In-plant, non-invasive spectral imaging for the prediction of lamb meat quality attributes

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Abstract

Muscle foods such as meat are a perishable, nutritious, relatively expensive food commodity, a great source of human nutrition and are a large part of the New Zealand economy, as well as overseas. Currently, New Zealand's meat producing companies measure meat quality attributes by using a different technology for every trait, with no overarching way to combine them, with many of the technologies requiring collection and destruction of the product. There is a desire by the meat industry to find a single way to measure and compare meat quality parameters in a single process or technology. The development of an in-line (within the normal production line of an abattoir or meat processor), real time, non-destructive quality control system could help define multiple meat traits in a way that can guarantee the product in terms of composition, safety and consistency. These guarantees not only help the producer to ask a higher premium for their product, but also give assurances to the consumer that they are getting exactly what they are expecting and paying for.

This thesis focussed on determining whether the spectral imaging technologies of near infrared and hyperspectral imaging, and relevant pre-processing and modelling techniques were suitable for use in an in-plant situation for the prediction of lamb meat quality attributes.

Data was collected on 2511 lambs from 10 separate kills. The lambs were slaughtered through three abattoirs owned by Alliance Group Limited with near infrared and hyperspectral imaging of intact *M. Longissimus thoracis et lumborum* muscle surface collected at 24 hours *post-mortem.* Traditional meat quality measurements were also collected; tenderness using a MIRINZ tenderometer, CIELab colour using a CR-400 colour meter, ultimate pH using an Eutech Cyberscan pH 300 meter, marbling using subjective scoring by trained personnel and intramuscular fat content using gas chromatography – flame ionisation detector. The resulting data were split and used to generate calibration and validation data sets. The calibration data was used together with the spectral data that was processed using a variety of chemometric techniques including partial least squares, variable selection and neural networks to generate predictive models. The accuracy of the predictive models was then tested using the validation data set.

This work found that not all meat quality traits were able to be predicted accurately and certain techniques worked better for differing traits. The best predictive models for ultimate pH using the near infrared and hyperspectral data achieved R^2 values (a measure of goodness of fit) from the validation data sets of 0.63 and 0.48 respectively. For near infrared the best predictive models were achieved using partial least squares with pre-processing (standard normal variate, orthogonal signal correction and mean centring) applied, while for hyperspectral imaging neural networks provided the best model using a decay of 0.00004 and a node size of 2. The best predictive models for intramuscular fat using the near infrared and hyperspectral data achieved R^2 values from the validation data sets of 0.56 and 0.75 respectively. For near infrared this was achieved using partial least squares with pre-processing (normalisation, multiplicative scatter correction and mean centring) applied, while for hyperspectral imaging neural networks provided the best model using a decay of 0.0009 and a node size of 4. This performance of these two traits in particular, shows that that the prediction abilities are of a quality that future work on implementing these into an in-line system at a pilot scale should be considered.

Overall, the use of novel modelling techniques such as neural networks showed potential to increase the predictive abilities of the resulting models, over more traditional modelling techniques. Additionally, it was demonstrated that the number of predictors needed to create a calibration model could be reduced, increasing the speed of analysis with only minimal loss in the accuracy of the resulting model.

Results obtained during this study suggest that the calibration models are not abattoir dependent and the transfer of one calibration model to multiple abattoirs could decrease the costs and allow for faster development and implementation of an in-line, in-plant system.

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Figures, tables and equations

1 Introduction

Adding value through quality: The need for monitoring quality in the meat industry

Muscle foods such as meat are a perishable, nutritious, relatively expensive food commodity, a great source of human nutrition and are a large part of the New Zealand economy, as well as overseas. A major push for the meat industry today is to find an in-line, real time, non-destructive quality control system that can help define meat properties in a way that can guarantee the product in terms of composition, safety and consistency. These guarantees not only help the processor and producer to ask a higher premium for their product but also give assurances to the consumer that they are getting exactly what they are expecting and paying for.

Defining the quality of a food product is one of the most common and complicated issues to solve when a new product comes onto the market. Food quality has many different meanings but can generally be described as "the characteristics of food that are between certain limits of acceptance in every step of manufacturing, from the raw materials to the acceptance of consumers" (Federico, 2013). The problem with this sort of definition is that the term quality is not immutable and can have a different meaning to people at different stages of the production/consumption chain. It also shows that quality refers to not just the physical product properties themselves but their perceptions by the consumer. Quality attributes can include carcass composition and conformation, palatability, freshness, health concerns and safety from disease and spoilage (Becker, 2001). For the majority of consumers the definition of quality is in regards to eating quality, with tenderness being the most important attribute (Koohmaraie & Geesink, 2006).

Monitoring quality: the challenges in the meat industry

Quality has many definitions and meanings making it very hard to define what explicitly quality is. This lack of definition has made it difficult for the meat industry to develop an objective measure. New Zealand is the world's largest lamb meat exporting country, exporting 95% of lamb produced annually (BLNZ, 2015). Meat processors use a number of different carcass features in an attempt to define the quality of the meat they are processing, with ovine carcass evaluation methods in New Zealand

including such measurements as carcass weight and GR fat scores (fat content based on measurement of total tissue depth over the 12th rib at a point 11cm from the midline of the carcass). Other measurements may include lean meat yield, shape and fat cover which are used to rank their product into groupings of best to worst quality and then indirectly relate that back to the eating quality the consumers will experience. The relationship between carcass grading and meat quality is tenuous but currently used as a proxy for quality, hence the need for better measurements that are as fast as the current grading system. Fat colour and marbling have also been considered and are being, or have been investigated also for this purpose.

These are all able to be measured or predicted in plant and in-line, creating the ability to quickly manage and utilise this information to sort the carcasses into the production lines that they are best suited for. The issue that arises is that these measures do not correlate well to actual meat eating quality as perceived by sensory and consumer panels (Moore *et al.*, 2012).

When looking to purchase meat, traditionally, consumers will look for indicators or clues as to how tender and delicious the meat will be once cooked. The most often used indicators are the colour of both the lean portion of the muscle and of the subcutaneous fat, as well as the amount of intramuscular fat (marbling) (Warriss, 2000). These attributes are often regionally specific so while one attribute might be favourable in one country, such as highly marbled meat in Kobe beef steaks in Japan, it may be undesirable in another that looks for more lean and low fat meat. As people are becoming more interested in where their food comes from, other factors that consumers consider are coming into focus (Figure 1) with things like grass-fed vs grain-fed, organic, ethically treated animals also playing a part in consumer purchasing decisions.

On farm practices, animal background and handling from farm to slaughter all have a significant impact on meat quality (Sutherland, Worth, Stuart, Dobbie & Clerens, 2016) and although the industry might have guidelines as to how the animals should be treated (NZFSA, 2004b) there will be natural variation from the geography and climate that the animals grew up on, the feed the animals received and the genetics of the animals themselves. These factors are all outside the ability of the processing

plant to control or know beforehand, meaning an objective measure would be beneficial for sorting and understanding the product that is being processed in plant and providing objective feedback to the animal producers.

