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Non-Destructive Discrimination of Beef-Blood Adulterated in Pork using NIR Spectroscopy Combined with Chemometrics

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In Partial Fulfillment for the Degree of
Bachelor of Science
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Department of Chemistry, Faculty of Science

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Abstract

Counterfeit beef is one of the most common issue affecting the Islam society in Thailand. Recently, counterfeit beef made from pork marinated in cow-blood has been used in Thai buffet restaurants in order to reduce cost. This study presents a feasible technique for rapid determination of counterfeit beef by using near-infrared spectroscopy (NIR) combined with multivariate data analysis. Prior to acquiring NIR spectra, the thermal phenomena of meat samples (pork, beef, and marinated pork) were investigated by thermogravimetric analysis (TGA). The thermal profiles of beef and pork show significant differences especially at degradation temperatures of 300°C - 400°C which correspond to the chemical compositions of free amino acids, long chains of peptides and lipids. Molecular information of the meat samples was collected by NIR spectrometer with reflection mode. The spectral pretreatments including Savitzky-Golay polynomial, standard normal variate (SNV) and outlier removal by interquartile range (IQR) were performed in order to remove the irrelevant information. By using spectral variance with 95% significance level, the significant NIR regions to discriminate the types of meat were selected at 5002 - 5503 cm^{-1} (the first overtone of O-H stretching of water or moisture content), 5981 - 6479 cm^{-1} (the first overtone of C-H stretching of fatty acids/fat), 6819 - 8207 cm^{-1} (the second N-H overtone of protein), and 8485 - 8797 cm^{-1} (the second overtone of O-H of water). Principal component analysis (PCA) with David-Bouldin index (DBI) was used to visualize the sample cluster. Score plots of PC4-PC5-PC11 were chosen as the best PCs to obtain high separation of meat samples. The correctly classified percentages (%CC) obtained from the Linear Discriminant Analysis was 79.48%and 88.64% for the prediction of pork and beef sample, respectively. From overall study, the developed NIR spectrometer with powerful data analysis is a feasible technique for determination of the counterfeit beef with acceptable accuracy.

Keyword: Counterfeit beef, Near-infrared spectroscopy, Principal component analysis

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LIST OF ABBREVIATIONS

NIR	:	Near infrared spectroscopy
PC	:	Principal component
PCA	:	Principal component analysis
LDA	:	Linear discriminant analysis
PLS	:	Partial least-square
DBI	:	David-Bouldin index
TGA	:	Thermal gravimetric analysis
FT-NIR	:	Fourier transform near-infrared spectroscopy
SOMs	:	Self-organizing maps
SNV	:	Standard normal variate
IQR	:	Interquartile range
NIPALS	:	Nonlinear Iterative Partial Least Squares
℃	:	Degree Celsius
min	:	minute
nm	:	nanometer
cm	:	centimeter
kcal	:	kilocalorie
kg	:	kilogram
g	:	gram
mg	:	milligram
µg	:	microgram
iu	:	International units

CHAPTER 1
INTRODUCTION

1.1 Introduction to the research problem and significance

Livestock are domesticated animals which are raised in an agricultural industry to produce several commodities. Livestock in Thailand is roughly divided into cows, pigs, chickens, ducks, and others. In 2018, beef cattle farming has become a large agricultural industry in Thailand. It plays a vital role in Thailand's economic market due to a huge demand of both domestic and foreign consumers. Beef cattle setting in Thailand[1] has been rising about 28% within 4 years (2017-2020). This involves the beef cattle of 4.9 millions increased to 6.2 millions. Diagram of the number of beef cattle in Thailand during 2017-2020 is shown in Fig 1.1. Besides, It has been reported from the Department of Livestock Development that the market value of beef is up to 41,810 millions Thai baht/year. However, it is still not adequate to the high demand of the beef trading market. Since beef consumption in Thailand and Asian is dramatically increasing. The Department of Livestock Development of Thailand[2] reported that ~1.06 millions of beef cattle were consumed in 2018 and ~1.08 millions in 2019 which is 1.89% increasing within 2 years.

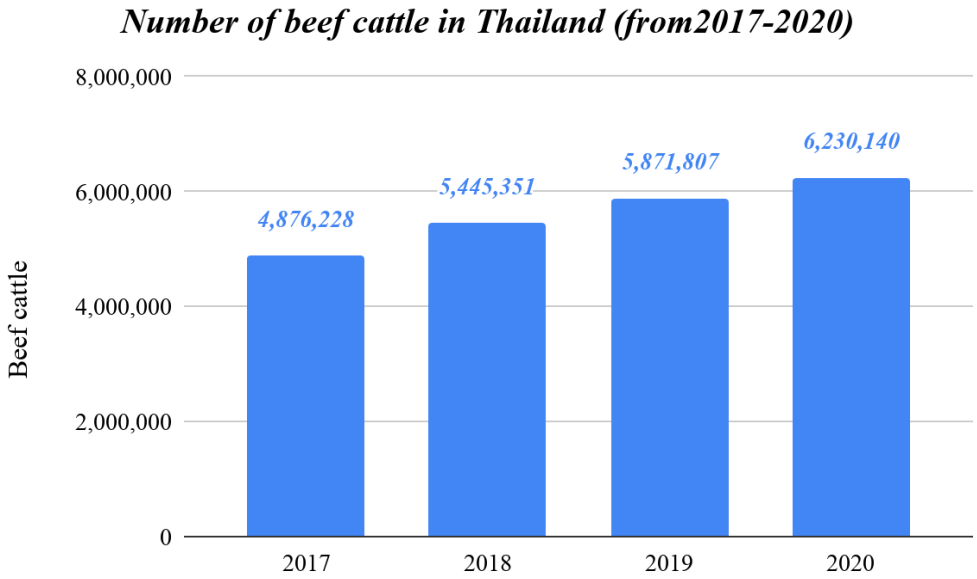


Figure 1.1 Number of beef cattle in Thailand between year 2017-2020 (Information and communication Technology Center)[1]

A few years ago, the price of beef was approximately 130-150 baht per kg, however the price was increased to 230-400 baht per kg in 2020 as shown in Table 1.1. Market price of beef is the greatest compared to pork and chicken. It is approximately four times higher than the price of chicken, and almost twice times the price of pork. According to an article from Bangkok Bank SME[3], the market price of beef tends to rise up to 400 baht per kg because of an in short supply

of beef cattle in Thailand even the beef cattle farming is rising. However, the rate of farm growth is not adequately supplying the growth rate of customer demands.

Table 1.1 Market price of beef and pork[4]

Meat cuts	Price (Thai Baht/kg)
Beef, meat, raw	230-250
Beef, loin/rump, raw	230-270
Beef, tenderloin, raw	300-400
Beef, belly, raw	210-230
Pork, rump, raw	145-150
Pork, shoulder, raw	145-150
Pork, loin, raw	150-155
Pork, tenderloin, raw	160-165
Pork, belly, raw	165-170
Chicken, wing, raw	70-75
Chicken, drumstick/ thigh, raw	60-65
Chicken, tenderloin, raw	60-70
Chicken, breast, raw	60-75

Accordingly, the high popularity of consuming beef can also be presented by the price of food in various known restaurants in Thailand such as Bar B Q plaza, Sukishi and local buffet restaurants. For example, BBQ plaza has a buffet promotion in which the price of a buffet including beef is more expensive than one that provides pork and other meats. Many restaurants in Thailand tend to add the market value of their dishes by including beef on the buffet. For buffet including beef, it will add up to 100 baht extra compared to the standard buffet. An advertisement poster of a buffet at Bar B Q plaza is shown in Fig 1.2.

Figure 1.2 Bar B Q Plaza and AKA buffet menu (Wongnai)[5]

In early 2020, there was an outbreak of counterfeit beef selling at a local market[6] in Thailand. Since the price of pork and beef is significantly different, some unscrupulous butchers sell “fake” beef that actually is marinated pork (pork with beef blood). The illustration of the

different prices of beef and pork is shown in Fig 1.3 which indicates that beef can be sold at a higher price compared to pork in a market.

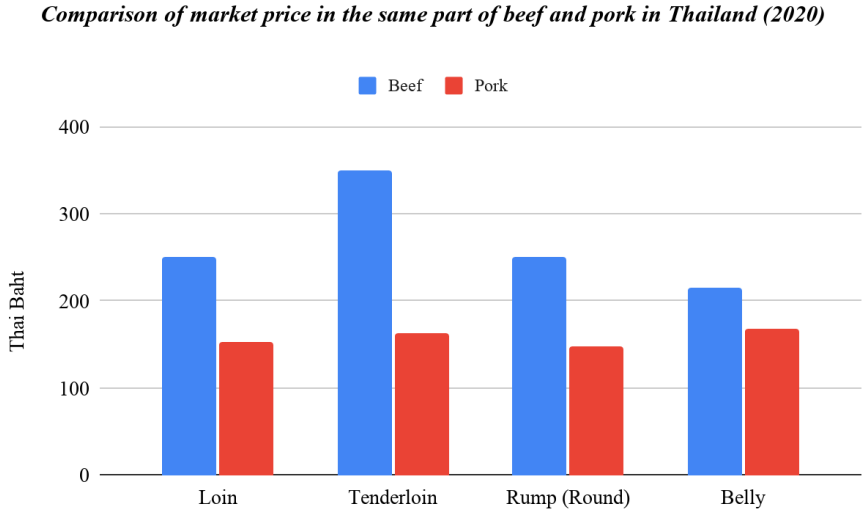


Figure 1.3 Comparison of market price in beef and pork.[1] (Information and communication Technology Center)

*The price mentioned is averaged.

On August 1st, 2020 The Halal Science Center Chulalongkorn University reported that 29 of 42 beef samples obtained from beef consumers were fake. They were pork marinated with cow blood, or dyed with food colour. Up to 70% of the collected samples (19 samples) were bought from the online market. Halal Science Center used Polymerase Chain Reaction or PCR technique[7] to detect pig DNA in suspected meat samples. This news posted by Muslim Thai Post, an online newspaper related to Muslims in Thailand[8]. Fig 1.4 displays beef, pork stained with cow blood and suspected beef that were reported on the news.

Figure 1.4 Beef, pork stained with cow blood, and suspected beef respectively. (Posttoday)[6]

The counterfeit beef sold in a local market impacts on various sectors. It infringes consumer rights, and has bad effects on consumer health due to its manufacturer process. Besides, there are several religions which prohibit the consumption of pork including Islam, and Judaism. In the Holy Quran of Islam mentions about halal and haram food, which halal foods are permitted to consume[9]. On the contrary, haram food is explicitly forbidden or not allowed to consume such as pork meat and products from pork. So mulisms require halal authentication to ensure their diet. This is also in accordance with the Jews. For Jews, there is a dietary law called "Kashrut"[10]. It states that only animals that chew their cud, have cloven hooves, and are free from disease are allowed to consume. Thus, pork is prohibited. The outbreak of counterfeit beef in Thailand put Muslim and Jewsish societies in a difficult situation and it affects to food exports and trust of the customer, especially, to the Jews and Muslim communities.

For physical appearance, the color of pork is paler compared to beef. Thus, using reddish meat accelerators[11] such as Clenbuterol, Zilpaterol, and Ractopamine that accelerates the conversion of fat into muscles, and reduces fat accumulation in tissues with old pork or pork can redden the pork color. These accelerator substances are harmful to human health. Alternatively, to make counterfeit beef can be done by food or synthetic dying pork with reddish colour. Furthermore, the easiest imitating beef method is to marinate pork with cow blood since cow blood reddens pork color and makes the scent of pork similar to beef. Therefore, it is difficult to discriminate between beef and counterfeit beef by its appearance.

Meat determination in food was important since it does not just damage to human health but also affects customer trust, especially the ones in religious restrictions. Nowadays, analytical methods and electrophoretic methods such as chromatography[12], immunoassay[13], polymerase chain reaction, and random amplification of polymorphic DNA[14] were used to differentiate types of meats. Although they provide high accuracy and prediction but these following methods were time-consuming and required advanced laboratory skills and they do not support a broad user community.

Near-Infrared spectroscopy (NIRS) was an alternative method that can determine various components such as fat, carbohydrate, and protein. It was used in a quantitative determination since it was a non-destructive and less time consuming analytical method. Moreover, NIRS sample preparation is uncomplicated compared to other chemical methods. NIRS detects the light that is scattered off the sample materials, this can be considered as NIRS advantages since the analysis process has no effect on the samples itself. Therefore, the sample material will not be damaged from the process. This technique will be based on the measurement of absorption in 800 - 2500 nm or 10,000 - 4000cm^{-1} which is the NIR region. However, the NIR spectra are complicated to interpret. The mathematical method or chemometric methods such as principal component analysis (PCA)[15] were used to interpret the data obtained from NIRS. Since PCA will reduce the number of data sets and categorize it into three main categories, principle component, scores, and loadings. This will make distinguishing all different types of meat samples possible. All these properties make NIRS an acceptable method in the food and beverages industry. There are several literatures proving the use of NIRS technique. Raw meat without grinding was analysed firstly in 1991. The qualities of beef cuts were determined by the physical and chemical characteristics using NIRS with multiple linear regression as a calibration method[16]. The outcome of the experiment concluded that the NIRS technique is feasible in meat quality determination. The first on-line NIRS application was used to determine fat, moisture, and protein content in ground beef was reported in 1996. The analysis was done to evaluate the meat quality. NIRS instrument was palace at the meat grinder, using the multiple linear regression as a

calibration method[17]. Tørgersen et al. (1999)[18] stated that NIRS technique can also be used during the mass production process for industrial factories. NIRS performance could be done contact-less with multilinear regression for the determination of fat, water and protein content in both beef and pork. The NIRS was considered a useful method for grinding meat.

