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Technical Paper

Rapid Assessment of Fresh Beef Spoilage Using Portable Near-Infrared Spectroscopy

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Abstract

The objective of this study was to develop a prediction model to assess fresh beef spoilage directly with the use of portable near-infrared spectroscopy (NIRS) without conducting a chemical method. Three fresh beef samples were bought from a slaughterhouse and traditional market on separate days. Spectra were acquired using a portable Scio spectrometer with wavelength 740-1070 nm. Two-third of the spectra were used for calibration sets and one-third for validation sets. Partial least square regression and cross-validation were used to develop a model and equation for predicting beef spoilage. The principal component analysis was used to classify changes in color, water loss, and muscle hardness in beef. The best predictive model was obtained from the original spectra (no pre-process) results as follows (R_C = 0.9, Rp = 0.86, SEC(%) = 0.61, SEP(%) = 0.69 and RPD = 3.53). Multiple Scattered Correlation (MSC) pre-processing method gave a good and acceptable model with results as follows; Rc = 0.89, SEC(%) = 0.66, SEP(%) = 0.83 and RPD = 2.91. NIRS showed variability of the samples and rate of spoilage, hence, was used to assess quality and safety. Further studies are needed to develop a robust model to predict fresh beef spoilage using a Scio portable NIRS.

Keywords: *portable NIR spectroscopy, fresh beef spoilage, pre-processing method, prediction model, and supply chain system*

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Introduction

The quality of meat harvested is influenced by several conditions, including pre-and post-slaughter activities, handling technology, storage duration, and environmental conditions. Meat quality comprises several sets of parameters categorized into physiochemical properties, sensory attributes, and nutritional values. These parameters are the criteria consumers considered when buying fresh meat (Grunert et al. 2004). Generally, consumers purchased fresh beef at the slaughterhouse and trade markets based on acceptable qualities and safety. Freshness is considered one of the most critical meat quality attributes, beneficial to producers and consumers regarding sales and consumption. Meat freshness is related to the amount of moisture content in beef and determines the internal constituents, processing method, and muscle characteristics (Liu et al. 2014). Fresh meat spoilage starts immediately after being harvested and advanced in the postharvest handling system, including slaughterhouses, transportation, processing, markets, and consumers' homes. Most often, the decline in quality occurs simply because of changes in freshness. It directly affects the pH, moisture content (MC), protein degradation, fat oxidation, flavor, juiciness, appearance, microbial growth, cooking time, and the degree of consumer acceptance (Dave & Ghaly 2011; Yang et al. 2017). Globally, consumers are now more concerned with the increased rate of fresh beef contamination and adulteration, threatening nations' public health (Kamruzzaman et al. 2016). The beef properties and contamination changes have prompted many stakeholders in the meat industries to search for appropriate monitoring and evaluation methods.

The two methods used to determine meat freshness, quality and safety are conventional and nondestructive. Many studies have been conducted in Indonesia to assess meat contamination and adulteration using the conventional method (Diyantoro & Wardhana 2019; Harlia et al. 2017; Yulistiani et al. 2019) compared to the nondestructive method. For instance, a polymerase chain reaction was used to evaluate the adulteration of meatballs with wild boar meat (WBM) (Guntarti et al. 2017). The disadvantages with the conventional method are; lengthy sample preparation, longer time to obtain a result, laborious, expensive, destructive (i.e samples are used only ones), massive use of chemicals, operated by an expert and performed in the laboratory. In contrast, the nondestructive method provides quick results, samples can be used more than once, minimal sample preparation, non-chemical, cost-effective, and on-line measurement (Prieto et al. 2006).