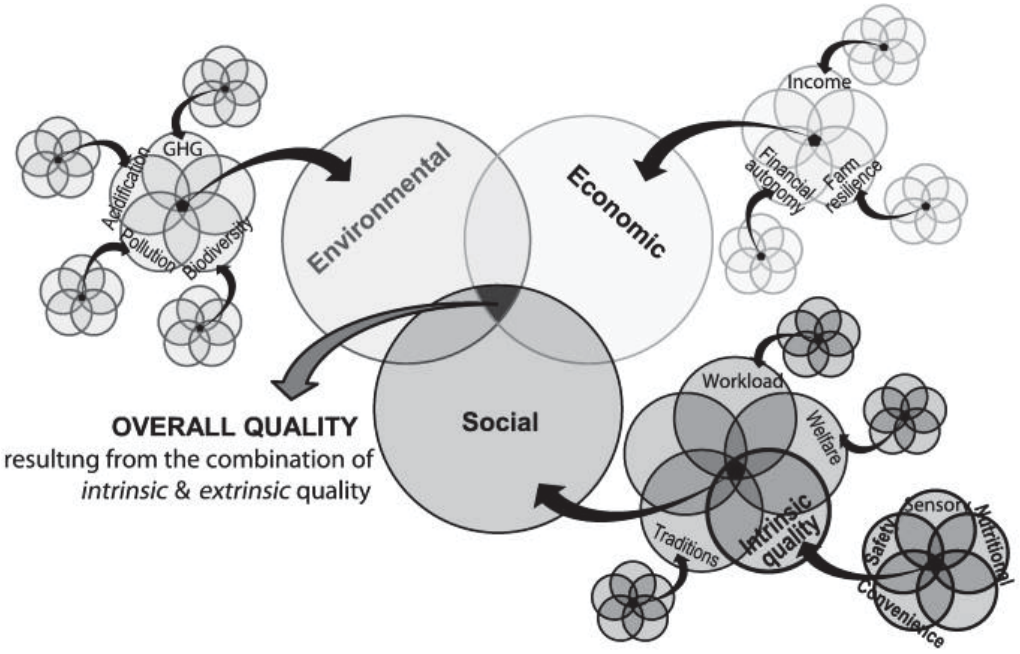


Figure 1: Representation of the complex relationship between intrinsic and extrinsic factors involved in defining what is meat quality, specifically beef in this example. GHG = greenhouse gas. Intrinsic quality includes physical (colour, shape, appearance, tenderness, juiciness, flavour) and nutritional. Extrinsic quality comes from factors affecting the product from external sources (Hocquette, Botreau, Picard, Jacquet, Pethick & Scollan, 2012)

In order to get a monetary and reputational premium for a product such as meat and animal tissues, a guarantee on its quality must be made to satisfy consumer demands and expectations. This can be problematic due to the complexity and heterogeneous nature of the product meaning robust systems must be in place to ensure quality or uniformity of any grading or guarantee system.

One of the downsides of attempting to implement a system based on quality attributes on a product subject to such variation as an animal carcass, is that certain parts or products may be downgraded for not meeting the quality guidelines set in place for consumer reassurances. Although this may

cause a loss of income to some farmers, it may be possible to partially mitigate any adverse effects by directing the animals to a different production stream or processing condition that is best suited for the product at hand or use the segregated ingredients to create a value added product where total carcass quality is no longer a factor.

Currently, to assess the eating quality of meat products within an industrial production system, guidelines such as Meat Standards Australia (MSA), Beef + Lamb NZ Quality Mark, New Zealand Meat Classification Authority are used to infer meat quality in regards to the consumers' expectations and experiences. While these guides are used to attempt to control the quality of meat within the meat processing environment they cannot control for external factors that may influence the animals before they reach the meat processing facilities. Outside of the production system, sensory assessment by trained panels could be used but is difficult to implement and requires a full time, highly trained team of people which can be very cost prohibitive. Quality control has also traditionally been performed using laborious, expensive, dangerous and destructive laboratory techniques such as mechanical tenderness testing, gas chromatography for intramuscular fat analysis and shelf life colour display that can take many days to find results and often happen outside the processing company. None of these methods are suitable for use at the speed at which the carcasses are processed in the abattoir, making them unsuitable for carcass evaluations. This means they do not allow for meat to be used for its most beneficial purpose as the meat processing company has had to make a decision from previous results, usually on different animals, along with educated guesses as to how the meat will behave in order to send it to the right type of manufacturing.

The ability to maintain and improve meat eating quality through advances in animal breeding (genetics) (Gao, Zhang, Hu & Li, 2007) and optimisation of on farm management and meat processing techniques is integral for red meat value chains wishing to increase market share and return on. Suitable measurement systems for meat eating quality that can be undertaken in real-time and non-destructively are essential to enable product differentiation in market. Furthermore, when linked to

individual animal identity, measurements can provide meat quality phenotypes to inform breeding and on-farm management decision making performance recording (Bindon, Burrow & Kinghorn, 2001).

Despite the need for suitable measurement systems at an industrial scale, there are currently no commercially produced, non-invasive direct measures of lamb meat quality available to the meat processing industry. This current work begins to evaluate the ability of spectral imaging to fill this role.

1.1 Meat Quality: a background

Before there can be an exploration into the abilities of spectral analysis there needs to be an understanding of what meat quality is, how it is measured and how the current New Zealand meat industry uses and implements the corresponding results. The following sections provide a background and summary of the current science of meat quality discovery from when the animal in question reaches the abattoir through to popular laboratory wet chemistry in current use.

Meat Quality Measurements used by Industry

1.1.1 Pre and during production

Due to the current inability to have timely meat quality measurements at the moment of production, the New Zealand sheep industry has produced its own standards on how to grade carcasses based on the type of animal, weight and the fat cover once the animal has been skinned and all internal organs, head and feet have been removed. Firstly, the animals are categorised based on their ages and sex; lamb, hogget, mutton or ram. Lamb is defined as a young sheep less than 12 months of age and/or does not have any permanent incisor teeth. A hogget is a young male (ram, cryptorchid or castrated male (wether)) sheep or female (ewe) that has not given birth yet, having no more than two permanent incisors, while mutton is classified as a ewe or a wether with more than two permanent

incisors. Lastly, a ram is an adult, uncastrated male sheep having more than two permanent incisors (NZMCA, 2004).

In respect to lamb, the industry standard is that the carcasses are then split into combinations of 5 weight classes and 7 fat classes which are used to give the carcass a final grade and decide to what use the meat is to be used for (Figure 2). Some meat companies, such as Alliance Group Ltd, do additional measurements on the carcass with two dimensional imaging systems called VIAscan® to estimate the lean meat yield in the three primal (leg, loin and shoulder) carcass regions (Hopkins, Safari, Thompson & Smith, 2004). Although these methods provide a good description of the size and fatness of the carcass, there isn't definitive evidence (Moore *et al.*, 2012) that these attributes are sufficiently correlated to final eating quality meaning there is a real need for a more accurate, online analysis of meat quality.

