN, pale, firm and nonexudative; PSE, pale pinkish gray, soft and exudative; RSE, reddish, soft and exudative) obtained at 460, 580, 661, 720, 850 and 950 nm, the colored images were obtained by combined images at 460, 580, and 720 nm (Qiao and others 2007).

reason, to a certain extent, this method cannot meet the demand of the industry which is in need of a rapid method for marbling degree classification.

- Chemical analysis is another conventional method for meat marbling evaluation, and it has been widely used as a standard method for the accurate determination of IMF content. However, this method is also tedious and time-consuming, in addition, it possesses the disadvantages of destructiveness and it also cannot reflect the visual appearance of samples. It thus cannot be considered as a fast grading method of meat marbling.
- NIR spectroscopy has been studied most extensively among the spectroscopic techniques due to the lower cost of the instrument and its high sensitivity to fat content in meat. It has

excellent prediction ability of IMF content for homogenized meat. However, a major drawback of NIR spectroscopy is its dependence to the reference method used for calibration. In order to develop a prediction model of IMF content, a large number of representative samples of known IMF content are needed. Thus, to a large extent, the predictive accuracy of NIR spectroscopy depends on the reliability of the reference method. Moreover, due to the limited spatial field of view, NIR spectroscopy cannot provide spatial information of the samples, which is essential for the grading of marbling degree, thus the predictive ability of marbling degree by NIR spectroscopy for an intact meat carcass is often not satisfactory (Prieto and others 2009).

- BI spectroscopy offers certain promise for the fast and objective assessment of marbling degree, however, it possesses the same problem as NIR spectroscopy, that is, it can provide good predictive accuracy of IMF content for homogenized meat, but for evaluating marbling degree of intact meats, the predictive accuracy needs to be greatly improved (Altmann and Pliquett 2006).
- NMR spectroscopy is a potential technique for the evaluation of IMF content, but, at present, both the high cost of the instrument and the strict testing environment required make it unsuitable for its application in the industry at this time.
- CIA has been considered the most promising technique for the objective grading of marbling degree due to the spatial information that it can provide and its best match with human eyes. By using CIA, accurate and repeatable predictions of marbling score are possible under commercial conditions. However, when the color contrast between fat flecks and lean muscle meat is not obvious, such as marbling in pork, the predictive accuracy of marbling degree by CIA cannot be guaranteed (Faucitano and others 2005).
- UI has the biggest advantage in that it can estimate the marbling degree in live animals, which is valued by the breeding and feeding industries. However, the disadvantage of UI is that its predictive accuracy is relatively low compared with other techniques, thus it offers limited potential for its application in carcass marbling evaluation.
- X-ray CT is a promising technique for the evaluation of IMF content of both carcass and live animals. With more information provided its prediction accuracy of live animals is expected to be better than UI. However, before it can be used in commercial applications, more investigations are still needed to improve the robustness of the system and to reduce the cost of the present detecting instrument by developing new adaptable instruments.
- HSI has great potential for the evaluation of meat marbling because it integrates the merit of both spectroscopic and imaging techniques, therefore it can provide rich information for marbling analysis which, however, is also a dilemma as it can lower greatly the evaluation speed. Therefore, in order to improve the efficiency of the system, the high dimensionality of the data must be significantly reduced, thus more advanced algorithms for proper data processing should be developed (Xiong and others 2014).

Conclusions and Future Trends

The determination and analysis of marbling plays an important role in the quality evaluation of meat, which is of great importance to the meat industry. The recent advances of the methods and techniques for marbling analysis are reviewed here. They cover a variety of conventional and instrumental techniques, including visual appraisal, chemical analysis, NIR spectroscopy, BI spectroscopy, NMR spectroscopy, CIA, UI, X-ray CT, and HSI. Visual appraisal and chemical analysis, as 2 traditional and useful methods for evaluating of marbling degree, have been used by the meat industry for many years. However, visual appraisal and chemical analysis have the disadvantages of being subjective and time-consuming, respectively. For this reason, modern instrumental techniques including spectroscopic and imaging techniques have been developed in the past few decades. Spectroscopic techniques such as NIR spectroscopy, BI spectroscopy and NMR spectroscopy are powerful in determining the IMF content of homogenized meat. CIA is suitable for the analysis of beef marbling degree, since the color contrast is more obvious. UI and X-ray CT techniques are capable of analyzing the marbling degree of live animals. As an emerging platform, HSI integrates the merit of spectroscopic and imaging techniques and is of great potential for meat marbling analysis with the rich spatial and spectral information that it can provide.

On the other hand, despite so many research efforts on marbling analysis by means of the above-mentioned modern instrumental methods, there are still some barriers to be overcome. With regard to spectroscopic and imaging techniques, further studies are still needed to improve the robustness of these techniques. In addition, the development of instruments that can be used in the commercial environment would be the future development direction. Besides, HSI as an emerging technique has promise for marbling analysis, however, before being widely applied to the industry much work is needed for the selection of optimal wavelengths to improve the computational efficiency and the exploitation of new algorithms for establishment of robust prediction models. In addition, some techniques that have not been applied for marbling evaluation, such as Raman spectroscopy and Raman imaging that are powerful in discriminating fat from proteins are also promising for assessing marbling degree.

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