Near-infrared spectroscopy (NIRS) is a nondestructive method, comprising electromagnetic radiation with long and short wavelengths for Fourier-transformed NIRS and portable NIRS, respectively (Manley 2014; Osborne 2000; Purwanto et al. 2015; Wiedemair et al. 2018). It is used to determine the physical, chemical, and biological features of agricultural commodities. These materials are made of bonds, namely oxygen to hydrogen (O-H), carbon to hydrogen (C-H), nitrogen to hydrogen (N-H), sulfur to hydrogen bond (S-H), which vibrates as they receive radiation energy. During vibration, material bonds formed overtones and combined bonds to evaluate a commodity's qualitative and quantitative characteristics (Manley 2014; Osborne 2000). Pre-processing uses mathematical methods to improve the clarity of chemical information in the spectra by removing background interference such as light, noise, and sound. Some pre-processing methods include multiple scattered correlation (MSC), normalization, and derivatives. The partial least square regression and principal component analyses are usually used to develop the model and classify samples according to levels. Portable Scio NIRS (Consumer Physics, America) is a short-wavelength device that ranges between 740-1070 nm and is used to evaluate food quality and safety (Peyvasteh et al., 2020). The smartphone assists the portable Scio NIR spectrometer for proper functioning. It has a cost-effective light source (e.g tungsten lamps) and detectors (e.g silicon diode arrays) which are used in the visible and NIR (750-1800 nm) spectral range (ElMasry & Nakauchi 2016). Portable Scio NIRS can be very helpful to rapidly assess meat quality and the rate of change in the supply chain system. Currently, in Bogor City no study has been reported to develop a predictive model to monitor and evaluate the rate of fresh beef spoilage. Therefore, developing a rapid, accurate, reliable, and repeatable model can benefit stakeholders in the meat industries and the general public. The objective of this study was to develop a prediction model to assess fresh beef spoilage directly with the use of a portable near-infrared spectrometer (NIRS) without conducting a chemical method.

Materials and Methods

Sample Preparation

Meat samples were harvested from the Brahman breed imported from Australia. In Indonesia, the Brahman breed is commonly known as BX. Three commercial cut samples (fresh striploin) were bought from the Bogor slaughterhouse and the traditional market in Bogor City. On the first day, 500g of fresh striploin steak was bought from the Bogor Slaughterhouse two hours after slaughter. While the samples for the second and third day were bought from the traditional market three hours after being slaughtered. Nonetheless the samples bought from both sites were fresh and had the same slaughtering effects but differ in post mortem. The difference in post mortem provided variability in samples, which relates to beef quality. Samples were vacuum packed in sterile polyethylene (PE) bags, placed in an icebox filled with gel packs, and immediately transported to the laboratory. Samples were removed from the icebox and placed on a sterile PE in a shallow plastic tray of dimensions; 50 cm x 20cm x 10 cm in length, width, and height, respectively. No pre-treatments such as the trimming of fat, removal of connective tissues, and washing was done on the samples. Instead, samples were kept at room temperature for ten minutes to stabilize the temperature before spectra measurements were carried out. In this study, the duration for measuring parameters was similar to the phenomenon occurring in traditional markets where traders sell from 2:00 am to 2:00 pm (Sundari et al. 2020). No chemical analysis was carried out because it was the main objective of the study.

NIRS Scio Instrument

The portable Scio near-infrared spectrometer (PNIRS) is a short-wavelength, ranging between 740-1070 nm with a resolution of 1 nm. PNIR spectrometer is a unit comprising a light source, optics dispersing constituents, and linear detector array. The spectrometer is a smartphone-assisted, palm-sized micro-sensor (Consumer Physics, America) with a weight of 54 g, dimensions of 8 cm × 4 cm x 3 cm in length, width, and height, optical window, and sensor window of 0.4 cm and 0.7 cm respectively. An optical nose, rectangular (2 cm x 4 cm), prevents direct contact between the sensor and the samples' surface (Figure 1). This help to avoid measurement error which may have been caused by dirt blocking the sensor. Additionally, the attached optical nose prevented scattering and penetrating of light from the background. Hence, optimized collection of reflected light radiation. Therefore, the spectrometer can be carried around by stakeholders in the meat industry for assessing fresh beef at processing sites. Spectrometer was calibrated with an Andriod version 4.9.1 with Bluetooth Technology connected to SCIO Lab software development Toolkit, Octave 4.2.1 (Consumer Physics, America).