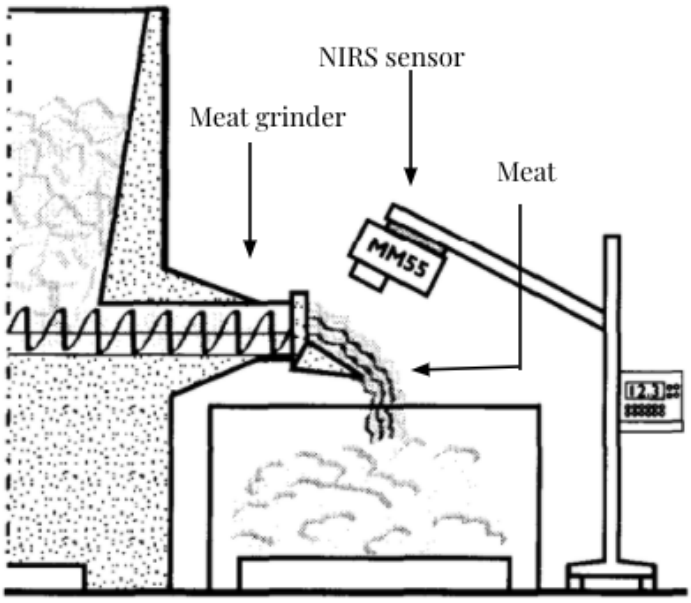


Figure. 1.5 Illustration of online NIRS system on the meat grinding process[17]

Adulteration was also a common issue in the modern era. Meat adulteration was the process of mixing one type of meat with another. The determination of pork adulteration in beef meatball by Kuswandi et al. (2015) was done using NIRS along with the chemometric methods such as partial least-square (PLS)[19]. PLS is an extension of the PCA method, it will extract principal components and correlate them with Y-block information to calculate the latent variables. The potential of NIRS was successful in distinguishing the pork adulteration in beef for halal authentication. NIRS has wide applications, determining the chemical composition of the sample is one of them. The study of Douglas et al. (2013) shows the efficiency of NIRS techniques in determination of chemical composition in intact pork. Key major chemical components in pork can be identified with PLS regression model support[20]. In 2016, Prieto et al. reported the use of NIRS on pig ears to discriminate the pork carcass[21]. This study was done to analyse the fatty acid composition and content. Hence, the NIRS has the potential to discriminate both the chemical composition and quality of meat carcasses. Fig. 1.6. demonstrated the overall NIRS system used in most studies. In this study, the NIRS was used for the determination of beef from pork adulterated with cow blood or counterfeit beef. From our far knowledge, there was no research using NIRS technique on this particular topic before.

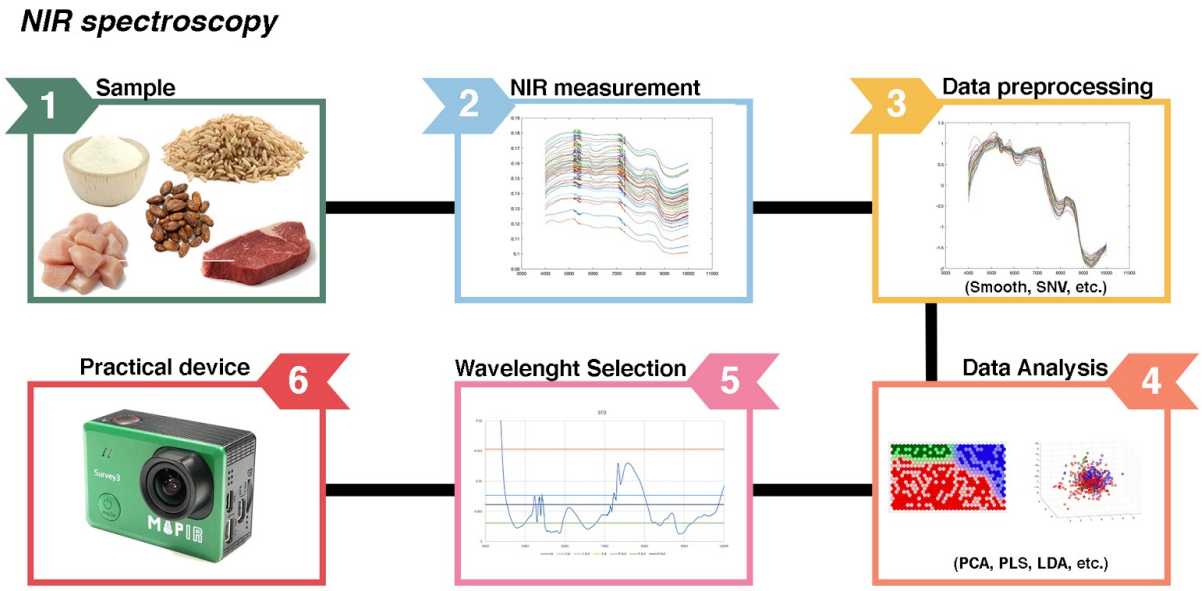


Figure 1.6 Overview scheme of applications of NIR combined with data analysis on agriculture products in order to access product quality

In this research, a rapid determination of counterfeit beef using NIRS combined with statistical analysis was developed. In the study, the beef samples including beef collar, beef round and beef loin, pork samples including pork collar, pork round, and pork loin, and pork marinated in cow blood was purchased from a certified market (Siam Paragon, Bangkok). Since different meats might contain different contents of chemical compositions. The degradation process of the meats was monitored by using Thermal gravimetric analysis (TGA). The molecular vibration patterns were acquired by using NIRS reflectance mode. The obtained NIR spectra was then interpreted using multivariate data analysis (called "Chemometrics"). This involves principal component analysis (PCA) with automated PC selection using David-Bouldin index (DBI) to visualize the cluster of meat based on NIR spectrum patterns. Furthermore, the classification model using Linear Discriminant Analysis (LDA) was constructed to demonstrate the performance of class prediction. This study will demonstrate the potential of NIRS combining with chemometric methods in order to discriminate counterfeit beef.

1.2 Research Objective

The aim of this study is to provide a feasible technique for rapid determination of counterfeit beef by using near-infrared spectroscopy along with multivariate data analysis

1.3 Scope of work

Generating rapid determination of counterfeit beef using NIRS with chemometric methods was the purpose of this study. Beef and pork samples used in this study were obtained from the certified supermarket. Cow blood was obtained from a local butcher. Both meat samples and cow blood were purchased in Bangkok, Thailand. The Thermo Scientific™ Nicolet™ iS5N FT-NIR spectrometer with extended range indium gallium arsenide (InGaAs) detector was used to analyse the sample. The analysing process was done at room temperature. Interpretation of the NIRS spectrum was done using the MATLAB version R2019B program. PCA analysis and SOMs visualize maps were performed using the MATLAB program.

CHAPTER 2
THEORETICAL BACKGROUND

2.1 Meat nutrition

Amount variations of nutrient contents in meat could be used as a guideline for the NIR spectral analysis. Since different nutrient composition could be generated the different patterns of vibrational spectra especially in the infrared regions. Beef and pork are meats which majorly distributed to consumers around the world. They are considered as red meat which is derived from mammals. Red meat is one of the most nourishing and energy-rich food products. It mainly consisted of water, proteins, vitamins, minerals, fats, and fatty acids. It is considered a good source of high-quality protein. A protein in meat contains all amino acids which are essential to the human body. It is also an enriched source of B-complex vitamins such as thiamine, niacin, vitamin B6, and B12. Moreover, vitamin A, D, E and K can be found in the meat organs. Aside from these nutrients, iron (heme iron) in meat is well absorbed, around 15-35%, which is better than plants, and intensifies absorption of iron from other sources. Furthermore, red meat also contains fats and fatty acids including saturated fatty acids, monounsaturated fatty acids, polyunsaturated as well as cholesterol[22]. The nutritional compositions of raw beef and pork is summarized in Table 2.1

Table 2.1 Nutritional compositions of raw beef and pork.[23 - 26]

Nutrients	Unit	Beef (per 100 g)	Pork (per 100 g)
Energy, by calculation	kcal	119	124
Moisture	g	72.6	72.9
Protein, total	g	20.3	21.13
Fat, total	g	4.2	3.97
Ash	g	3.1	1.07
Calcium	mg	4	6
Phosphorus	mg	216	245
Sodium	mg	85	100
Potassium	mg	354	349
Iron	mg	2.99	1.01
Copper	mg	0.12	0.1
Zinc	mg	2.4	1.83

Nutrients	Unit	Beef, lean, raw (per 100 g)	Pork, lean, raw (per 100 g)
Selenium	µg	17.8	17.1

Thiamin	mg	0.05*	0.84
Niacin	mg	5.59*	5.7
Fatty acids, total saturated	g	1.91	1.52
Fatty acids, total monounsaturated	g	1.15	1.98
Fatty acids, total polyunsaturated	g	0.19	0.65
Fatty acids, total omega-3 polyunsaturated	g	0.03	0.01
Cholesterol	mg	65	45

* the value thiamin, and niacin provided by beef, loin, raw per 100g[26]

2.1.1 Protein and amino acids

Protein, one of a macronutrient, essential for the growth and maintenance of the body and also providing energy which is vital for the human body. Chemically, protein is a polymer of amino acids which consists of nitrogen, carbon, hydrogen, oxygen and some protein might contain sulfur and phosphorus in the structure[27]. Generally, the average protein content in raw red meat is approximately 20-24 g per 100 g. Protein in cooked red meat is slightly increased to 27-35 g per 100 g because the moisture content decreases during the cooking process, therefore, the protein becomes more concentrated[28]. According to Table 2.1, Beef has less protein contents than pork. This could be caused by different amounts of amino acids. There are 190 known amino acids, however only 20 are required to generate protein structures. All essential amino acids including isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine as well as arginine and histidine described as semi-essential amino acids could be found in red meat[23]. From Table 2.2, the contents of leucine and lysine are significantly higher than the others and they mostly present in beef rather than pork. Studies reported that contents of isoleucine, methionine, phenylalanine, valine, and arginine in the meat increased proportionally with its age[29]. Furthermore, the essential amino acid contents diverge in different parts of the carcass. In the non-essential amino acids part, glutamic acid or glutamine presenting in red meat has the highest contents (about 15%).

Table 2.2 Essential and non-essential amino acids presented in beef and pork. The data has been reported by Ahmad et al (2018)[30]

Essential amino acids		
Amino acids	Beef	Pork
Histidine	2.8	3.1
Isoleucine	5	4.8
Leucine	8.5	7.6

Lysine	8.2	7.9
Methionine	2.2	2.6
Phenylalanine	4.1	4.3
Threonine	4.2	5.2
Tryptophan	1.3	1.5
Valine	5.6	5.2
Arginine	6.4	6.6
Cystine	1.5	1.2
Non-essential amino acids		
Amino acid	Beef	Pork
Proline	5.2	4.4
Glutamic acid	14.3	14.6
Aspartic acid	8.9	8.8
Glycine	7.2	6
Tyrosine	3.3	3.1
Serine	3.9	4.1
Alanine	6.3	6.4

2.1.2 Fats

Fats are also one of the three major macronutrients, as well as carbohydrates and proteins. Fats are lipids consisting of tri-esters of glycerol and fatty acids or triglycerides. There are three main types of fats existing in meat; intermuscular fat which is fats found between the muscles, intramuscular fat or fats marbling within muscles, and subcutaneous or visible fats that are found below the meat skin[28]. Different types of fats could be a specification in order to distinguish between beef and pork by appearance.