Export Carcass Classes

Fat Class	Weight Class									
A Light weight and almost devoid of external fat	**A**		**L**		**M**		**X**		**H**	
	A	None								
		Up to but not including 9.1kg								
		Less than 9.0kg								
Y Low fat content			**YL**	Up to and including 6mm	**YM**	Up to and including 7mm	**YX**	Up to and including 9mm		
				9.1kgand up to but not including 13.3kg		13.3kg and up to but not including 17.1kg		17.1kg and over		
				9.0kg to 12.5kg		13.0kg to 16.0kg		16.5kg and over		
					YME	Well muscled YM	**YXE**	Well muscled YX		
P Medium fat content			**PL**	Over 6mm, up to & including 12mm	**PM**	Over 7mm, up to & including 12mm	**PX**	Over 9mm, up to & including 12mm	**PH**	Over 9mm, up to and including 12mm
				9.1kg and up to but not including 13.3kg		13.3kg and up to but not including 17.1kg		17.2kg and up to but not including 21.3kg		21.3kg and over
				9.0kg to 12.5kg		13.0kg to 16.0kg		16.5kg to 20kg		20.5kg and over
					PME	Well muscled PM	**PXE**	Well muscled PX	**PHE**	Well muscled PH

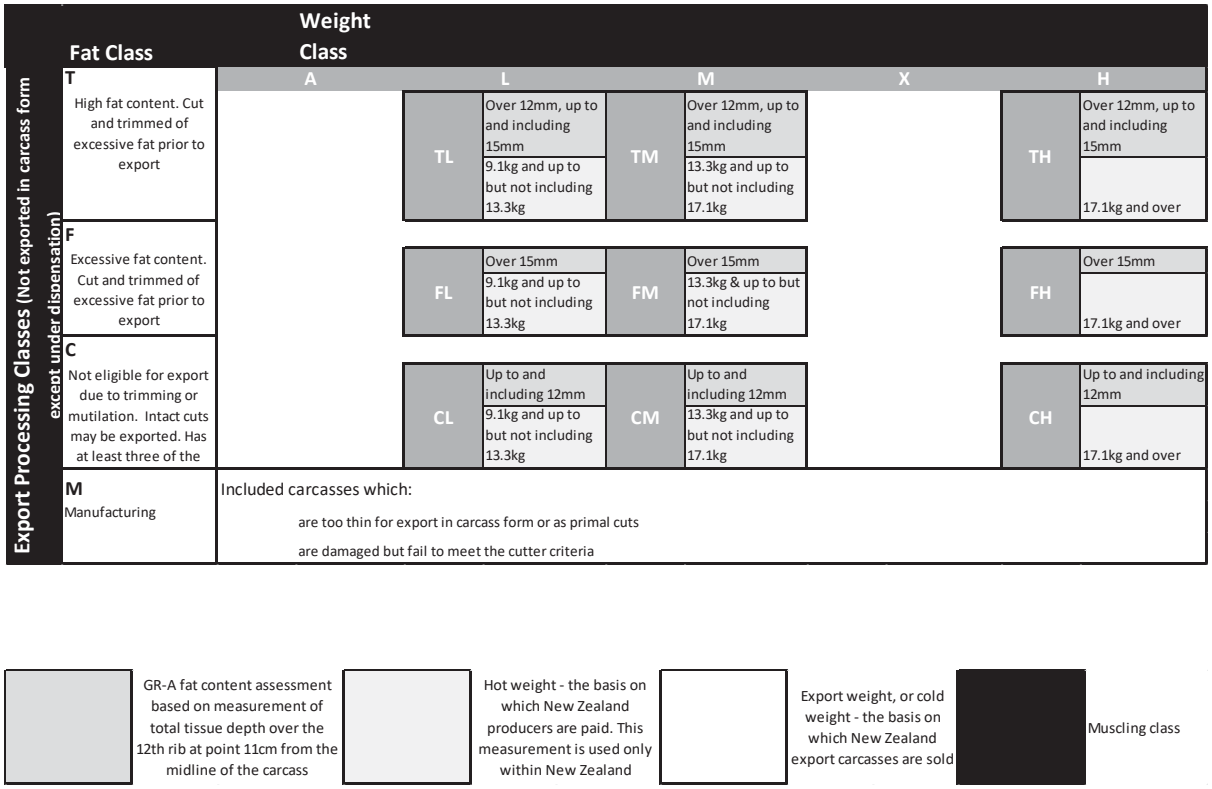


Figure 2: Current New Zealand abattoir grading system for lamb carcass quality assessment (NZMCA, 2004)

1.1.2 Post production

Current grading systems within an abattoir only categorise animals into various weight and fat groups, with any meat quality measurements made subsequently. To generate more accurate meat quality information, it is currently required to remove a sample of meat from individual carcasses of interest and test it using off-line techniques such as discussed below. Whilst some of the meat quality measurements described below can be carried out within the processing floor, others do require the product to be removed from the controlled processing room for testing in a separate laboratory, with the product rendered not-fit for human consumption. Specific meat quality traits commonly measured currently include pH, meat colour, meat tenderness and intramuscular fat. These are described in more detail below.

pH

What is pH?

The pH scale is a representation of the ⁻log measure of the amount of free hydrogen ions (H^+) in an aqueous solution measured in moles per litre and is an often used as a surrogate measurement for meat quality. The pH scale is ranked from 0 to 14 with 0 being extremely acidic, such as hydrochloric acid and 14 being extremely basic, or alkali, such as sodium hydroxide (Figure 3).

Figure 3: Example of common household items and their corresponding pH levels and corresponding H^+/OH^- concentration (Brunning, 2015)

What influences pH: aerobic and anaerobic metabolism

The aerobic system—which includes the Krebs cycle (also called the citric acid cycle or TCA cycle) and the electron transport chain—uses blood glucose, glycogen and fat as fuels to resynthesise adenosine

triphosphate (ATP) in the mitochondria of muscle cells, used as energy for metabolism. When using carbohydrate, glucose and glycogen are first metabolised through glycolysis, with the resulting pyruvate used to form acetyl coenzyme A (acetyl-CoA), which enters the Krebs cycle as shown in Figure 4. The electrons produced in the Krebs cycle are then transported through the electron transport chain, where ATP and water are produced (a process called oxidative phosphorylation). Complete oxidation of glucose via glycolysis, the Krebs cycle and the electron transport chain produces 36 molecules of ATP for every molecule of glucose broken down (Robergs & Roberts, 1997).

During glycolysis, carbohydrate in the form of either blood glucose or muscle glycogen (the stored form of glucose), is broken down through a series of chemical reactions to form pyruvate (glycogen is first broken down into glucose through a process called glycogenolysis). For every molecule of glucose broken down to pyruvate through glycolysis, two molecules of usable ATP are produced (Brooks & Fahey, 2000). This means very little energy is produced through this pathway but the energy is produced very quickly. Once pyruvate is formed, it can be converted to either lactate or to acetyl-CoA, which enters the mitochondria for oxidation and the production of more ATP (Robergs *et al.*, 1997). When there is enough oxygen available to meet the muscles' needs (i.e., during aerobic exercise), pyruvate (via acetyl-CoA) enters the mitochondria and goes through aerobic metabolism. Conversely, conversion to lactate occurs when the demand for oxygen is greater than the supply. The aerobic system produces 18 times more ATP than does anaerobic glycolysis from each glucose molecule.