NIR Spectrometer Measurement

The portable NIR spectrometer was used to measure spectra data in fresh beef by reflectance mode. Before measuring spectra, the spectrometer was calibrated on-line with the SCIO Lab software assisted by Bluetooth connection. The device was calibrated while in the case with the sensor end directly against the sunlight. Three samples weighing 500 g were used for this study. The samples of 500 g were used whole rather than cutting into 100g to obtain five samples. The use of whole beef was to elucidate the actual phenomena occurring at the slaughter and market sites where consumers usually buy fresh commercial cuts. Additionally, it was to evaluate the monitoring ability of the spectrometer to assess a whole sample. Moon et al. (2020) reported using 50g of meat and fish to evaluate the microbial, *Staphylococcus aureus* growth in culture. Samples were scanned at three points, the geometric center, and the two edges to optimize the variability in the muscle characteristics in the orientation. The scan lasted for 6s and was performed 58 times per sample at an interval of 15 minutes. A total of 174 spectra (58 times x 3 scanned) were measured per sample (Moon et al. 2020). The samples were scanned many times to minimized measurement error and assess the optimum variability in beef particle orientation. The spectra were handled on-line using the SCIO Lab software and later downloaded from the same website. The spectral data were downloaded as csv file, converted and saved as an excel file (xlsx) for further analysis using a statistical software package. Spectra were orientated in a matrix (x) format, whereby the columns represented wavelengths and the row represented the samples.

Data Analysis

Data analysis involves the conversion of reflectance to absorbance $log = \frac{1}{R}$, whereby R stands for reflectance, pre-processing of spectra, development of calibration and validation model, and interpretation of results. The total spectra obtained from the three samples were 522. Two-third of the spectra were assigned for calibration sets and one-third for validation sets. These spectra were selected at an interval of one hour to identify the levels of spoil, change in color, and change in texture in storage for 14.5 hours. Pre-processing is the removal of unwanted information from the background. Some of these background interference include noise, background light, and the signal-to-noise ratio (Geesink et al. 2003; Rinnan et al. 2009; Wiedemair et al. 2018). During pre-processing, spectra are transformed from reflectance to absorbance, followed by using a mathematical transformation method (MTM). In this study, we used MSC as an MTM to correct the multiplicative and additive effects of the spectra (Hasnah Ar et al. 2019). Pre-processing was performed because beef is a heterogeneous substance comprised of different particle sizes, shapes, and densities. Hence, defects in muscles can influence the spectra and the chemical information.

Partial least square regression (PLSR) was used to analyze the calibration sets combined with a cross-validation setup of leave-one-out to develop calibration and predictive models. Cross-validation grouping was performed with a total of twenty segments, five segments per sample. Hence, the pre-processing method was used to improve the predictive model's accuracy, repeatability, and reliability of the PLS model. PLSR is a powerful and widely accepted chemometric method for developing meat spoilage and contamination (Lin et al. 2004; Sahar et al. 2019). PLSR is a model used to determine the linear relationship existing between spectra and the attributes under investigation. Often, it is used whenever the number of variables exceeds the number of samples. PLSR predicted the calibration sets (dependable variable) from a group of validation sets (independent variables). While the prediction was carried out by selecting from the validation sets that differ from calibration sets but the same species. The new sets of latent factors were selected to obtain the best predictive power on the data. The optimum PLS factors were selected based on the standard error of calibration (SEC), standard error of prediction (SEP), coefficient of calibration (R_C), coefficient of prediction (R_P), coefficient of covariation (CV), and the ratio of performance to deviation (RPD). The performance to develop prediction models was based on squares error of prediction, coefficient of determination (R^2), and RPD, using the equations 1 to 6.