Intermuscular fat

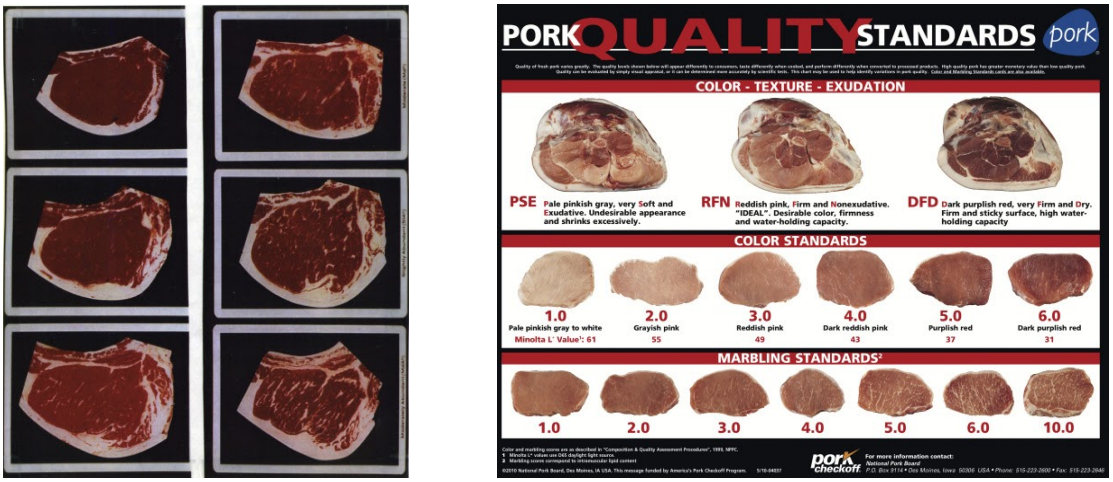
Fats that are located between the muscles, and generally surround the moving muscles area. The area between bone and points of muscle attachments is also filled with fats which are described as intermuscular fats. It acts as a buffer and reduces friction from muscle movement. Therefore, it is particularly found in the legs, neck, and thorax part of animal meat[31]. Fig 2.1 shows the intermuscular fat presented in beef and pork.

(A) (B)

Figure 2.1 Intermuscular fat presents in (A) the chuck part of the beef carcass[31] and (B) the neck part of the pork carcass[32]

Intramuscular fat

Intramuscular fats are located between muscle cells, and in the later stage of the growth process its deposition increases. Marbling describes intramuscular fats in the meat industry, it outstandingly impacts on fresh meat marketing, especially beef cuts. Marbling variation depends on their genetic traits, sex of animals, hormones, and growth stage[31]. Marbling is the last type of fat to be deposited, it will be deposited on the 4th growth stage of animals. It takes a long time to accumulate in animals. As the result of slow-growing breeds of cow, marbling in beef is clearer than pork[33]. Fig. 2.2 shows the marbling in beef and pork.



(A) (B)

Figure 2.2 An example of different deposition of marbling presents in (A) beef and (B) pork[31]

Subcutaneous fat

Subcutaneous fat is located under the skin of animals and is considered as the greatest amount of fat in the pork carcass. In pork, subcutaneous fat is deposited in layers and is separated by connective tissue into the three layers of subcutaneous fat[31] as shown in Fig 2.3. These fat layers are clearly distinguished when the animal grows. The outer layer is deposited in the early growth stage, and more essential to pig for physiological functions and adjustment to

the environment. Aside from it, subcutaneous fat also exists in cattle as beef. The largest fat deposition of fat cattle (850 pounds) is in the rump, hind flank, lower loin, brisket, and center of the shoulder. Deposition of fats in these areas protects the animal from bruises. Throughout the growth of cattle from 850 to 1000 pounds, fats are deposited in the lower flank.

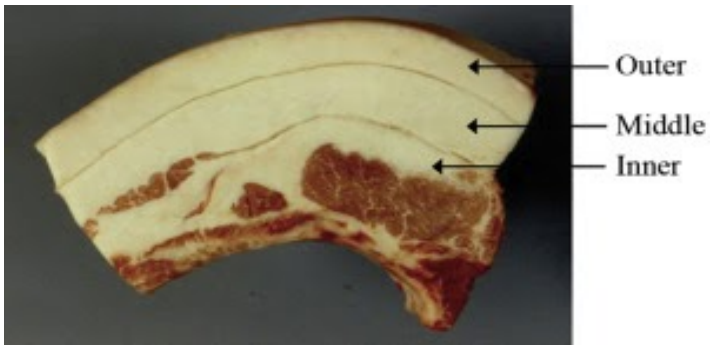


Figure 2.3 example of subcutaneous fat in pork[31]

2.1.3 Fatty acids

Red meat consists of unsaturated fatty acids; oleic (C-18:1), alpha-linoleic (C-18:2), linolenic (C-18:3) and the long-chain fatty acids including arachidonic (C-20:4) acid which is reported as essential fatty acids[34] presenting in Table 2.3. Variation on fatty acids compositions depends on species whether it is a ruminant or not. The digestive characteristics of ruminant affect fatty acid compositions. As during digestion in the ruminant like cows, most of the dietary unsaturated fatty acids are isomerised and hydrogenated to saturated fatty acids (stearic acid) by rumen microbial enzymes[35]. Therefore, the contents of saturated fatty acids in ruminant meat (beef) are present in larger amounts than non-ruminant pork as shown in Table 2.3. A well-known trans fatty acid as conjugated linoleic acid (CLA) is also presented in beef because of the biohydrogenation of bacteria in rumen although the amount of it is minimal[23]. On the contrary, pork seems to have higher contents of unsaturated fatty acids compared to beef, but its composition is still determined by the fatty acid profile of the animal feed.

Table 2.3 Composition of fatty acids in beef and pork[36]

Fat and total fatty acids (g)	Beef (per 100 g)	Pork (per 100 g)
Total fat	4.3*	4
Saturated Fat	1.74*	1.36
Cis - Monounsaturated fatty acids	1.76*	1.5
Total Cis-PUFA	0.2	0.69*
n-6 PUFA	0.17	0.61*
n-3 PUFA	0.07	0.09*
Total Trans	0.14*	0.02

Total Branched	0.08*	0.01
Caprinic acid (C10:0)	0	0
Lauric acid (C12:0)	0	0
Myristic acid (C14:0)	0.1	0.04*
Pentadecylic acid (C15:0)	0.02*	Trace
Palmitic acid (C16:0)	0.97*	0.83
Stearic acid (C18:0)	0.59*	0.01
Arachidic acid (C20:0)	0	0.01

*The values present a greater amount.

2.1.4 Vitamins

Red meat is an excellent source of five of the B-complex (B1, B2, B3, B6 and B12). About two-third of the daily requirement of vitamin B12, together with 25% of recommended daily intakes for riboflavin (vitamin B2), niacin (vitamin3), vitamin B6 and pantothenic acid per 100 g of red meat is provided[25]. Moreover, vitamin B12 is important for the replication process of DNA. The composition of vitamin contents are illustrated in Table 2.4. Folic acid amount in beef is greater than pork while the contents of thiamine (vitamin B1) in beef is less against pork. Vitamin A and folate can mostly be found in red meat organs, especially, liver.

Table 2.4 Vitamin compositions in beef and pork[30]

Vitamin units / 100 g of raw meat	Unit	Beef	Pork
Vitamin A	iu	Trace	Trace
Vitamin D	iu	Trace	Trace
Thiamine (vitamin B1)	mg	0.006	1.2
Riboflavin (vitamin B2)	mg	0.21	0.21
Niacin (vitamin B3)	mg	5.1	5.2
Vitamin B6	mg	0.2	0.4
Vitamin B12	mg	2	2
Pantothenic acid	mg	0.5	0.5
Folic acid	µg	9	2
Biotin	µg	2	5

2.1.5 Minerals

An important dietary source of bioavailable minerals, and trace elements can be found in red meat. The contents of minerals in beef and pork is illustrated in Table 2.5. Red meats such as

beef and pork are a good source of iron and zinc. Since dietary iron exists in two forms: heme iron and non-heme iron, which heme iron is derived from the hemoglobin and myoglobin so it could only be found in meat products. It is able to easily be absorbed in the intestinal lumen, and utilized by the body[37]. Although iron is available in many food products, heme iron form presents only in meat. In beef, iron, and zinc contents are greater than in pork. Selenium, copper and potassium in beef are also slightly greater. On the contrary, calcium, phosphorus, sodium, and magnesium contents in pork appear to be higher compared to beef.

Table 2.5 Mineral composition in beef and pork [26, 30]

Minerals (per 100 g of raw meat)	Unit	Beef	Pork
Calcium	mg	4	6
Phosphorus	mg	216	245
Sodium	mg	85	100
Potassium	mg	354	349
Iron	mg	2.99	1.01
Copper	mg	0.12	0.1
Zinc	mg	2.4	1.83
Selenium	µg	17.8	17.1
Magnesium	mg	24.4	26.2

2.2 Near-Infrared Spectroscopy (NIRS)

Near-Infrared spectroscopy (NIRS) is a non-destructive and efficient method with simplicity in sample preparation that has been proved to be the most promising on/in line detection methods in food and many industries. NIRS analysis is capable of rapid determination of fat, water, protein, and many other components simultaneously. The main components of the NIRS system are light source, light-isolating mechanisms, detector, and sampling devices (Figure 2.4).

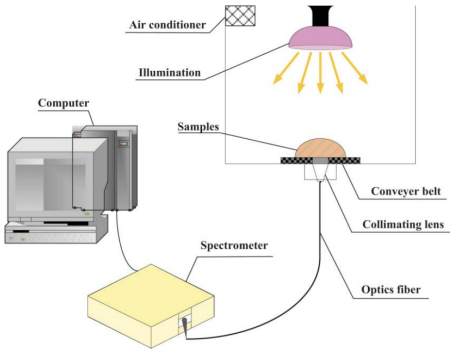
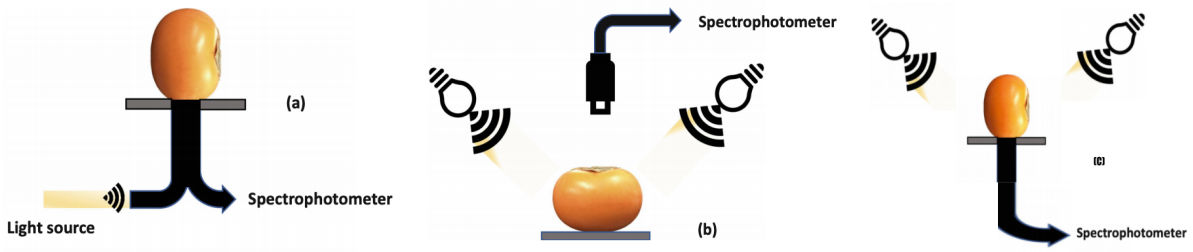


Figure 2.4 Component parts of a NIRS measurement system [38]

Data collection modes of the NIRS system consist of three different modes, transmittance, interactance, and reflectance. Transmittance mode was suitable for liquid sample analysis since the detector was placed in the opposite side of illumination, it will measure the amount of light that is transmitted through the sample. Interactance mode was the combination reflectance and transmittance mode. This mode is suited for large samples. Reflectance modes are suited for solid and powdered solid samples. The detector in reflectance mode was placed at the same side of the light source to measure the reflected light from the sample surface. These different modes could be used differently according to the type and component of sample[38] as shown in Figure 2.5.



(A) (B) (C)

Figure 2.5 Schematic representation of (A) interactance (B) reflectance and (C) transmittance mode [43]

NIRS has based on the absorption of electromagnetic radiation[39] at 780 - 2500 nm, along with the principle that the light of the sample carries a wide range of information related to its molecular overtone and combination vibration which can be served as a sample component such as protein (N-H), fat or water content (C-H/O-H)[40]. The combination of vibration was caused by the interaction of two or more vibrations. In a given molecule, a normal mode following the internal atomic motions will allow all the atoms to move in phase with the same frequency but different in amplitude. Aside from the normal mode transition will be considered as an overtone. This sort of transition is forbidden by the selection rules of quantum mechanics. Hooke's law or the basic law of vibrational spectroscopy was used in the calculation of fundamental vibrations for diatomic molecules in IR[41]. Hooke's law state that, for two harmonic oscillator, frequency of vibration can be express as

$$\bar{\nu}\ (cm^{-1}) = \frac{1}{2\pi c}\sqrt{\frac{k(m1+m2)}{m1m2}}$$

(C = speed of light, K = 5×10^5 dynes/cm)

The overtone bands are multiples of fundamental absorption frequency which will occur when a vibrational mode is excited from ground state ($v = 0$) to second excited state ($v = 2$), third ($v = 3$) or fourth ($v = 4$) excited state (Figure 2.6).

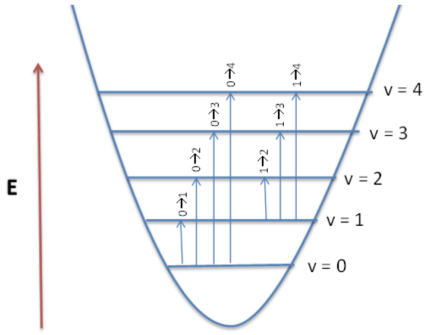


Figure 2.6 Energy levels of the vibration transition state of diatomic molecule[41]

The transition of ground state (v = 0) to second excited state (v = 2) will result as first overtone. The transition of ground state to third, fourth, and fifth excited state will also result in the second overtone, third overtone, and fourth overtone, respectively (Figure 2.7).