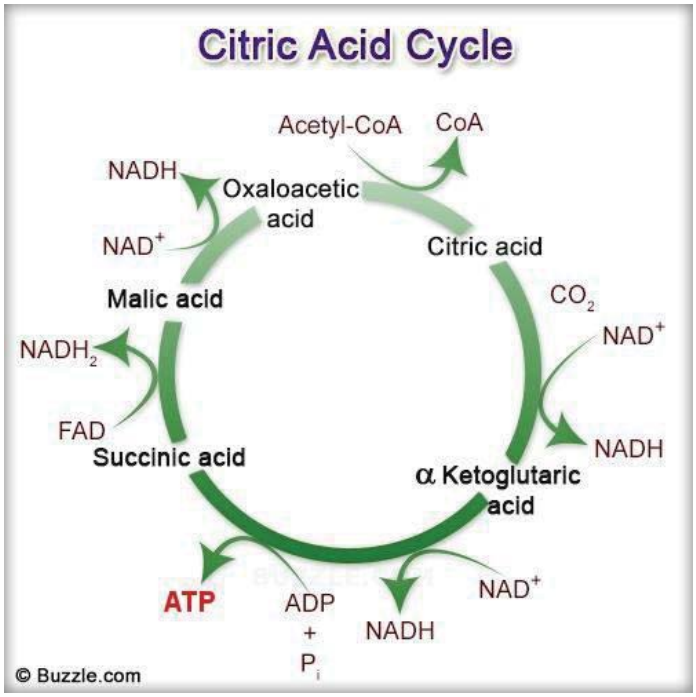


Figure 4: Simplified diagram explaining the Krebs or citric acid cycle showings its major products and by-products

Why is pH important?

When an animal experiences high stress or significant exercise before slaughter, it will deplete the glycogen which is found within its muscles and therefore *post-mortem* lactic acid production is diminished. It is the lactic acid produced within the muscle after slaughter that lowers the muscle pH. In normal situations this glycogen is efficiently turned into ATP aerobically. However, under stressful conditions, the anaerobic energy production system of glycolysis is used due to the lack of available glycogen. This depletion of glycogen results in high ultimate pH (pH_u = the pH of the meat once it has gone into and through rigor mortis). High pH affects meat colour with a pH_u being greater than 6.0, creating dark, firm and dry (DFD) meat (Young, Reid & Scales, 1993) which is considered unattractive (Figure 5). Meat with a pH_u of less than 5.8 has the ideal bright cherry red appearance, and is desirable. DFD meat has a higher water holding capacity and tighter structure than meat with a normal pH, and it has been suggested that this may decrease the rate of oxygen diffusion into the muscle and

consequently inhibit the formation of oxymyoglobin, resulting in the darker colour (Young & West, 2001).

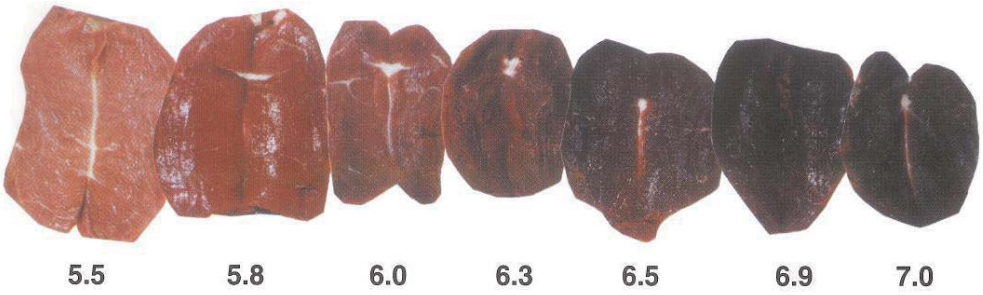


Figure 5: The colour of beef meat at various pH levels (MIRINZ, 1999)

As well as an unattractive look and inferior cooking characteristics, the meat is also at a far more favourable pH for high levels of bacterial and other microflora growth (Hedrick, Aberle, Forrest, Judge & Merkel, 1994). All of this can have severe consequences from a New Zealand perspective as a lot of lamb meat is exported chilled for long periods of time (possibly up to 12 weeks depending on the market). A long chilled storage time allows for an increased chance of contamination in terms of food safety but also a reduced colour stability of the final product, meaning a decreased window of opportunity for the meat to be sold to consumers.

Tenderness is also affected by pH_u. There is a curvilinear relationship between meat pH_u and tenderness in beef and lamb (Bouton, Harris & Shorthose, 1971; Devine, 1994; Jeremiah, Tong & Gibson, 1991) and on average meat with the lowest tenderness has an intermediate pHu — that is, meat with a pHu between 5.8 and 6.19 (Lomiwes, 2012) (Figure 6).

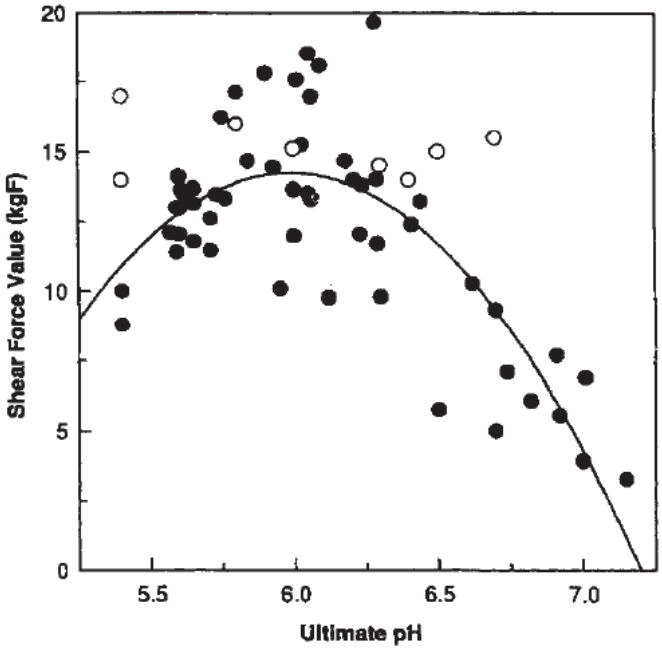


Figure 6: A graph showing the shear force (objective value of tenderness measured by the force needed to break through muscle fibres) values in kgf of low, intermediate and high pH_u lamb meat after 1 day of ageing.Taken from Watanabe, Daly and Devine (1996). See meat tenderness section for further explanation.

The issues associated with meat having a high pH_u are particularly applicable to beef, venison and lamb. Low ultimate pH meat can be a problem in pork, where a high rate of *post-mortem* glycolysis results in pale, soft and exudative (PSE) meat, due to stress prior to slaughter (James & James, 2002), but is considerably more rare in other red meats such as beef and lamb.

How is pH measured?

Traditionally, pH is quantified using a pH meter to measure the hydrogen ion concentration as described above, shown in Figure 7. The downside of using a traditional pH meter to find the pH_u is that rigor mortis (the stiffening and subsequent relaxation of muscles after death caused by chemical changes that effect the ultimate pH and tenderness of the meat) in sheep carcasses can take up to 24 hours to complete, by which time it is usually too late to change where the carcass is to be directed in

the production chain or to remove any carcasses that are outside of specifications. Further limitations, include that the meter and probe itself must be cleaned regularly and must always be carefully handled due to its glass tip that is easy to break and a person must manually take multiple readings along the length of a muscle due to its heterogeneous nature and then record the reading in a way to allow transcription into a database to occur either through electronic logging within the meter itself or by written record.

Figure 7: A typical electronic pH meter as in use in the majority of food industry and research situations (Thermo Fisher Scientific, Massachusetts, USA)

Meat colour

What is meat colour and why is it important?