The principal component analysis (PCA) is a multivariate statistical method used to identify the hidden pattern in samples or spectra to observe the variabilities. PCA operates the multivariate data matrix to classify samples in groups or clusters based on their similarities and differences. Also, PCA is used to reduce the dimensionality of data by transforming the number of related variables into a set of unrelated

Figure 1. Spectra collection from fresh beef using a smartphone-assisted portable NIR spectrometer.

variables while retaining much information of the sample variation as possible (Kadegowda et al. 2008; Hanasil et al. 2020). The transformed variables known as the principal component (PC) are the linear combination of the original variables. In this study, PC was used to differentiate the levels of spoilage, change in color, and change in muscle hardness (i.e change in texture). Regarding the classification, the different levels of spoilage, spectra were selected in one hour, pre-processed using MSC pre-processing method, and later analyzed with PCA. The results of the different sub-stages of spoilage is presented in a score plot. PCA reported being effectively separate beef quality such as a change in color, drip loss (spoilage), and change in texture (Byrne et al., 1998; Peyvasteh et al., 2020).

RPD is the ratio of the standard deviation of the reference to the standard error of prediction of the validation sets. It is calculated to assess the practical potential of the prediction model. A value of RPD>3 is considered suitable for screening purposes; >5 is good for quality control and >8 is deemed to be excellent for all analytical tasks (Olivieri 2018). According to Williams (2001) calibration model with RPD<2.3 is a poor model which is not good to predict new samples and >8 indicates a good prediction model and can be used for any purpose.

$$Bias = \frac{\sum(x_n - y_n)}{n} \quad (1)$$

$$SEP(\%) = \sqrt{\frac{\sum\left(x_n - y_n - Bias\right)^2}{n-1}} \quad (2)$$

$$SEC(\%) = \sqrt{\frac{\sum\left(x_n - y_n\right)^2}{n-1}} \quad (3)$$

$$R = \frac{\sum(x_n - \bar{x}_n)(y_n - \bar{y}_n)}{\sqrt{\sum(x_n - \bar{x}_n)^2 \sum\left(y_n - \bar{y}_n\right)^2}} \quad (4)$$

$$RPD = \frac{SD}{SEP} \quad (5)$$

$$CV\ (\%) = \frac{SEP}{\bar{x}} \times 100 \quad (6)$$

Where; n was the number of total spectra data, x_n was the reference validation sets and y_n was the prediction validation set (Purwanto et al., 2015). Spectra data were analyzed by using an Unscrambler X version 10.4 (CAMO, Norway) and Microsoft Office Excel.

Results and Discussion

Assessment of Spectra Characteristics

Figure 2a shows reflectance spectra transformed to absorbance (Figure 2b). In this study, absorption peaks observed are; 756-770 nm, 956-1000 nm, and 756-768 nm and 953-978 nm for unprocessed and pre-processed, respectively. The peaks indicated regions of strong absorption along the muscle fibers. The wavelength range of 953-978 and 956-1000 nm are second and third stretch of overtones attributed to moisture content (O-H group). In beef, Cozzolino et al. (2002) and Grau et al. (2011) identified two absorption bands of a water molecule (O-H) at 980 and 970 nm using NIR spectroscopy. Similarly, Andrés et al. (2008) observed five absorption bands in the NIR region, including 760, 980, 1200, 1450, and 1950 nm using a sensor of 750-2500 nm. Our results were in agreement with those reported by Cozzolino and Murray (2004), Mamani-Linares et al. (2012) and Ishikawa et al. (2016).