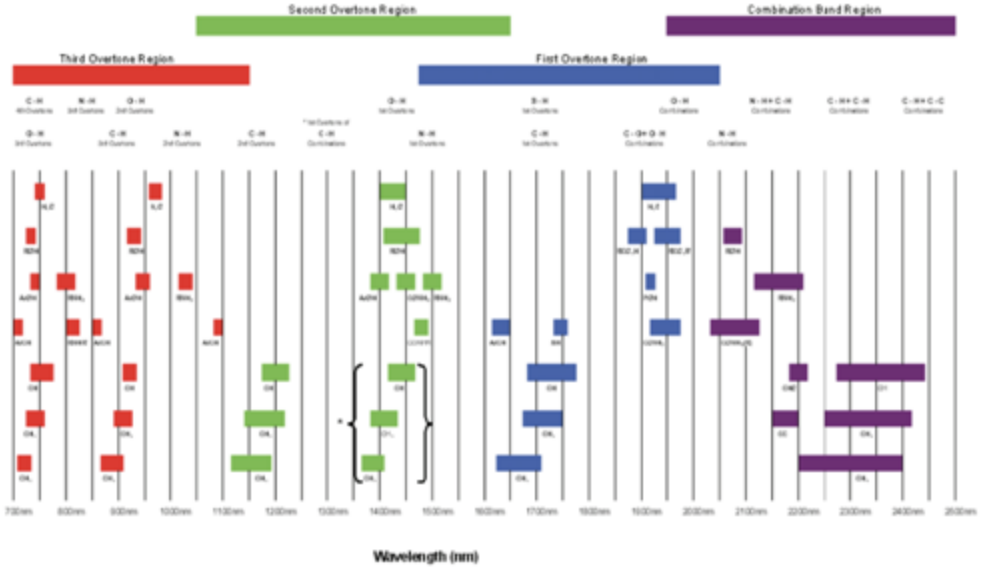


Figure 2.7 Diagram of NIR regions corresponding to overtone absorption of chemical functional group[42]

The overtone and combination bands correspond to C-H, N-H, O-H, and C=O groups whose molecular stretching and bending absorb in the IR region. These band assignments responded to specific chemical composition as shown in Table 2.6

Table 2.6 Chemical composition that correspond to the band assignment

Wavenumber (cm^{-1})	Band assignment	Structure	Reference
4545	=C-H, C=C	Polyunsaturated fatty acids	43
5102 - 5190	O-H 1st stretching	Water content (Moisture)	43
5190	O-H deformation	Water content (Moisture)	44
5620	C-H stretch + H-O-H deformation combination	Cellulose + Water	45
5753 - 5830	C-H 1st stretching	Fatty acids/ Fat	43, 46
6369 - 9200	N-H 2nd overtone	Protein	46
6890	O-H 2nd stretching	Water content (Moisture)	20
7790	O-H 1st overtone (ROH)	Oil	45
9700	C-H 3rd overtone	Aromatic	47
10204	O-H 3rd stretching	Water content (Moisture)	43
17857	O - binding protein	Oxymyoglobin	48
19047	Fe-, O- binding protein	Myoglobin	48

This overtone transition will exceed the fundamental transition ($v = 0 \rightarrow 1$) in terms of both energy and wavenumber. In contrast, the overtone was anharmonic oscillator which gives out weak absorption bands making NIR spectra complex and hard to interpret. Therefore, chemometrics methods such as principal component analysis (PCA) were applied in order to clarify all of the information.

2.3 Chemometrics

Chemometrics is the tool for extracting information from multivariate chemical data that uses mathematical and statistical methods. Chemometrics methods have become a powerful data analysis method used in many research fields since it is capable of analysing a wide variety of data types.

2.3.1 Principal Component Analysis (PCA)

PCA is an unsupervised pattern recognition technique used to display patterns in multivariate data. The aim of PCA is to find the pattern and display the relative positions of data points in fewer dimensions to summarize the information while filtering out noise. PCA will reduce the large data matrices by capturing the variance in terms of principal components (PCs). Principal Components (PCs) are a set of variables that are uncorrelated (orthogonal) and ordered by the maximum amount of variance in the data. The first PCs describe the greatest source of variation within the data and the axis of first PCs will lie along the greatest line of variation[49]. The second PCs or second axis will lie along the next greatest line of variation and lies at the right angles of the first. This makes all axes independent from each other, or orthogonality. In a PCA algorithm, matrix form can be express as

$$X = TP^{T} + E$$

(X = data matrix, T = score matrix, P = loading matrix, and E = residual matrix)

This PCA model structure was illustrated in Fig. 2.8. All data spectra can be analysed using PCA without any preprocess method being held. The score will show the apparent variation in concentration. Loading will demonstrate the mean spectra.

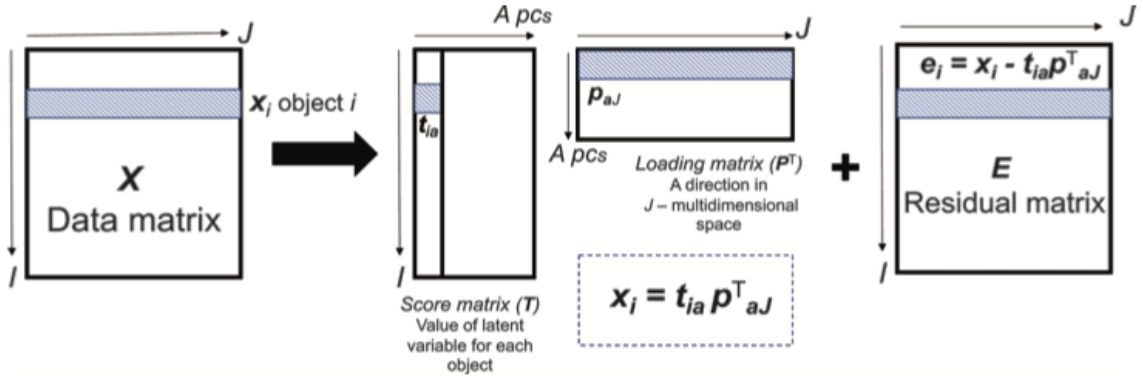


Figure 2.8 illustrate the PCA model[49]

To perform PCA, column scaling on the data is important due to the assumption that the variations in the data always deviate from the origin. Several algorithms can be used to perform PCA modeling, and should give out the same results when the variance in the data is unchanged. Singular value decomposition (SVD) and non-linear iterative partial least squares (NIPALS) were common algorithms used. In SVD[49], data matrix (X) was used to produce three matrices which can be express as

$$X = UDV^{T} + E$$

(U, V = matrices, D = diagonal matrix)

where U contains the same column vectors as the score matrix (T), V^T is identical with the loading matrix (P^T) but it is normalized to length one and D is a diagonal matrix containing the square roots of eigenvalues extracted from X^TX. SVD algorithm will extract all possible PCs in a single calculation including undesired information. Due to this, a large data matrix will require longer analysing time and more storage space on the computer.

In NIPALS algorithms[49], only a single component will be calculated at a time until a certain number of PCs is reached. NIPALS is suitable for a large data matrix since only the desired PCs can be chosen for calculation. This following steps must be follow to calculated the PCs using a data matrix $(X)^x$:

1. A column of X with the greatest sum of square (variance) is determined and used as the initial score vector ($t_{initial}$)
2. Loading vector p^T_{unnorm} is calculated by $p^T_{unnorm} = t^T_{initial}X/(t^T_{initial}t_{initial})$
3. Normalized the loading vector to unit length: $p^T_{norm} = p^T_{unnorm}/p^T_{unnorm}p_{norm}$
4. Calculate the new score vector by $t_{new} = Xp^T_{norm}$
5. Check for convergence by comparing the $t_{initial}$ and t_{new} . The sum of squared differences between all elements of the two consecutive score vectors is calculated. If the value meets the criterion (small enough), this indicates that the PC has been extracted; otherwise, replace $t_{initial}$ with t_{new} and return to step 2, repeating until con- vergence is achieved.
6. Residual data matrix (X) was calculated by subtracting the extract PC from X: $E = X - tp^T$
7. If it is desired to compute further PCs, substitute the data matrix X with residual matrix E and return to step 2

Various properties for each component could be determined after the scores and loading was calculated. Eigenvalue (ξ) was one of the properties representing the importance of the component. The eigenvalue was calculated by the sum of the scores vector all I samples:

$$\xi_a = \sum_{i=1}^{I} t_{ia}^2$$

Where the sum of all eigenvalue is equal to the sum of square of the matrix:

$$\sum_{i=1}^{I} \xi_a = \sum_{j=1}^{J} \sum_{i=1}^{I} x_{ij}^2$$

The percentages of the total amount of variance was used to determine the significance of each PC:

$$\%\xi_a = \frac{\xi a}{\sum_{j=1}^{J} \sum_{i=1}^{I} x_{ij}^2} \times 100$$

In PCA, the first few PCs will be considered as the most important pieces of information since the later PCs may contain noise. However, some biological samples might influence the experiment conditions making the first few PCs correlated with the background of the samples. Therefore, the useful information may be presented in the later PCs.

2.3.2 Linear Discriminant Analysis (LDA)

Linear discriminant analysis (LDA) is a supervised pattern recognition used as a dimensionality reduction technique. It was an extension method of euclidean distance that pooled variance covariance matrix (S_p) in the distance calculation. LDA will display the dataset into the lower-dimensional space with good class separation to avoid overfitting[50]. The distance between the samples to the class centroid is weight according to the overall variance of each variable. Mahalanobis distance[49] was used to calculated the LDA distance to the class centroid g,

$$d_{ig} = \sqrt{(x_i - \bar{x}_g) S_p^{-1} (x_i - \bar{x}_g)^T}$$

Where S_p is the pooled covariance matrix, the two classes can be calculated as follows:

$$S_p = \frac{\sum_{g=1}^{G} (I_g - 1) S_g}{\sum_{g=1}^{G} (I_g - 1)}$$

Where I_g is the number of samples in class g and S_g is the variance covariance matrix for group g. In addition, the pooled covariance matrix is valid if the classes contain similar variance-covariance matrices, otherwise this matrix will not be accurate.

2.3.3 Davies-Bouldin Index (DBI)

Davies-Bouldin index (DBI) is one of the existing indices used in cluster separation. Clustering was a technique used to group data points that are similar according to the chosen similarity metric and well separated. DBI was based on the concept of dispersion of a cluster and diversity between the clusters. DBI will estimate the distance between clusters and their dispersion resulting in the value that represents the quality of the partition. DBI can be expressed according to the following equation[51]:

$$DB_k = \frac{1}{k} \sum_{i=1}^{k} max \left\{ \frac{diam(c_i) + diam(c_j)}{\|c_i - c_j\|} \right\}$$

(max = maximum ($j = 1, ..., k, i \neq j$))

Where, the diameter of the cluster is defined as

$$diam\,(c_i) = \left(\frac{1}{n_i} \sum_{x \in c_i} \left| \left| x - z_i \right| \right|^2 \right)^{1/2}$$

(n_i = number of points, z_i = centroid of the cluster c_i)

A smaller DBI value indicates better cluster quality due the ratio of intra-cluster and inter-cluster similarity[52]. Therefore, this index was minimized when the best number of clusters were defined.

CHAPTER 3
EXPERIMENT

3.1 List of Equipment and Instrument

1. Near-Infrared spectrometer (Thermo Scientific™ Nicolet™ iS5N FT-NIR spectrometer)
2. Perkin Elmer Pyris 1 TGA thermogravimetric analyzer
3. Cutting Board
4. Kitchen utensil
5. Box Container

3.2 List of chemical and materials

1. Pork (Loin, round, collar part)
2. Beef (Loin, round, collar part)
3. Cow Blood

3.3 Sample collection & preparation

Raw beef and pork samples were collected from certified supermarkets including Gourmet market and Tops supermarket in Bangkok, Thailand. There were 3 different parts of beef and pork samples which involved neck part, Loin part, and round part. The chosen meat parts in the study were determined based on the similarity of their physical appearances between pork and beef. Cow blood was purchased from a local butcher (Sapanmai market butcher) in Bangkok, Thailand. It was further used to marinate pork making “fraud” beef.

Sample of the three different parts of fresh beef and pork were prepared prior to further data acquisition. Each part of both beef and pork was cut into 3 square pieces with 3 cm in length but different in thickness which is 0.5 cm, 1 cm, and 2 cm. A total of 9 beef samples and 9 pork samples were used to represent meat of beef and pork, respectively. To generate “fraud” beef, pork samples were marinated preserved in cow blood and stored in a refrigerator at 4^{o}C. The marinated time varied from 6 hours to 72 hours. After marinating, the excess cow blood was eliminated using kitchen tissue papers several times. All the samples further be measured by NIR spectrometer. An overview diagram of sample collection and preparation is shown in Fig 3.1.