Of the various quality attributes of fresh meat, colour is the most important factor at the moment of purchase (Mancini & Hunt, 2005). Consumers can only judge the quality of meat on the colour of the external surface of the meat at the point of sale, with a bright cherry-red colour being associated with fresh meat (Suman & Joseph, 2013). During retail display, meat undergoes discolouration, although the extent of which depends on the method of processing and packaging which is discussed subsequently. Discoloured meat will be either discounted or the whole product will be discarded (Kim & Hunt, In Press). As a result, for example, in the US nearly 15% of retail beef is discounted in price

due to surface discolouration, resulting in an annual loss of $1 billion (Smith, K. E. Belk, J. N. Sofos, Tatum & Williams., 2000).

Myoglobin (MB) is the protein largely responsible for meat colour. In the living cell it has two functions, serving as both an oxygen-storage and oxygen-delivery molecule (Livingston, 1983). The capacity of MB to bind oxygen depends on the presence of a haem co-factor. As shown in Figure 8, a haem co-factor is a non-polypeptide prosthetic group consisting of protoporphyrin and a central iron atom. Of the six bonds available, four connect the iron atom to the haem ring, the 5th attaches to the proximal histidine-93, and the 6th site is available to reversibly bind ligands, including oxygen, carbon monoxide and nitric oxide (AMSA, 2011). The nature of the group attached to the iron, and the state (covalent or ionic) of the iron determines meat colour. Since MB contains haem iron, it is rather susceptible to oxidation. MB oxygenation, also known as blooming, depends on time, temperature, pH and competition for oxygen by mitochondria. More specifically, the competition for oxygen between MB and mitochondria determines oxygen penetration beneath the meat's surface, which significantly affects the intensity of surface colour (Kropf, 2008).

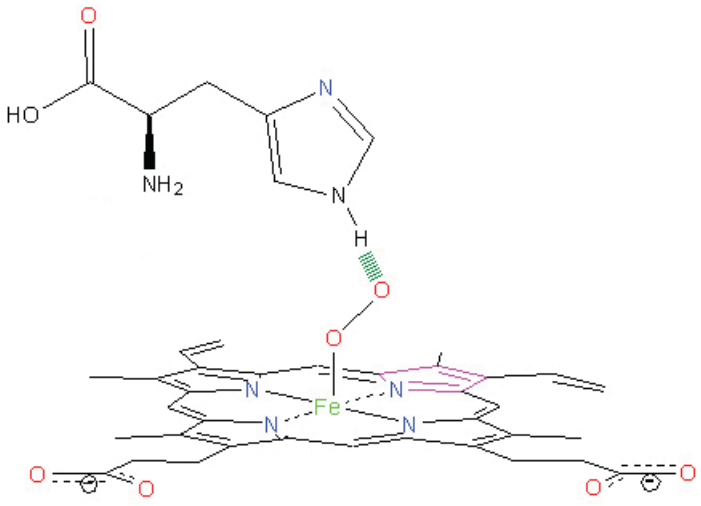


Figure 8: Diagrammatical representation of the haem myoglobin (By Mrbean427 [CC BY-SA 3.0 via Wikimedia Commons)

In uncut meat which is not exposed to the oxygen in air, MB exists in its reduced ferrous state (Fe^{2+}) with no bound oxygen. Meat in this state is purple-red in colour. This form of MB is known as deoxymyoglobin (DMB) (AMSA, 2011). This purple-red or purple-pink colour is also typical of vacuum packed meat due to the absence of oxygen. However, by opening the vacuum bag or by cutting the meat, DMB reacts with oxygen and forms a pigment called oxymyoglobin (OMB), leading to a bright red colour consumers associate with fresh meat (Mancini *et al.*, 2005) (Figure 9, Rx 1). At low oxygen concentrations, OMB tends to undergo oxidation (where the Fe^{2+} in the haem is oxidised to Fe^{3+}) to form the undesirable brown pigment metmyoglobin (MMB) (Figure 9, Rx 2a and 2b). This change is prevented if no oxygen is present. The change from OMB to MMB occurs quite readily, but the reverse is more difficult due to reducing agents such as NADH no longer being formed, lessening the effectiveness of residual enzymatic activity (O'Keeffe & Hood, 1982).

Different packaging conditions can influence the appearance of the meat as they can potentially delay the development of MMBs characteristic brown colour. When meat is packed in a high-oxygen modified atmosphere packaging (MAP), oxygen binds to the haem ring and forms OMB. When muscle is stored in high oxygen conditions (80% oxygen and 20% carbon dioxide), oxygen penetrates deeper into the meat surface and thus creates a much thicker layer of OMB and consequently a more stable red colour (King & Whyte, 2006).

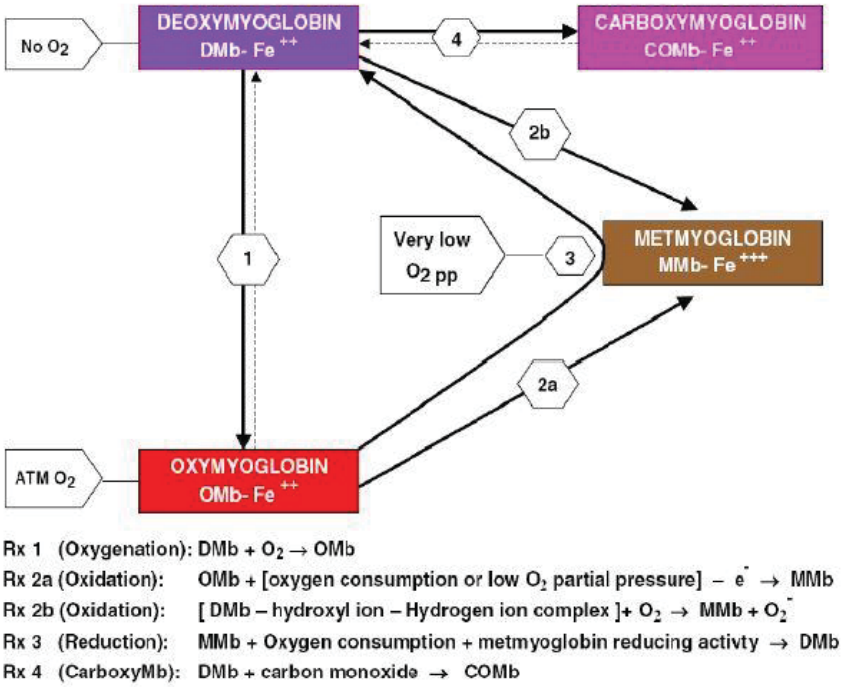


Figure 9: The different chemical states of myoglobin, the colour of the meat is regulated by the oxidative state of iron in the haem group (Mancini et al., 2005)

When low oxygen is present in meat, a thin third layer of MMB forms between the surface OMB and the internal DMB layers (Figure 10). This intermediate layer of MMB becomes thicker and moves toward the meat surface with time, while the OMB layer becomes thinner. A thinner layer of OMB and a more pronounced layer of MMB dulls the meat's red appearance and eventually the OMB layer is completely replaced by MMB, resulting in total discolouration of the meat surface (MIA, 2013)(Figure 10).