As the post-mortem time increases after slaughter, muscle segregates, thus releasing water from the intra-myofibril compartment to the extra-myofibril muscle on the surface, enhancing absorbed spectra. Release of water from the muscle was attributed to rigor mortis at the early stage of post-mortem, and the muscle hardness increase as the shelf life increased (Li et al. 2016). Drip loss is a process of moisture lost from the fibers of fresh beef to the environment without applying external force or thermal energy. High drip loss is related to high absorption bands in regions such as 969, 972, 973, and 975 (Barbin et al. 2012; ElMasry et al. 2012; Su et al. 2018). Results depicted that beef freshness declined as the storage time increase.

Regarding fat, the C-H group was most likely within the range 953-1000 nm in the fresh beef. Su et al. (2018) identified fat molecules in red meat at a spectrum value of 940 nm using hyperspectral imaging and attributed it to third overtones. Prieto et al. (2008) revealed that intramuscular fat is related to C-H chains of fatty acids found between the absorbance band of 1300-1400 nm. The high variability of the O-H and C-H groups correlated to beef heterogeneous with complex structures and compositions. As mentioned earlier, samples were not pre-processed against intramuscular fat, probably the reason while fat was identified. The bands of 756-770 nm and 756-768 nm were considered to be color changes because the portable NIR spectrometer is within the visible light range (Figure 2a and b). The color change was further classified using principal component analysis (PCA).

Principal Component Analysis (PAC)

The PCA is a multivariate statistical tool used in data analysis to classify spectra data according to their variability in the co-linear information, reducing the dimensionality of the variables while retaining much of the valuable information as possible. PCA pre-processed spectra by MSC to align the data for their score component in the axis. The alignments in different component axis show the different levels of spoilage. The beef spoilage, change in color, and change in muscle were categorized into five groups according to principal components (PC1 and PC2), as shown in Figure 3. PC1 shows a 95% clear separation of the different change levels, with the negative values representing fresh sample and positive values spoil sample. PC2 explained only 3% of the differences in the parameters. Figure 3 shows the grouping of beef levels such as blue cluster representing fresh sample (0-1 h), red cluster for less fresh (2.5-4 h), green cluster for slightly spoiled (4.5-7.5 h), gray cluster for spoiled (8-11 h) and deep red cluster for dark firm dry (11.5-14 h).

We observed a positive correlation ($p<0.05$) with the decline in freshness regarding the color change. The change in color was classified into five clusters, including blue for purplish red (deoxymyoglobin), red for bright cherry red (carboxymyoglobin), green for cherry red (oxymyoglobin), gray for brownish-red (metmyoglobin), and deep red for dark firm dry (Figure 3). The progressive change in color was due to blooming with the beef surface interacting with oxygen. Regarding color change, the results of this study showed a similar trend with that of Peyvasteh et al. (2020). Andrés et al. (2008) revealed that metmyoglobin discoloration in fresh beef occurs at wavelength 780 nm using visible spectroscopy. Damez and Clerjon (2008) observed a change color by using a visible-light spectrometer operating with polarized light with a 400-800 nm wavelength, widely used to detect color and collagen. Color is determined by the amount of myoglobin change based on post-mortem duration from bright-red, cherry-red, purplish-red, and brownish-red (Liu et al. 2003). Spectra acquisition began at 3 and 4 post-mortem of beef derived from the slaughterhouse and traditional market respectively.

The muscle was tender with much free water at the start of spectra measurement. But as storage time increases, the surface muscles became compact and hard, and offensive odor. Probably due to increase drip loss (i.e the water holding capacity reduces) as the post-mortem period increased at room temperature. Further investigation is needed to ascertain the observation. The unpleasant odor perceived indicated possible oxidation of subcutaneous fat.