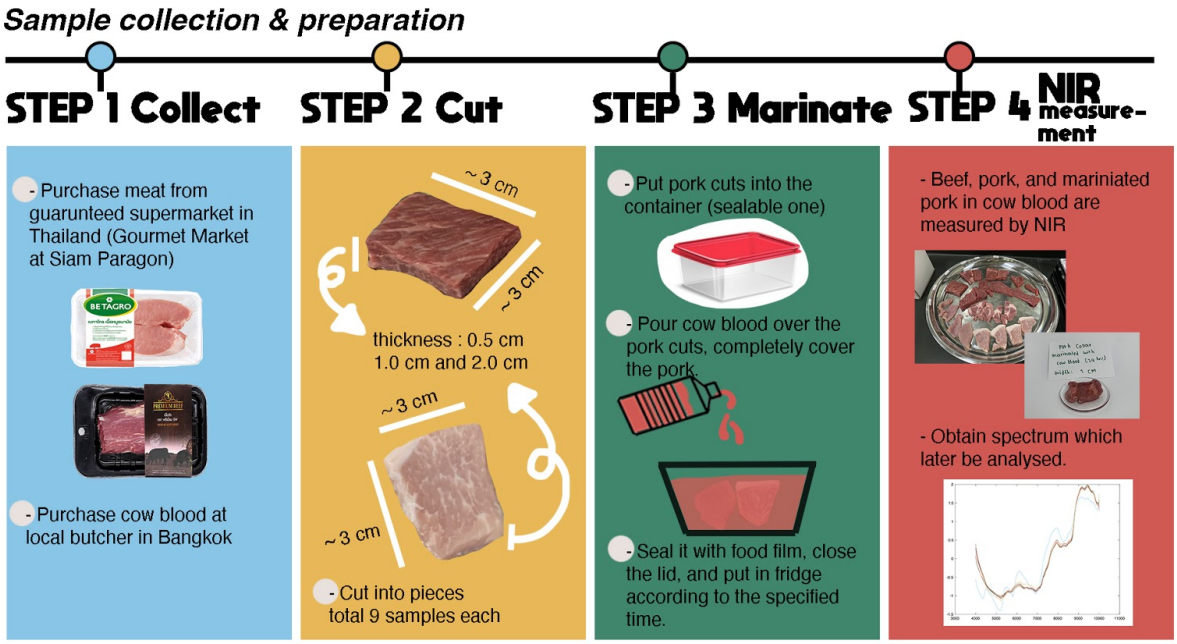


Figure 3.1 Overview scheme of sample collection and sample preparation for beef, pork and "fraud" beef (pork marinated in beef blood)

3.4 Thermogravimetric analysis (TGA)

The thermogravimetric experiments were conducted by using the Perkin Elmer Pyris 1 TGA thermogravimetric analyzer to determine the thermal behaviors of meat samples involving beef, pork and pork marinated with beef blood. All meat samples were prepared in the range of 25 - 60 mg. The TGA system was carried out under the inert condition with nitrogen flow of 20 mL/min. The absorbed water and moisture on the sample were eliminated primarily by isothermal heating the sample at 50°C for 2 minutes. After the isothermal scan, the samples were continuously heated from 50°C to 800°C with the rate of 20°C/min to visualize the thermal degradation behaviors.

3.5 Near infrared spectral data acquisition

The NIR spectra of raw beef, raw pork, and adulterant pork with cow blood were obtained by using Thermo Scientific™ Nicolet™ iS5N FT-NIR spectrometer with extended range indium gallium arsenide (InGaAs) detector, high intensity halogen light source and temperature stabilized solid-state Near-IR diode laser purchased from Thermo Fisher Scientific. The NIR spectra were collected with reflection mode with -log 1/R unit. Each sample was scanned 10 repeated times using a spectral resolution of 16, with reflection mode, and wavenumbers from 4000 to 10000 cm^{-1}. The acquired data were smoothed by the Savitzky-Golay filter (3 windows) and Standard Normal Variate (SNV) in order to eliminate multiplicative interferences of scatter and particle size. After smoothing, the data were treated by chemometric modelling.

3.6 Data analysis

Chemometrics methods were applied to extract out only the interesting variations from the acquired data, and their relations for establishing optimal calibration methods to distinguish the difference between samples. In this study, MATLAB software with in-house programs was used for calculations, visualizing underlying data distribution, classification and prediction. The acquired spectral data were decomposed to divide the variation of NIR spectra into smaller parts, and the effective variations were selected preparatory to further analysis. The steps of data analysis are summarized in Fig 3.2.

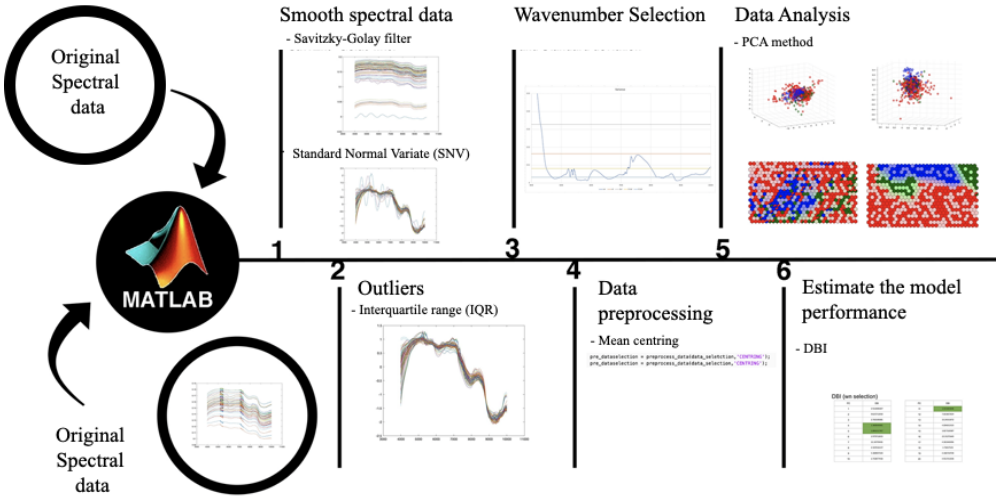


Figure 3.2 Steps of data analysis in the study involving data preprocessing, outlier elimination, wavenumber selection, data visualization and classification.

Step 1: Smoothing spectral data

The raw obtained spectral data were smoothed by the Savitzky-Golay filter with 3 windows sizes. Then, using standard normal variate (SNV) to eliminate the influence of background shift. The standard normal variate is calculated by:

$$X_{i,j}^{SNV} = \frac{(X_{i,j} - \bar{x}_i)}{\sqrt{\frac{\sum_{j=1}^{p} (x_{i,j} - \bar{x}_i)^2}{p-1}}}$$

$X_{i,j}^{SNV}$ = the element of the transformed spectral data

$X_{i,j}$ = the corresponding original element of the spectrum i at variable j

$\bar{X}$ =the mean of spectrum i

P = the number of variables or wavelengths in spectrum

Step 2: Outliers

Due to the variability in the measurement, the data point that is significantly different from others should be eliminated. In this study, the outliers are indicated by using the interquartile range (IQR) which is a measurement of statistical variability based on dividing a

data set into quartile so that is defined as the difference between the 25- and 75-percentile marks. Those data which has values outside 1.5 × interquartile range (IQR) is described as outliers.

Step 3: Wavenumber Selection

Variance and standard deviation was calculated to optimal selection of the appropriate wavenumber for further analysis. Standard deviation and variance are both calculated by using the mean of a group of numbers in obtained data. Standard deviation is calculated as the square root of variance by figuring out the variation between each data point relative to the mean. Variance is a measurement of the broadening between numbers in a data set. It measures how far each number in the set is from the mean and from every other number in the set.

$$S^2 = \frac{\Sigma(x_i - \bar{x})^2}{n-1}$$

S^2 = sample variance
X_i = the value of the one observation
$\overline{X}$ = the mean value of all observations
n = the number of observations

In this study, the wavenumber range selection was based on the SD 0.2. The ranges of selected wavenumber are shown in Fig 3.3

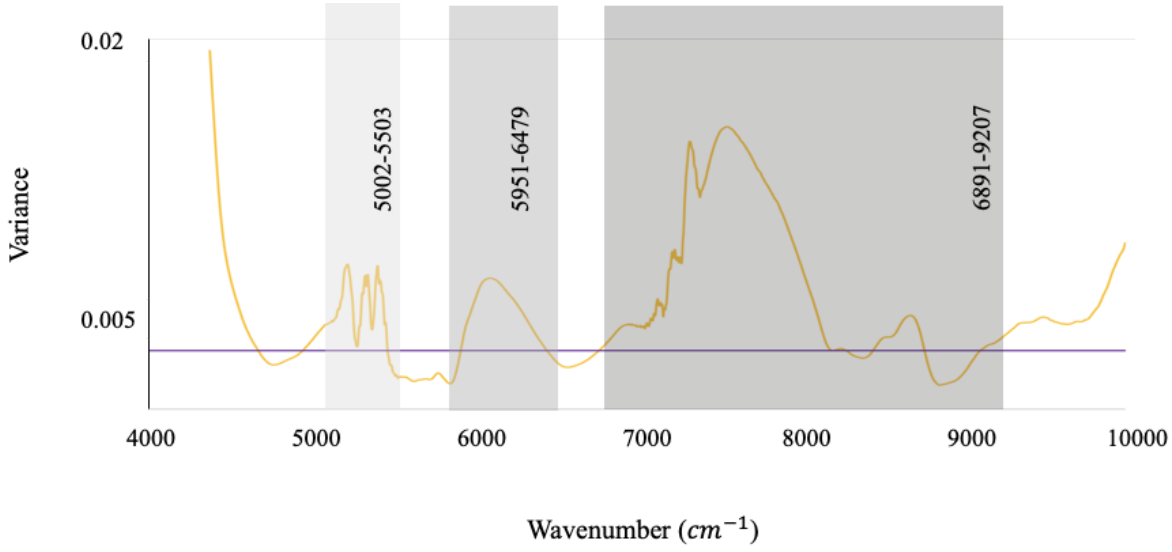


Figure 3.3 Variance of spectral data (cm^{-1})

Step 4: Data preprocessing

There are different types of data preprocessing, such as Standardisation, Log scaling, and Mean centring etc. In this study, Mean centring was used to preprocess the data set. Mean centering subtracts a variable's mean from all observations on that variable in the data set that the variable's new mean is zero. The mean centring in this study is calculated by:

$$(X_{ij} - \overline{X_j})$$

Step 5: Chemometrics analysis

Chemometrics analysis, PCA method with NIPAL algorithm was performed in MATLAB software. It was used as a mathematical transformation of a large set of variables into a smaller one that still contains most of the information, which brings out the loading and scores matrix. The maximum number of principal components were set up to 20 PCs since the first few PCs didn't contain enough information due to the biological samples. In addition, to classify the data set between beef, pork, and marinated pork with cow blood, LDA methods such that a frequently used method for data classification was also performed. It maximizes the ratio of between class variance to the within-class variance in any particular data set.

Step 6 : Estimate the model performance

The calibration model performance was determined using David-Bouldin Index (DBI). Distance between the clusters was measured to specify the quality of partition. Lower DBI indicated better cluster separation. DBI value can be calculated by:

$$DB_k = \frac{1}{k} \sum_{i=1}^{k} max \left\{ \frac{diam(c_i)+diam(c_j)}{\|c_i - c_j\|} \right\}$$

Then the classification accuracy rate of each clustering was evaluated by the correctly classified percentages (%CC). This %CC was obtained using the LDA process which resulted in four indicators, true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The data will be categorized into class data (Cred) and test data (ts). These percentages were denoted as

$$TP = \frac{ts\,(+)}{Cred\,(+)} \times 100$$
$$FP = \frac{ts\,(- \rightarrow +)}{Cred\,(+)} \times 100$$
$$TN = \frac{ts\,(-)}{Cred\,(-)} \times 100$$
$$FN = \frac{ts\,(+ \rightarrow -)}{Cred\,(-)} \times 100$$

Where $ts\,(+)$ is the number of correctly classified for positive cases
$ts\,(-)$ is the number of correctly classified for negative cases
$ts\,(- \rightarrow +)$ is the number of negative cases that were classified as positive cases
$ts\,(+ \rightarrow -)$ is the number of positive cases that were classified as negative cases.
$Cred\,(+)$ is the total number of positive cases
$Cred\,(-)$ is the total number of obtained

CHAPTER 4
RESULT AND DISCUSSION

4.1 Physical appearance and properties of meat

The physical appearance is the simplest choice to be used to discriminate between types of meat. In the case of beef and pork, from the physical appearance, it is clearly seen that the color of pork is paler and more pinkish while the color of beef is cherry red. Moreover, the fat layer permeated beef and pork is significantly different. In the case of beef, fat is marbled and infiltrated in the meat whereas fat in pork has less marbled and less infiltrated fat. Generally, the fat is separately layered on the outer shell of pork as already discussed in Chapter 2. The overview of pieces of beef and pork used in the study is shown in Fig 4.1. Different parts of meat could have different appearances including colours, texture and amounts of fats. Thus, in this study, three different parts of pork and beef including loin, collar, and round are discussed together with marinated pork with cow blood.