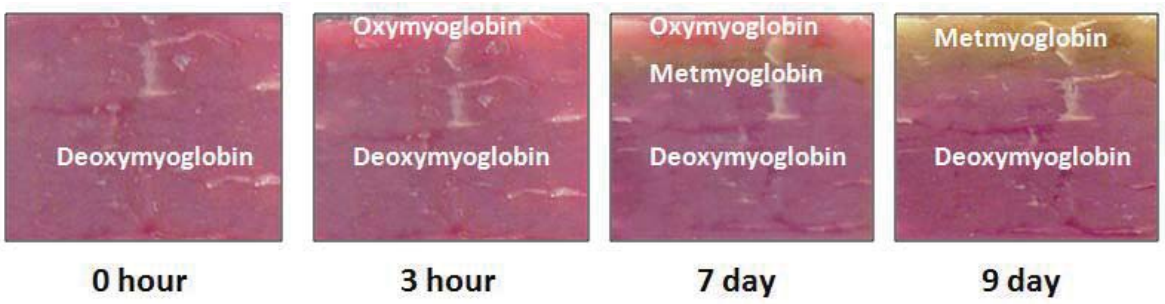


Figure 10: Illustration of DMB, OMB and MMB formation in the surface layer of a cut of meat by increasing oxygen exposure (Photo courtesy of Dr. D.H. Kropf, Kansas State University).

The existence of an enzyme system capable of reducing MMB back to MB was proposed by Livingston and Brown (1981) and was termed metmyoglobin reducing activity (MRA). In fresh muscle the enzyme is very active and the MMB formed is quickly reduced to DMB and oxygenated back to OMB, thereby retaining the bloomed colour. However, as the meat ages or is frozen, the activity of the MRA is decreased, and MMB begins to accumulate rapidly on the surface of the meat (Ben Abdallah, Marchello & Ahmad, 1999).

Different muscles have been shown to have different colour profiles and stabilities. Kim, Keeton, Smith, Berghman and Savell (2009) reported that the *M. Longissimus thoracis et lumborum* muscle of beef had the highest level of MRA (and subsequently the most stable colour), followed by the *Semimembranosus* muscle and finally the Psoas major muscle. Mancini, Suman, Konda and Ramanathan (2009) reported that every muscle with a different colour profile would respond differently to packaging under modified atmosphere (MAP) and therefore have different discolouration profiles.

How is meat colour measured?

Meat colour can be measured either visually or instrumentally. Human judgement better reflects the total impression of the whole meat surface being evaluated; however, the consistency and repeatability of visual evaluation can be influenced by personal preference, external lighting and visual deficiencies within the eye and as a result are subjective. Instrumental measurements are more

objective. The most common method for measuring meat colour instrumentally is to use a colorimeter such as the Minolta CR-400 using the colour scale CIE L* (lightness), a* (redness) and b* (yellowness) with a 10° observer angle, which is more representative of what the consumer sees than some other methods such as the HunterLab or RGB colour scales or tighter observer angles such as 0° or 2°that have been used in the past (Hedrick *et al.*, 1994) .

Hue angle is a measure of meat colour as calculated from the recorded a* and b* values (see methods section 2.3.3). It is used as a representative measure of meat discolouration and corresponds well to colour changes seen by the human eye over time (Kim, Stuart, Rosenvold & Maclennan, 2013).

Meat tenderness

What is meat tenderness and why is it important?

Although the initial purchase decision of raw meat is primarily affected by colour, the likeability, and subsequent choice of the consumer to repurchase the meat is markedly affected by the tenderness of the cooked product. Tenderness is also linked to other meat attributes such as juiciness, texture and fat content. The evaluation of other factors such as flavour and juiciness can only be independently made when cooked meat is at an acceptably tender level so that it doesn't interfere with other meat quality characteristics (Dumont, 1981). Meat tenderness is not simple to define and is more than just a measure of biting effort. The perception of tenderness involves ease of fragmentation, mealiness, texture and the adhesion of muscle fibres during mastication (Hendrick & Hartl, 1993). The tenderness of meat is not always consistent following cooking due not only to the muscle fibres themselves but also to many other intrinsic properties of meat that determine tenderness such as pH_u, cold shortening and connective tissue content (Purchas, 2004a). The abundance of connective tissue surrounding the muscle fibres, bundles and the entire muscle is an important source of variation in the tenderness of meat (Purchas, 2004b). Of this connective tissue, the proteins collagen and elastin are of particular interest. Collagen in meat becomes less soluble with increasing age of the animal, and results in a

chewy texture to meat, while elastin has elastic properties and is extremely insoluble, which may contribute to meat toughness (Purchas, 2004a).

How is tenderness measured?

There are numerous ways to measure meat tenderness objectively, all of which are based on measuring the amount of force required to shear a piece of cooked meat giving an indication of how hard it would be for a consumer to bite and chew the meat and so how tender they would perceive it. The most common measurement techniques being Warner-Bratzler shear force, slice shear force and the MIRINZ Tenderometer. Warner-Bratzler is generally considered the 'gold-standard' of meat tenderness testing and works by taking circular core samples of 12.7 mm diameter from a cooked piece of meat, parallel to the muscle fibre direction. These cores are then placed in a texture machine equipped with a triangular slotted blade and sheared. This method, although considered accurate, is slow to execute due to the equipment used and the accuracy needed for the sample preparation.

Slice shear force works by taking a single slice 5 cm long, which is removed by placing the cooked meat sample in a mould and then using a special double bladed knife with the blades 1cm apart, to cut a slice at a 45° angle to the length of the loin. This slice is then sheared using a wide, blunt ended blade. This method requires a lot of uniform meat, such as whole lamb loins, to generate the required size for sampling each time, limiting its usefulness as a testing option. The tenderness is taken as the highest peak of the force deformation curve output, i.e. the maximum amount of force needed to break through the muscle fibres.

The MIRINZ Tenderometer works by taking a cooked piece of meat and cutting ten 1 x 1 cm sub-samples parallel to the fibre direction (Figure 11) and then placing each sub-sample individually into the tenderometer where it is sheared by a blunt v-shaped tooth. The shear force is given as the kilograms of force being needed to break through the muscle fibres.

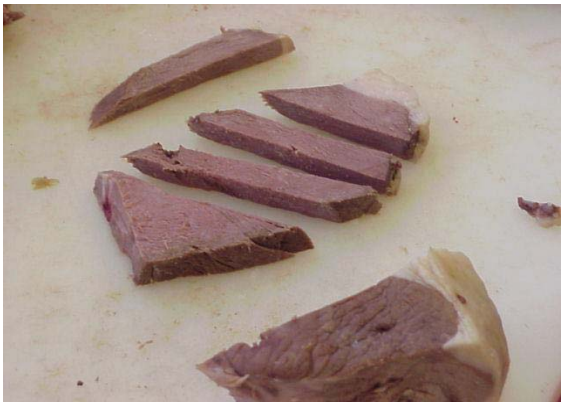


Figure 11: A Photo of a cooked lamb loin sample cut perpendicular to fibre direction in a 1 x 1 cm sub-sample ready for tenderness testing

The major difference in sampling method between the Warner-Bratzler, slice shear force and MIRINZ tenderometer is that the former two use a constant time and variable pressure while the latter uses constant pressure increase and variable time.