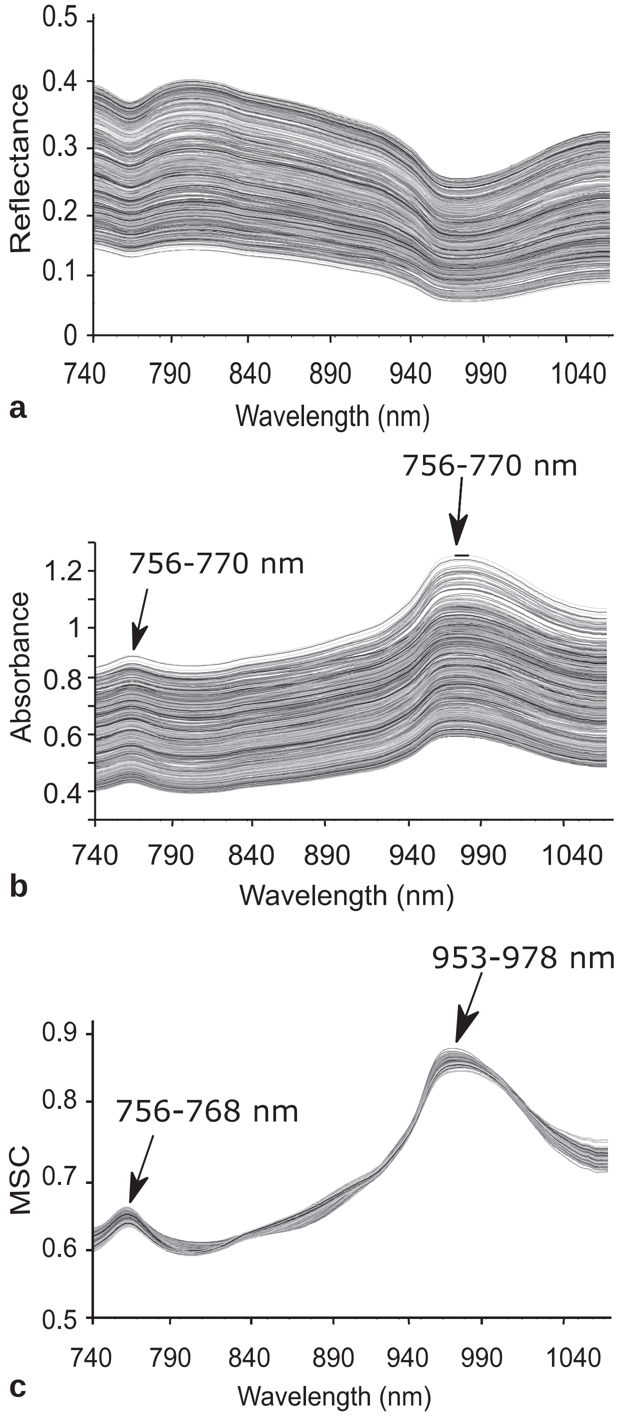

Figure 2. The NIR spectra of fresh beef spoilage showing absorption ranges (b) absorbance, (c) MSC pre-processing except for (a) reflectance.

Effect of Pre-processing Method and The Selection of Accurate PLS Model

While developing a predictive model using the PLS algorithm, the selection of pre-processing method and the number of PLS factors was essential. The selection of an accurate, and reliable pre-processing method was achieved through several trial-by-errors. Generally, fewer factors are essential for developing a robust model, especially when dealing with small sample sets (Rødbotten et al. 2000). In this study, the maximum PLS factor used was 9, and factor 8 was selected as the best prediction model. The best PLS model was developed from the original spectra to predict spoilage, change in color, and muscle hardness (Table 1). The value of CV(%) depicted that the model can be used in the future to guarantee calibration.

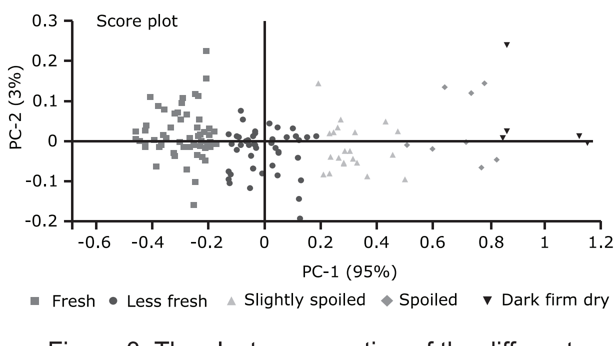

Figure 3. The cluster separation of the different levels of beef spoilage, change in color, and change in muscle texture. For instance, blue for fresh beef, red for less fresh, green for slightly spoiled, pink for spoiled, and deep red for dark firm dry.