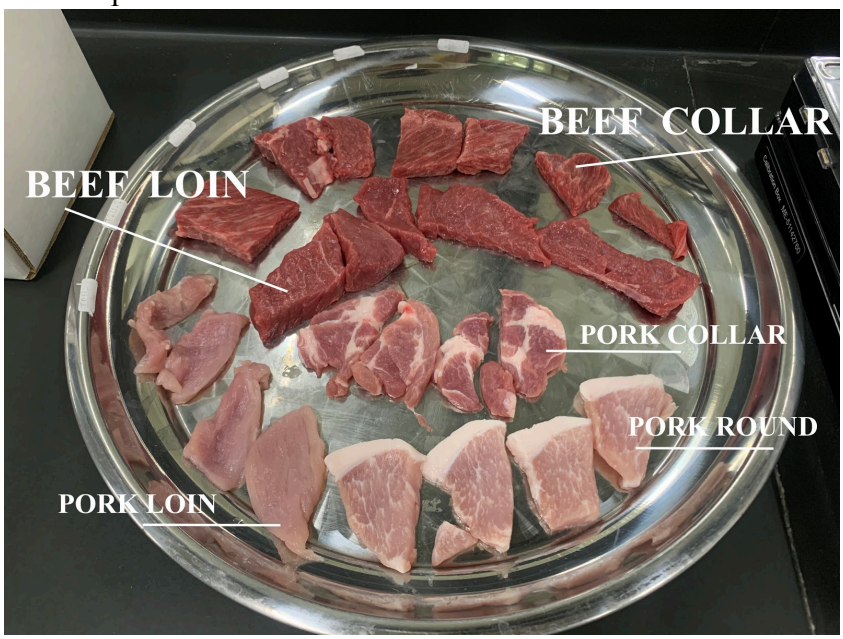


Figure 4.1 Plate with pieces of beef loin and beef collar (top) and pork loin, pork round and pork collar (bottom)

4.1.1 Comparison of meat in loin part

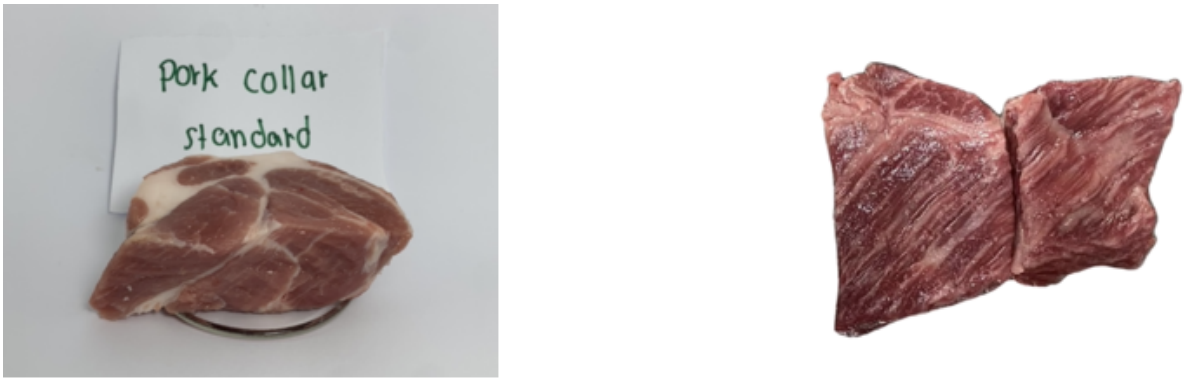
The loin was the part of meat collected from the cut which runs along from the shoulder to the legs. This loin part is the leanest area which composes less amount of fat compared to the other part. Beef and pork loin cuts in the study are shown in Fig 4.2. As seen in the illustrated figure, both of the meat contains less fat. The prominent difference is the color of meat that color of pork is paler and more pink compared to beef. Also, the intermuscular fat mostly appears in both beef and pork.

(A) (B)

Figure 4.2 (A) Piece of pork loin and (B) piece of beef loin

4.1.2 Comparison of meat in collar or chuck part

For pork, it is called a collar which is located between the neck and shoulder part of the pig. It has a darker pink color in comparison to the other parts. Pork collar is one of the meat part consisting of the most filled fat including both intermuscular (between muscles) and intramuscular (marbled in muscles) fat. Pork collar is shown in Fig 4.3 (A) On the other hand, a similar cut area to pork collar in cows is normally called a chuck. The piece of beef is cut including parts of the neck, shoulder blade, and upper arm of cows. The colour of the chuck part presents as a dark cherry red. Beef chuck is illustrated in Fig 4.3(B). It is clearly seen that a large amount of fat is marbled in the chuck part especially for intramuscular fat.



(A) (B)

Figure 4.3 (A) Piece of pork collar and (B) piece of beef chuck

4.1.3 Comparison of meat in round part

The round part of both beef and pork is the rear leg of pig and cow. Generally, this round part was frequently used to move the animal body. Therefore, the meat of the round part is quite lean but tougher. However, it still contains some fats infiltrated in tissue both in beef and pork but they are in very small contents. From physical appearance, pork round has a pink colour while the beef round is darker red as shown in Fig 4.4.

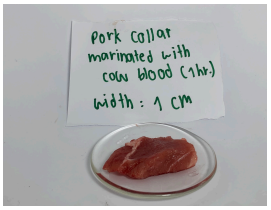
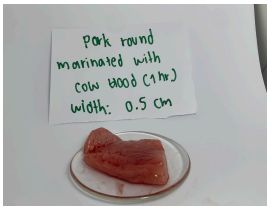
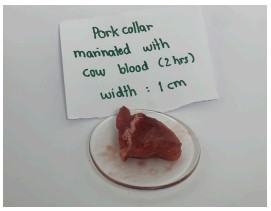
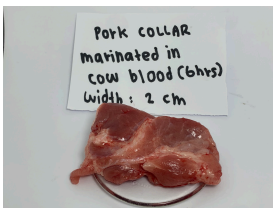
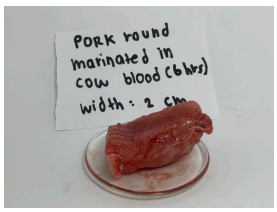
(A) (B)

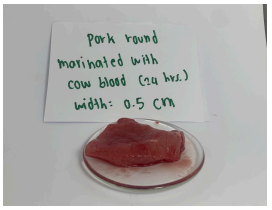
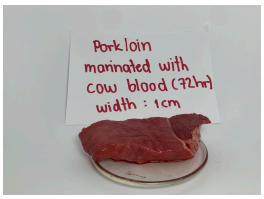
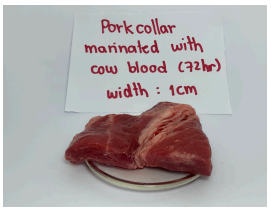
Figure 4.4 (A) Piece of pork round and (B) piece of beef round (beefitswhatsfordinner.)

4.1.4 Difference between marinated pork at various times and beef

As mentioned in the previous section, the significant difference between beef and pork is colour. In order to make the "artificial" beef from pork, the cow blood is used to marinate pork at various times. The comparison of physical appearance of the marinated pork with cow blood and beef at different preservation times is shown in Table 4.1. In the case, the excess cow blood stained on pork is removed by using kitchen tissue paper several times before capturing photos.

Table 4.1 Marinated pork with cow blood at various times.

Preservation time	Loin part	Collar part	Round part
1 hour			
2 hours			
6 hours			

24 hours			
72 hours			

As shown in Table 4.1, the colour of marinated pork tends to change over a prolonged time. The longer time used to incubate pork in cow blood, the darker red colour pork becomes. Color changes in meat could be classified into 3 periods. In the first period, 1-6 hours of the preservation time, the colour of pork initially turned to red-wine. However, the colour was changed, especially on the surface of meat. It suggests that the beef blood did not completely diffuse in the meat, therefore, it looks like blood-coating on pork rather than looking like "artificial" beef. Following the second period (6 -12 hours), the meat colour tends to be darker red as the beef blood seems diffused in the meat not only on the surface. However, the beef blood still did not diffuse into the fat layer of pork. Lastly, in the third period of time (> 24 hours), it shows that the beef blood was completely diffuse in the pork meat and fat layer. Therefore, the physical appearance of marinated pork is similar to the beef as shown in Fig.4.5. In the study, a marinated pork with beef blood for 72 hours is used as "artificial beef" in the further analysis.

Figure 4.5 Marinated pork with cow blood for 72 hours.

4.2 Thermogravimetric Gravity Analysis (TGA)

As mentioned in section 2.1, the different meat shows the different pattern of chemical compositions and also different chemical contents. In the section, thermogravimetric analysis is used to reveal the thermal degradation profiles of beef, pork, and marinated pork as shown in Fig.4.6. The thermal profiles are directly correlated to the amount of chemical contents represented in the meats. From the thermal profile, it shows 3 degradation steps involving the moisture, amino acids and polymeric chain of fats and proteins. In the first state of degradation at 100-200°C, over 60% of sample weight relates to moisture on the meat surface and inside the meat. A slightly different weight loss in samples during the first state of decomposition could be caused from the difference of the amount of water adsorbed in meat. In the second degradation state, it involves the degradation of free amino acids which was initially observed at 300°C. During the stage, the co-existed degradation of the polymeric chain of proteins and fats was also observed at ~400°C. The thermal profile at 300-400°C shows significantly different patterns between meats. The thermal decomposition of meat samples is stable at 450°C such that all the samples turn into carbon black with ~10% of sample weight. From the thermal profile, it shows high possibility that the spectroscopic technique could be used to differentiate the chemical contents presented from different meats.

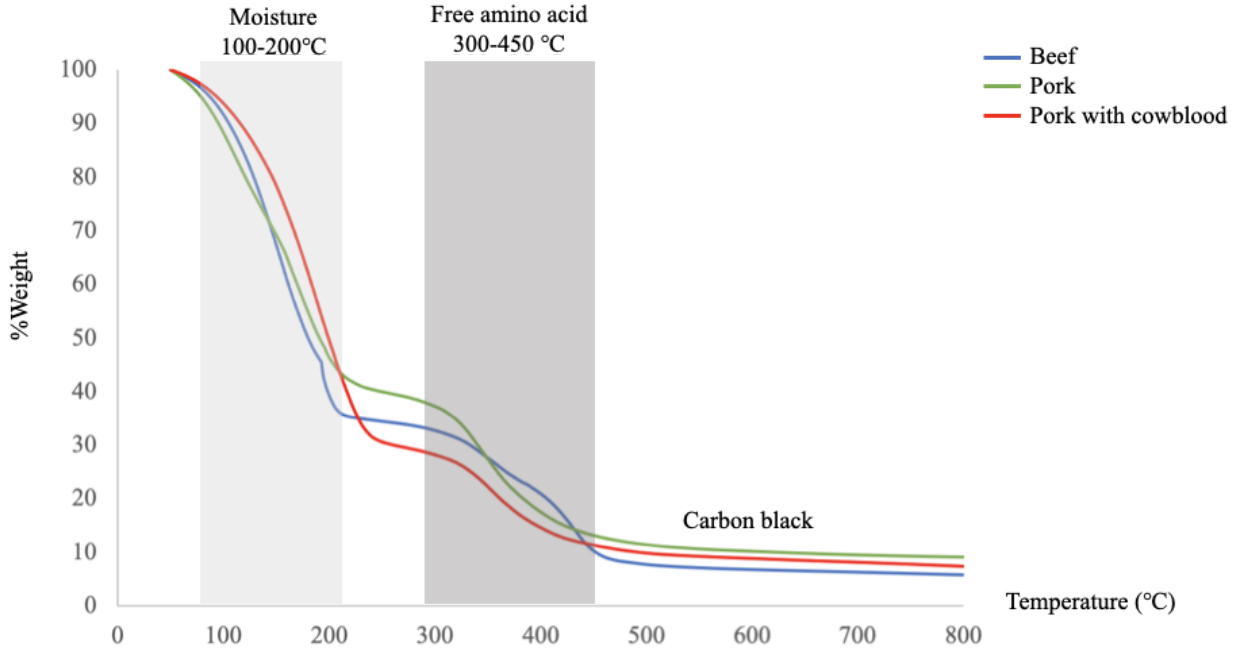


Figure 4.6 Thermal degradation profiles of meat samples beef, pork and pork marinated with cow blood representing in blue, green and red solid line, respectively

4.3 NIR spectra of meats

The acquired NIR spectral data in reflection mode not only contain the molecular information from samples but also include the irrelevant external effects (e.g. human errors, sample placement, and outside incident light). Therefore, the original NIR spectral data must be preprocessed in order to minimize the irrelevant information and maximize the sample information. The step is necessary and is required prior to further analysis by chemometric

modelling. There are various preprocessing techniques which are appropriate to different kinds of data. In this study, the Savitzky-Golay filter, Standard Normal Variate (SNV), interquartile range (IQR) criterion, and Mean Centring are applied to pretreat the original spectral data. Firstly, the original spectral data is smoothed by the Savitzky-Golay filter with 3 windows sizes (~size of ~12 cm^{-1}) to reduce the random noises. The smooth data is shown in Fig 4.7(A). However, it still represents the strong background shift which is incompatible for the data analysis therefore standard normal variate is applied to eliminate the scattering effects from the non-uniform surface roughness of the sample. Figure 4.7(B) presents the data after pretreating with smoothing and standard normal variate. Nevertheless there still contain a few outliers appearing on the data which significantly show different spectral patterns compared to others, the interquartile range (IQR) is performed to remove the outliers. Figure 4.7(C) illustrates the preprocessed spectral which is ready for further data analysis by several chemometrics methods.