Comparisons between the various methods have been made by Graafhuis, Honikel, Devine and Chrystall (1991) and Bekhit, Devine, Morton and Bickerstaffe (2003) showing that the results are not identical but can be comparable with simple conversion formulas. While these comparisons and machines give their results in kgf, the international system of units (SI units) for force is the Newton with one kilogram of force equal to 9.80665 Newton.

Intramuscular fat

What is intramuscular fat and why is it important?

Fat tissue is formed by adipogenesis, which can be stimulated by insulin and glucocorticoid hormones and by insulin-like growth factor. In the carcass, three main types of adipose tissue can be found: subcutaneous fat, intermuscular fat and intramuscular fat (IMF). Intramuscular fat is deposited between muscle fibre bundles mainly as adipocytes but also in lesser amounts within the cytoplasm of the myofibres. Intramuscluar fat is a depot fat that is deposited later in the animal's development and has different characteristics when compared to intermuscular and subcutaneous fat in terms of

cellularity and metabolic capacity. These two types of fat present in muscular tissue are also known as depot fats and structural fats. Depot fats are composed mainly of triglycerides, although small amounts of monoglycerides, diglycerides and fatty acids can also be present. When these fat depots increase in size and number, the droplets can be visible in muscle surface itself between the muscle fibres and close to the capillary beds, showing as white markings or lines which is commonly known as marbling (Harper & Pethick, 2004). Structural fats are found in the cell membranes and comprise of phospholipids and cholesterol. Membrane fats are important for muscle structure and function. Intramuscular fat is mainly composed therefore, of triglycerides, phospholipids and cholesterol.

Intramuscular fat is related to the amount of other fat types present in the carcass but it is not dependent on them (Yang, Albrecht, Ender, Zhao & Wegner, 2006). It is, however, dependent on the variation of adipocytes in the muscle in terms of quantity and metabolic activity and also depends on the muscle growth rate and metabolic activity off other organs such as the liver (Hocquette, Gondret, Baza, Mdale, Jurie & Pethick, 2010).

Although IMF has been shown be related to perceived tenderness of pork in consumer trials (Font-i-Furnols, Tous, Esteve-Garcia & Gispert, 2012; Fortin, Robertson & Tong, 2005), it appears to have a more significant impact on juiciness and flavour due to its ability to lubricate the mouth during chewing (Thompson, 2004), though this is not substantiated in some studies (Channon, Kerr & Walker, 2004; O'Mahony, Cowan & Keane, 1991).

The minimum amount of IMF to achieve acceptable consumer satisfaction for lamb is about 5% (Hopkins, Hegarty, Walker & Pethick, 2006). A confounding problem for producers is trying to reach this level of IMF while also keeping the total animal fatness levels to below 30%, which is the point where it no longer becomes a profitable production system and also goes against current consumer expectations of a low fat amount surrounding retail cuts (Pethick, Hopkins & McPhee, 2007). This is becoming a major industry concern where a balance between satisfying eating experience, health

concerns in regards to fat and a positive retail display appearance must be met (Hocquette *et al.*, 2010).

The fatty acids observed in IMF typically include saturated and monounsaturated C12 to C24+ fatty acids; polyunsaturated omega 3 C18, C20 and C22; as well as polyunsaturated omega 6 C18 and C20. The short chain fatty acids tend to be present within the depot lipids, whereas the long chain fatty acids are the structural lipids. The relative proportions of the different fatty acids depend on the total level of IMF observed. When IMF levels are low, and the main lipids are the structural lipids, the long chain fatty acids are at a higher proportion of total lipid. As the amount of depot lipids increases, the amount of structural lipid does not correspondingly increase and therefore the proportion of the total lipid made up of structural lipids decreases.

Different types of fat have different benefits and disadvantages in terms of both health, eating quality and production values meaning the ability to know the total amounts of fat as well as its constituent parts would allow for producers and consumers the ability to choose differing options and products depending on what their specific markets and needs are (McCurry, 2011).

How is intramuscular fat measured?

Fats can be extracted from a matrix (such as a sample of meat), using a non-polar solvent, and saponified to produce salts of the free fatty acids. Fatty acid salts are derivatised to form fatty acid methyl esters, to increase volatility, improve peak symmetry, and decrease sample activity, and thus provide more accurate analytical data. After derivatising the free acids to form methyl esters using an alkylation agent, the mixture can be analysed by gas chromatography (GC), due to the volatility and thermal stability of the Fatty acid methyl esters (FAMEs). Gas chromatography is an important technique in fat analysis because accurate results can be obtained for box complex and simple sample matrices as shown in example (Figure 12).

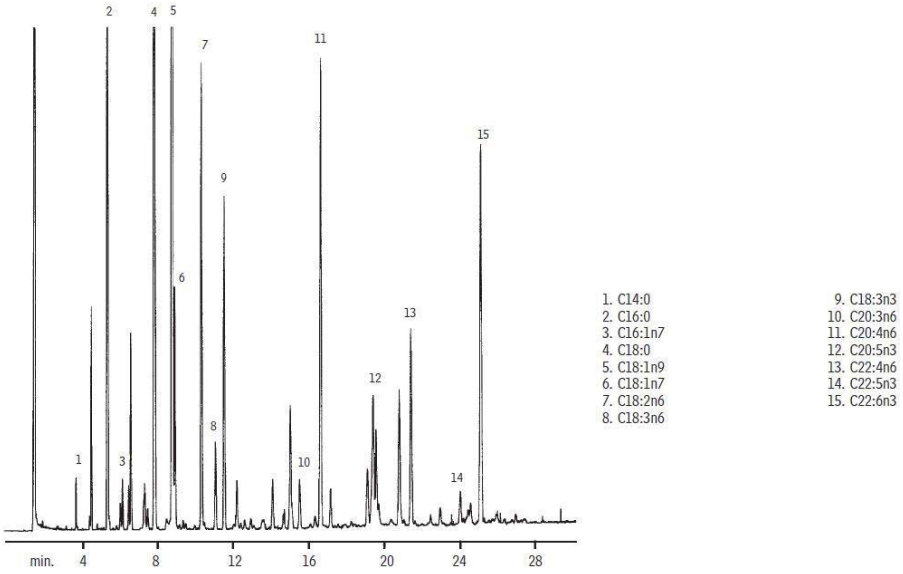
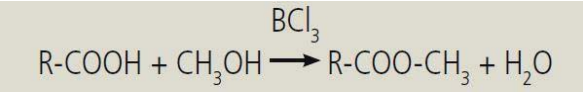


Figure 12: Example of gas chromatography results showing peaks correlating to different fatty acids. The total fat content can be found by addition of all the peaks

FAMEs analysis is an important tool both for characterising fats and oils and for determining the total fat content in foods such as meat. FAME analysis is often used instead of directly sampling the underived fatty acids because, in their original form, fatty acids are more difficult to analyse due to their highly polar compounds which tend to form hydrogen bonds meaning a decreased volatility and so harder to analyse using gas chromatography. Reducing this polarity using derivatisation as shown in Equation 1, allows for more accurate analysis. Another reason is that the small differences exhibited by unsaturated fatty acids are hard to distinguish and so the carboxyl functional groups must first be neutralised. This allows column chemistry to perform separations by boiling point elution, degree of unsaturation, position of unsaturation and the cis/trans configuration of the unsaturation.