Table 1. The result of PLS model for fresh meat spoilage.

Spectra pre-processing	PLS Factor	CV (%)	Rc	SEC (%)	Rp	SEP (%)	RPD
0riginal	8	16.51	0.90	0.61	0.86	0.69	3.53
MSC	8	22.46	0.89	0.66	0.79	0.83	2.91

According to Andrés et al. (2005), the acceptable CV(%) level should be no less than 20%. The original depicted CV(%) of 16.51 compared to MSC with 22.46. The best model had an acceptable calibration of Rc = 0.9 and its ability to predict training sets was good and acceptable (Rp = 0.86, SEC(%) = 0.61, SEP(%) = 0.69 and RPD = 3.53) (Table 1). Nevertheless, its application on-site could be limited because the model has some degree of background interference. MSC calibration result was accepted with Rc = 0.89 and the prediction model on the cross-validation sets was good and acceptable which may be assigned to guarantee significant calibration (Rc = 0.89, SEC(%) = 0.66, SEP(%) = 0.83 and RPD = 2.91) (Table 1). The results of this study was different from that reported by Su et al., (2018) to predict physical parameters of beef using NIR reflectance spectroscopy (R_C = 0.731-0.833, R_P = 0.576-0.661, SEP(%) = 1.324-1.483 and RPD = 1.17-1.31). The low model developed by Su et al. (2018) could most likely be because they used homogenized muscle. However, similar results of beef were found by S. Andrés et al. (2008). MSC corrected the multiple additives and reduced the signal-to-noise ratio, thus provided accurate chemical information compared to the unprocessed spectra. Therefore, pretreated model is the most appropriate to predict future samples for quality and safety. Nonetheless, to improve the robustness of the model, more samples are needed.

Prediction of Fresh Striploin Spoilage

In this study, the equation $y=0.84x+61.26$ was developed by the best PLS model (Figure 4a). This predictive model showed a low value of SEC(%) and SEP(%), indicating a fitting model for predicting the rate of muscle hardness, color change, and drip loss that led to dark firm dry. Furthermore, the values of R_C = 0.9, R_P = 0.86, and RPD = 3.53 ascertained that this model could be used in the supply system to monitor and evaluate fresh beef quality. This study revealed that the model could be accurate and reliable to predict samples with different post-mortem periods. In addition, it explains the variability of samples bought from different processing sites and environmental conditions. Those are some of the factors to be considered before conducting engaging in any study. For example, Huff-Lonergan & Lonergan (2005) explained that an early increase in post-mortem temperature led to serious denaturing of the protein muscle, particularly the intra-myofibrils, and cause a decrease in water-binding ability. However, the equation efficacy is limiting because it was developed from the original spectra comprised of unwanted background information.

In contrast, the developed model by MSC pre-processing method is the most appropriate prediction model. Because all multiplicative and addictive impurities from the background have been removed, living the actual chemical characteristics of the investigated parameter. The equation for the preferred development model is $y=0.82x+106.23$. The equation can predict the levels of beef spoilage, color change, and muscle tenderness. Also, the MSC can be used to monitor fresh beef spoilage at the slaughterhouse,