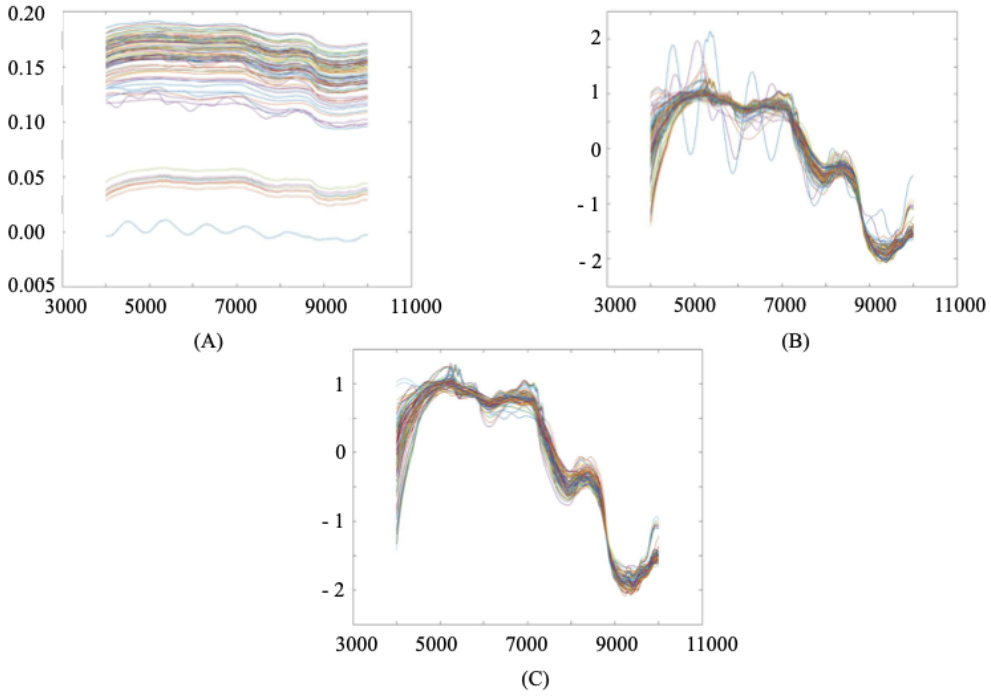
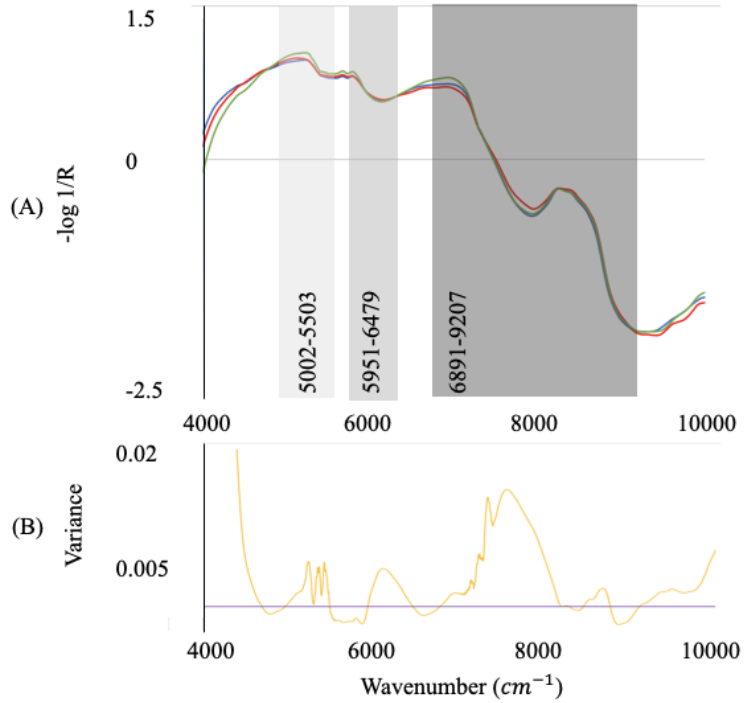


Figure 4.7 (A) Smoothed NIR spectral data (B) NIR spectral data after performing standard normal variation(SNV) (C) NIR spectra after removing outliers using interquartile range (IQR) criterion

4.4 Data Visualization

NIR spectra of all meat samples (beef, pork and marinated pork) after applying the preprocess method was shown in Fig. 4.7C. It can be clearly seen that there are some regions which show the different NIR patterns due to the sample chemical compositions. These differences of chemical compositions in each type of meat were already proved by TGA in section 4.2. To determine the significant NIR regions, the variance of the NIR spectra (from 3 meats) was calculated as shown in Fig 4.8B. The overtone regions which provide a high variance with 95% significant level was selected as regions for meat discrimination. Fig. 4.8A illustrated the region corresponding to a specific band assignment. The reflection bands at 5002 - 5503 cm^{-1} are the first overtone of O-H stretching of water (moisture content)[49]. The reflection bands at

5981 - 6479 cm^{-1} are the first overtone of C-H stretching of fatty acids (fat)[49, 52]. The reflection bands at 6819 - 8207 cm^{-1} and 8485 - 8797 cm^{-1} were corresponding to the second N-H overtone of protein and the second overtone of O-H [24, 53].



Wavenumber (cm^{-1})	Band assignment	Reference
5002-5503	O-H 1st stretching (moisture)	43
5951-6479	C-H 1st stretching (Fatty acids/ Fats)	43,46
6891-9207	N-H 2nd stretching (Protein)	46
8485-8797	O-H 2nd stretching (moisture)	20

(C)

Figure 4.8 (A) average NIR spectrum of beef (blue), pork(green) and marinated pork (red). (B) the variances calculated from NIR spectra of all meat samples. (C) Table summarize the band assignment of NIR spectral regions for meat discrimination

To visualize the cluster of meat samples, principal component analysis (PCA) was used. In samples with complex structures such as biological samples, food, meat, agricultural product, it is not necessary that the first few PC components can be used to demonstrate the cluster of samples. In these cases, the first few PCs might represent the common chemical compositions in the sample, for example, polymeric peptide chain and protein are the major chemical components in all three types of meats. It might not be possible to use them to discriminate the meat samples. It is more appropriate to use the latter PC to cluster the samples as they might correspond to minor components such as free amino acid, lipid, which are high potential to discriminate class of samples. In the study, the DBI index was used to determine the potential PC to discriminate type of meat from NIR spectra. The lower DBI value , the better discriminator presents. Figure 4.9A shows the DBI value of each PCs. It was found that PC4, PC5 and PC11 have the lowest values with 1.36, 1.08 and 1.53, respectively, compared to the other PCs. Therefore, they were chosen to be used for visualization.

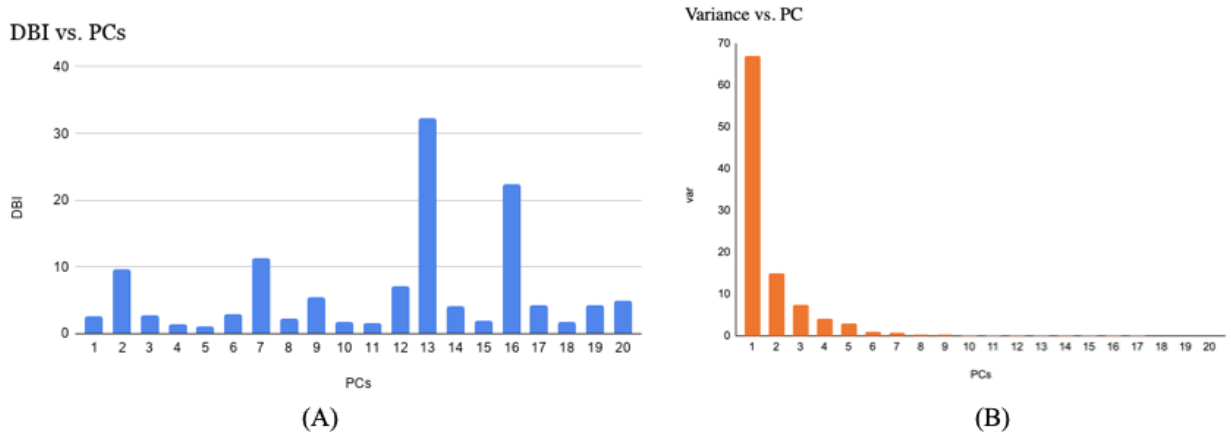


Figure 4.9 The histogram demonstrate (A) DBI values of each PC (B) Variance of each PCs

The PC score plots were used to classify the pork samples and beef samples in the form of 3 dimensions map, the map was built based on PC [4 5 11]. The comparison of cluster separations of the samples on the score plot from PC[1, 2, 3] represent the PC with highest variances corresponding to major chemical components and the score plot from PC[4, 5, 11] relating to minor components with the lowest DBI value are shown in Fig. 4.10

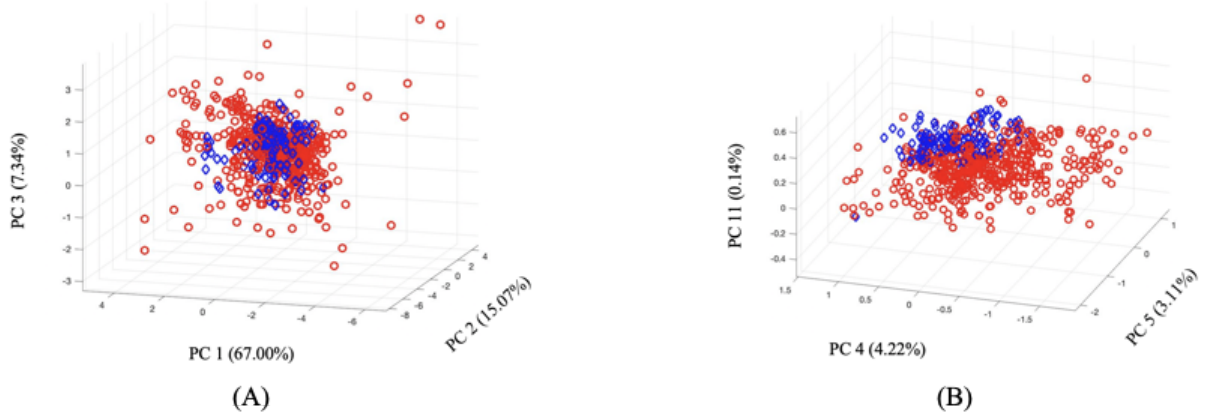


Figure 4.10 PC score plot calculated form NIR spectra of beef (blue) and pork (red) sample using (A) PC1, PC2 and PC3 (the highest variance) and (B) PC4, PC5 and PC11 (the lowest DBI value)

In PC score plot, the blue spots represent beef samples and red spots represent pork samples including both marinated in cow blood and pure standard pork meat. It can be seen that pork and beef samples could be differentiated as the clusters were clearly separated from each other in PC4, PC5 and PC11 rather than using PC1, PC2 and PC3. This suggests that the developed method to select the appropriate PC by DBI value is successfully used to visualize clusters of the samples. The correctly classified percentages (%CC) obtained from the LDA process were used as classification rate index. The %CC will indicate the accuracy of the sample prediction. The pork samples were indicated as the positive cases and beef samples were

indicated as the negative cases. The four indicators will be calculated, true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The number of correctly classified for positive cases was defined as TP, and the number of negative cases that were classified as positive cases was defined as FP. For TN is the number of correctly classified for negative cases, and FN is the number of positive cases that were classified as negative cases. Table 4.2 showed the correctly classified percentages for PC[1 2 3], and Table 4.3 showed the correctly classified percentages for PC[4 5 11].

Table 4.2 Contingency table to demonstrate the classification performance of the data using PC1, PC2 and PC3 using Linear Discriminant Analysis (LDA) as a discriminator

		TRUE	
	PC [1 2 3]	**Pork (+)**	**Beef (-)**
Prediction	Pork (+)	65.14% (TP)	34.86% (FP)
	Beef (-)	25.76% (FN)	74.24% (TN)

Table 4.3 Contingency table to demonstrate the classification performance of the data using PC4, PC5 and PC11 using Linear Discriminant Analysis (LDA) as a discriminator

		TRUE	
	PC [4 5 11]	**Pork (+)**	**Beef (-)**
Prediction	pork(+)	79.48% (TP)	20.52% (FP)
	Beef (-)	11.36% (FN)	88.64% (TN)

The %CC of the PC[4 5 11] was higher than PC[1 2 3] on both TP and TN value. Higher TP and TN value indicated higher classification performance of NIR and proved that the chosen PCs were appropriate PCs needed in beef and pork classification. This could suggest that the NIR technique combining with chemometrics method might become a feasible method in discriminating counterfeit beef.