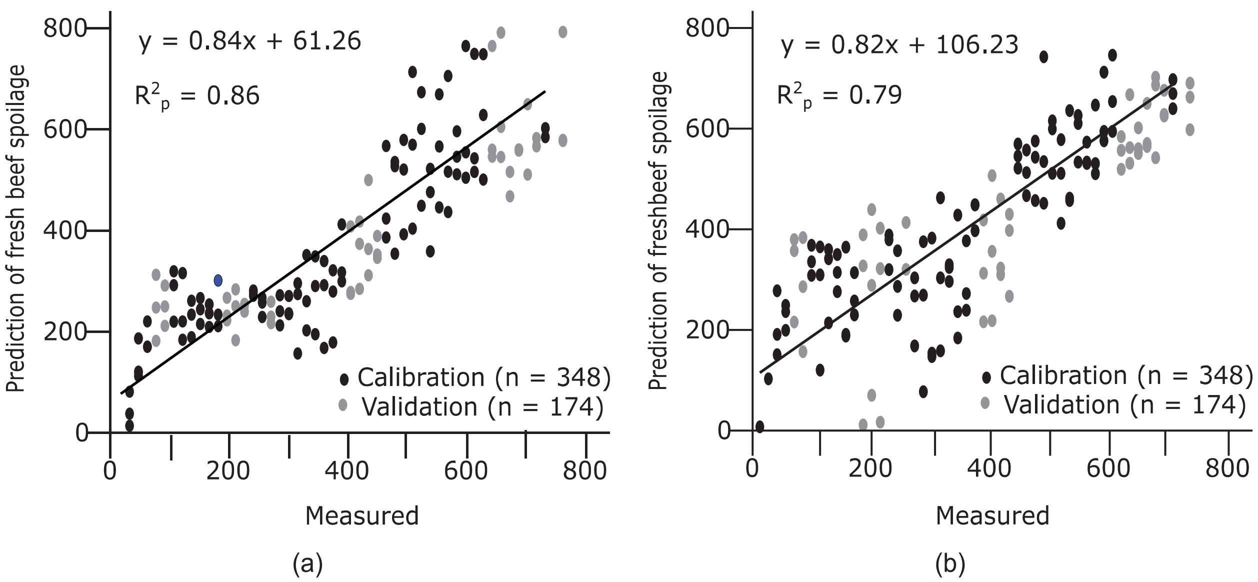

Figure 4. The prediction of fresh beef spoilage (a) no pre-processing and MSC pre-processing method.

markets, during distribution, and at homes because of its good predictive features (Rc = 0.89, Rp = 0.79, and RPD = 2.91).

The performance of develop models was evaluated using the RPD value. The RPD values of this study range between 2.5 and 3.0 which are considered good and very good respectively for qualitative and quantitative evaluation. Cen & He (2007) reported that the RPD values above 1.5 and 2 should be used to predict quantity and quality parameters. Manley (2014) stated that RPD values >3 are used for screening, >5 can be used for quality control, and >8 for any application system.

Based on the results of this study, the spectrometer provided satisfactory results that collaborated with the objective of this study, to develop a prediction model to assess fresh beef spoilage directly with the use of a portable Scio NIR spectrometer without conducting chemical analysis. This study further revealed a good prediction model developed from original spectra (without pre-processed method).

Conclusion

In this study, the original spectra gave a good prediction model though limited because it is composed of unwanted background information. The parameters of the PLS model includes Rc = 0.90, Rp = 0.86, SEC = 0.61%, SEP= 0.69% and RPD = 3.53. The most appropriate and acceptable model for prediction for on-line monitoring and evaluation of spoilage, change in color and muscle hardness was derived from pre-processed spectra by using MSC pre-process method. This method provided the essential chemical information by removing unwanted background information. The parameters of the PLS model includes Rc = 0.89, SEC(%) = 0.66, SEP(%) = 0.83 and RPD = 2.91. The portable Scio NIR spectrometer can be used at different processing sites to assess quality and safety directly. However, more research is needed to develop a robust prediction model for on-line assessment of fresh beef spoil coupled with increased sample size. Further study is needed to ascertain the changes of pH, water holding capacity and microbial status of the beef.

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