CHAPTER 5
CONCLUSIONS

Due to the spreading of counterfeit beef (pork marinated in beef blood) in the local buffet markets, this is a severe problem especially for Islam and Judaism persons who are unpermitted to consume pork. The high performance methods such as chromatography and DNA gel electrophoresis have been used to differentiate types of meat. However, these methods involve complicated sample preparation, consuming long analysis time and require a high-cost instrument. In the study, we propose the combination of NIR spectroscopy and multivariate data analysis (Chemometrics) to feasibly discriminate types of meat and to classify counterfeit beef out of "real" beef. The counterfeit beef was generated by marinating pork with cow blood for 72 hours until the physical appearances of the meat are insignificantly different. Thermal Gravimetric Analysis (TGA) was performed to obtain the thermal profile of the degradation of chemical compositions in meat. It was found that the thermal profiles of beef are significantly different from the pork at 300-400ºC which are corresponding to the thermal degradation of free amino acid and long chain of peptide and lipid. The chemical patterns of meat were collected by using NIR spectrometers with reflection mode. The NIR spectra were collected using a scale of -log(1/R) in the range of 4000 – 10000 cm^{-1} with resolution of 8 cm^{-1} and the number of scans equal 16. Prior to data analysis, the NIR spectral data was pre-treated with Savitzky-Golay smoothing and standard normal variate in order to eliminate the random noise and the scattering effect from the non-uniform roughness of sample surface, respectively. Moreover, the outliers were removed by using interquartile range (IQR). The significant wavenumbers were selected at 5002-5503 cm^{-1} (the first overtone of O-H stretching of water or moisture content) , 5951-6479 cm^{-1} (the first overtone of C-H stretching of fatty acids/fat), 6819-8072 cm^{-1} (the second N-H overtone of protein), 8485-8789 cm^{-1} (the second overtone of O-H of water) by using variance.

To visualize the clusters of meat, principal component analysis (PCA) was used to reveal the underlying relation of the NIR spectra data. In the study, we develop the automatic algorithm to select the best PC as the discriminator by using David-Bouldin index (DBI) value. The PC4, PC5 and PC11 were selected from the top 20 PCs as the best discriminator PC to reveal the clusters of meat. From the score plot, the clusters presented in PC4-5-11 are more promising than the clusters presented in the top 3 PCs (PC1-2-3). Moreover, the linear discriminant analysis (LDA) was used to estimate the class (type of meat) of an unknown sample. It shows that the correctly classified percentages (%CC) of the PC4-5-11 are raised up to 79.48% and 88.64% for prediction of pork and beef, respectively when the score data of PC4-5-11 was used. This could be considered that the developed method by NIR coupled with data analysis is a practical technique to differentiate counterfeit beef.

REFERENCES

1. Agricultural and livestock department, Thailand. Beef cattle farmers in Thailand. *Agricultural and livestock statistics 2017*. **2017**
2. Prachachat news. Northern Beef Cattle species development. *Economic news* [online]. Feb 22, 2010, https://www.prachachat.net/local-economy/news-293006. (accessed Nov 1, 2020)
3. Bangkok Bank. SME in focus. **2019**. https://www.bangkokbanksme.com/en/cow-aec (accessed Nov 1, 2020)
4. Ministry of commerce, Thailand. Thai beef cattle, an opportunity for Thailand livestock in AEC. *Meat and agricultural products prices.* **2020**
5. Promotion to you page. https:// www.promotiontoyou.com/2017/02/promotion-bar-b-q-plaza- refill-5-bbq-plaza-buffet/ (accessed Nov 1, 2020)
6. Post Today. How to Determine Counterfeit beef. *Lifestyle news* [online]. July 6, **2020**, https://www.posttoday.com/life/healthy/627731 (accessed Nov 1, 2020)
7. Jitnupong W.; Rungmee S.; Penchan N.; Thalungraung W. Alternative: Pork detection method by real time PCR. *49th Conference of Kasetsart university (Agricultural department)*. **2013**, 665-672
8. Muslim Thai Post. Outbreak of Counterfeit beef. *Lifestyle news* [online]. July 2, **2020**, http://news.muslimthaipost.com/news/34356 (accessed Nov 1, 2020)
9. The National News. Ask Ali: Why pork is forbidden for Muslim. *Art & Culture news* [online]. Dec 14, **2020**, https://www.thenationalnews.com/arts-culture/ask-ali-why-pork-is-forbidden-for-muslims-1.444442#:~:text=The%20main%20reason%20pork%20is,declared%20haram%2C%20which%20means%20forbidden. (accessed Nov 1, 2020)
10. Garage A.M. Eating according to religious Practices: Kosher and Halal. *Gordon Food Service* [online]. https://www.gfs.com/en-us/ideas/eating-according-religious-practices-Kosher-and-halal (accessed Nov 1, 2020)
11. National Food Institute, Thailand. Beta-Agonist. http://www.nfi.or.th/foodsafety/upload/damage/pdf/beta-agonist.pdf (accessed Nov 1, 2020)
12. Jorfi R.; Mustafa S.; CheMan Y.B.; Hashim D.B.M.; Sazili A.Q.; Farjam A.S.; Nateghi L.; Kashiana P. Differentiation of pork from beef, chicken, mutton and chevon according to their primary amino acids content for halal authentication. *African journal of Biotechnology.* **2012**, *11 (32),* 8160-8166
13. Mandi J.; Fatimi I.El.; Seddaoui N.; Amine A. Enzyme immunoassay (ELISA/immunosensor) for sensitive detention of pork adulteration in meat. *Food Chemistry.* **2018**, *255*, 380-389
14. Arslan A.; Iihak O.I.; Calicioglu M.; Karahan M. Identification of meats using random amplified polymorphic DNA (RAPD) technique. *Journal of Muscle Foods.* **2005**, *16,* 37-45
15. Dearing T. Fundamentals of Chemometrics and Modeling [online]. https://depts.washington.edu/cpac/Activities/Meetings/documents/DearingFundamentalsofChemometrics.pdf (accessed Nov 1, 2020)
16. Mitsumoto M.; Maeda S.; Mitsuhashi T.; Ozawa S. Near-infrared spectroscopy determination of physical and chemical characteristics of beef cuts. *Journal of food science.* **1991**, *56,* 1493-1496

17. Isaksson T.; Nilsen B.N.; Tørgersen G.; Hammond R.P.; Hildrum K.I. On-line, Proximate analysis of ground beef directly at a meat grinder outlet. *Meat Science.* **1996**, *43*, 245-253
18. Tørgersen G.; Isaksson T.; Nilsen B.N.; Bakker E.A.; Hildrum K.I. On-line NIR analysis of fat, water and protein in industrial scale ground meat batches. *Meat Science.* **1999**, *51,* 97-102
19. Kuswadi B.; Cendekiawan K.A.; Kristiningrum N.; Ahmad M. Pork adulteration in commercial meatballs determined by chemometric analysis of NIR spectra. *Journal of food measurement and characterization.* **2015**, *9,* 313-323
20. Barbin D.F.; ElMasry G.; Sun D.W.; Allen P. Non-destructive determination of chemical composition in intact and minced pork using near-infrared hyperspectral imaging. *Food chemistry.* **2013**, *128,* 1162-1171
21. Prieto N.; Juárez M.; Dugan M.E.R.; Zijlstra R.T.; Aalus J.L. Near infrared spectroscopy on ears to classify pigs based on carcass and fat composition. *Meat Science.* **2016,** *112,* 183
22. Bender A. Meat and meat products in human nutrition in developing countries. *Food and Nutrition paper 53 (FAO).* **1992**
23. Pereira P.M.C.C; Vincente A.F.R.B. Meat nutritional composition and nutritive role in human diet. *Meat Science.* **2013**, *93,* 586-592
24. Agriculture and Consumer Protection department, USA. Composition of meat [online]. http://www.fao.org/ag/againfo/themes/en/meat/backgr_composition.html#:~:text=Meat%20is%20composed%20of%20water,and%20small%20quantities%20of%20carbohydrates (accessed Nov 1, 2020)
25. Williams P. Nutritional Composition of red meat. *Nutrition & Dietetics*. **2007**, *64,* S114-S119
26. Judprasong K.; Puwastien P.; Rojroonwasinkul N.; Nitithamyong A.; Sridonpai P.; Somjai A. Thai Food composition database. *Institute of Nutrition Mahidol university.* **2015**
27. Szalay J. What is protein. *Live science contributor.* **2015**
28. Wyness L.; Weichselbaum E.; O'Connor A.; Williams E.B.; Benelam B.; Riley H.; Stanner S. Red meat in diet: an update. *Nutrition Bulletin.* **2011**, *36*
29. Sakomura N.K.; Ekmay R.D.; Mei S.J.; Coon C.N. Lysine,methionine, phenylalanine, arginine,valine, isoleucine, leucine, and threonine maintenance requirements of broiler breeders. *Poultry Science.* **2015**, *94,* 2715-2721
30. Ahmad R.S.; Imran A.; Hussain M.B. Nutritional composition of meat. *Meat Science and Nutrition.* **2018,** 61-74
31. Lonergan S.M.; Topel D.G.; Marple D.N. Chapter 5 - Fat and fat cells in domestic animals. *The science of animal growth and meat technology (second edition).* **2019,** 51-69
32. Boxgum Grazing. Pork neck [online]. https://boxgumgrazing.com.au/products/pork-neck (accessed Nov 1, 2020)
33. Dixon J. What is marbling. *The telegraph lifestyle* [online]. Mar 11, **2017,** https://www.telegraph.co.uk/food-and-drink/scotch-beef/what-is-marbling/ (accessed Nov 1, 2020)
34. Enser M.; Hallett K.G.; Hewett B.; Fursey G.A.; Wood J.D.; Harrington G. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Science.* **1998,** *49*, 329-410

35. Woods V.B.; Fearon A.M. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: a review. *Livestock Science.* **2009,** *126,* 1-20
36. Ministry of Agriculture, Fisheries and Food (MAFF). Fatty acid compositions. **1998**
37. Simpson R.J.; Mckie A.T. Regulation of intestinal iron absorption: The mucosa takes control? *Cell Metabolism.* **2009,** *10,* 84-87
38. Qu J.H.; Liu D.; Cheng D.W.S.; Ma J.; Pu H.; Zeng X.A. Applications of near infrared spectroscopy in food safety evaluation and control: a review of recent research advances. *Critical reviews in food science and nutrition.* **2015,** *55*, 1939-1954
39. Huang H.; Yu H.; Xu H.; Ying Y. Near-Infrared spectroscopy for 0n/in-line monitoring of quality in foods and beverages: A review. *Journal of food engineering.* **2008,** *87*, 303-313
40. Kucham C.T.; Ngadi M.O. Rapid assessment of pork freshness using miniaturized NIR spectroscopy. *Journal of food measurement and characterization.* **2000**
41. Reddy H.; Dinakaran S.K.; Srisudharson; Parthiban N. Near-infrared spectroscopy - an overview. *International journal of ChemTech research.* **2011,** *3*, 825-836
42. Eldin A.B. Near infrared spectroscopy. *Sigma Pharmaceutical Crop. (Egypt)*. [online] https://cdn.intechopen.com/pdfs/23738/InTech-Near_infra_red_spectroscopy.pdf (Accessed Nov 1, 2020)
43. Cozzolino D.; Murray I. Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. *Food science and Technology.* **2004,** *37,* 447-452
44. Morsy N.; Sun D.W. Robust linear and non-linear models of NIR spectroscopy for detection and quantification of adulterants in fresh and frozen-thawed minced beef. *Meat Science.* **2013,** *93,* 292-302
45. Shenk J.S.; Workman J.J.; Westerhaus M.O. Application of NIR spectroscopy to agricultural products. *Handbook of Near-infrared analysis.* **2001,** 419-474
46. Dumalisile P.; Manley M.; Hoffman L.; William P.J. Near-Infrared (NIR) spectroscopy to differentiate longissimus thoracis et lumborum (LTL) muscles of game species. *Food analytical methods.* **2020**
47. Zu X.; Zhao J.; Povey M.J.W.; Holmes M.; Hanpin M. Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta.* **2010,** *667,* 14-32
48. Fan Y.; Liao Y.; Cheng F. Predicting of intramuscular fat content in pork using near infrared spectroscopy and multivariate analysis. *International Journal of foddering properties.* **2018,** *21*
49. Wongrevee K.; Ishigaki M.; Ozaki Y. Chapter 10 Chemometrics as a green analytical tool. *Challenges in green analytical chemistry: 2ndd edition.* **2020,** 277-336
50. Raschka S. Linear Discriminant analysis {online] https://sebastianraschka.comArticles/2014_python_lda.html (accessed Nov 1, 2020)
51. Saitta S.; Raphael B.; Smith I.F.C. A bounded index for cluster validity. *Lecture note in computer science*. **2007,** 174-187
52. Singh A.K.; Mittal S.; Malhotra P.; Srivastava Y.V. Clustering evaluation by Davies-Bouldin Index (DBI) in cereal data using K-means. *Fourth international conference on computing methodologies and communication.* **2020,** 306-310

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