The official methods of the Association of Official Agriculture Chemists (2012) and the American Oil Chemists Society (AOCS, 2009) contain procedure Ce 1b-89 for the derivatisation reaction. In general, the glycerides are saponified by refluxing with methanolic sodium hydroxide. The esterification is

affected with a reagent such as boron trifluoride (a catalyst) in methanol and the FAMEs are extracted with a non-polar solvent (e.g., heptane) for analysis by GC.



Equation 1: showing the esterification reaction of a carboxylic acid with methanol using a boron trichloride catalyst.

For some samples, trimethyl-sulfonium hydroxide (TMSH), an alternative derivatisation reagent, can be used for transesterification. A major advantage of this approach is that the derivatisation can be performed in a single, fast reaction step.

Sensory evaluation by panel

What is sensory evaluation and why is it important?

Eating quality testing by instrumental assessment can only be an approximation of the actual eating experience as no current machine can test for interacting characteristics and instead measures only one variable at a time and makes inference towards its influence on meat quality. When making a measure for tenderness using a MIRINZ tenderometer, for example, it gives only one value in isolation but when a piece of meat is eaten its tenderness is perceived not only as the pressure it takes to break through the meat fibres but also the 'mouth feel' due to juiciness and fat content which cannot be objectively tested at the same time. To counter this problem, taste panels using actual people to eat and score the meat can be used.

How is sensory evaluation measured?

Two main types of sensory panel are trained and consumer. Trained panels are used to taste meat under controlled conditions and sampling preparations. These panels usually consist of up to ten people who have been screened for their tasting abilities and trained to give repetitive and comparable results. Testing is carried out in controlled conditions with each panellist separated from

their peers and known values of lighting and ventilation used. Results from this panel are reported as objectively as possible using a number or line scale with questions asking for defined values such as rating the saltiness or firmness of the product from 1 to 10 without asking if it is preferable or liked. These panels take a long time to train and must be kept consistent for as long as possible, preferably years, with regular monitoring and retraining required. Most panels are for only specific products or within a single company so a trained meat panel would need to be retrained again if it were to be testing for non-meat foods such as potato crisps or carrots. All of which takes a lot of time, resources and management, making it an expensive, ongoing cost (Warriss, 2000).

The other type of sensory panel is consumer based, which can be further split into subsections. One such subsection is the consumer being able to take the product home and prepare it themselves before filling out and returning a questionnaire about the product. This approach is cheaper and easier in the respect that special facilities and staff are not required to perform the cooking and sensory testing while giving a better idea of how a product would fair in a real-world situation such as the home kitchen cooked by the general population. The drawbacks to this include that the conditions are far less controlled in terms of cooking and preparation and the panellists could be influenced by what else they have eaten with it, or family members who they may discuss the product with at the time of eating (Warriss, 2000).

Another version of a consumer panel is when the products are cooked by the research team and then taken to the general public who are asked questions about it at the time of consumption. This type of panel has large amounts of variation and needs many people participating, often over 100, to get a statistically relevant sampling. It can also be subject to bias if the participants aren't selected randomly such as only using people from a certain business or on the street at a certain time of day. This can also be performed in a booth type situation where people are invited into a controlled booth such as in a trained panel and provided samples to eat and comment on with questions of subjective thought such as "How does it taste to you?" or "Do you like this product?" more likely to be used.

This line of questioning and using everyday people who are likely to buy the actual product can be beneficial to gauge the satisfaction or how liked the product would be in a specific market and would give guidance on possible areas for the manufacturer to concentrate on that would give the greatest increase in desirability. The downside of consumer panels is that they can require a lot of the product being tested and can be relatively expensive and time consuming. This allows for the opportunity of an objective, instrument based method of testing sensory attributes to be developed to give consistent, objective, faster and potentially cheaper results.

1.2 Spectroscopic based sensors

The previous sections have identified and discussed the range of attributes of meat that ultimately contribute to the visual and eating experience of customers whom purchase it with every attribute measured using a different technology and no overarching way to combine them. There is a desire by industry to find a single way to measure and compare meat quality parameters in a single process or technology.

Spectroscopic-based sensors coupled with multivariate analysis techniques have been applied to predict meat quality parameters from a scan of the meat surface at a pilot scale. This type of sensor presents a major advantage over traditional methods with the ability to perform measurements rapidly and non-invasively, allowing online monitoring of processes.

Using this type of online tool allows processors to optimise their production and manage meat products of various grades by providing immediate feedback, allowing them to send the carcasses and/or parts thereof to the value added products or processing lines they are best suited for.

Spectroscopy in the visible and near infrared spectral ranges is recognised as a low cost, rapid and non-destructive tool for determining food chemical composition. For over 40 years, it has been evaluated, tested and applied experimentally in the meat industry for the prediction of meat quality by measuring and attempting to predict the chemical and physical composition of different meat cuts

from varying meat producing species such as beef, pork and lamb. Recently, large collaborations such as the European Union's COST Action programme, which involves over 120 scientists and technical personnel from 19 European countries, have been developed with the aim of optimising non-destructive, *post-mortem* imaging and spectroscopic methods for the measurement of meat quality with the hopes of developing a value-based payment system to reimburse farmers and suppliers for producing the type of meat needed by both industry and the consumer markets with this programme confirmed the applicability of spectroscopic based methods for quality assessment in the meat industry. This sort of value-based system may be beneficial for NZ Inc. as a way of guaranteeing product quality in markets where only the best and most consistent products are accepted, such as high end restaurants and top of the line supermarket lines where there a premium for guaranteed tenderness and flavour is possible. This would go some way towards helping mitigate the competition from low-cost producers from much larger countries which rely on bulk commodities in trade. While much work has been and is being done in this area, such as the EU COST Action programme mentioned above, very little work has been done on lamb meat and almost none has been performed on lamb specifically from a New Zealand genetic and geographical standpoint using spectroscopic based sensors.

1.2.1 Spectroscopy in the Visible and Near Infrared spectral range

In these spectral ranges the incident radiation interacts with matter to produce a response which is dependent on chemical composition and structure of the matter. In the visible spectral range, the radiation is absorbed depending on electronic transition of specific molecules. In the near infrared spectral range the radiation is absorbed according to changes in the vibrational status of molecules. This depends on the degree, and way, in which the bonds between atoms of dissimilar mass, i.e. those atomic pairs with a large dipole moment, are deformed. The molecular vibrations are subject to bending or stretching in response to the light energy that impinges on them. The bond vibrations may be altered in a variety of ways in response to light energy. Figure 13 gives examples of simple

combinations which give rise to absorbance in the near infrared (Murray & Williams, 1987).

−C−C−	H−−−O	N−H	−O−H	=P−P
C=C	intramolecular	$-NH_2$	−O−O	=P−C−
−C−H	and	$-NH_3$	−O−S−	=P−H
CH_2	intermolecular	NH_2	S=O	
$-CH_3$	hydrogen	−NH	−O−N=	
$-CH_2$-phenyl	bonds	−N=N−	O=N−	−S−H
$-CH=CH_2$-vinyl		N−N−	−O−C−	−S−S−
−C−N=		N−P	−O−C−	−S−C−
=C=N−		−N−P−	−O−P	S=C
−C=N−		=N−S−	O=P−	−S−P=
		−N=S−		

Figure 13: Simple bond combinations which give rise to VIS-NIRS absorbance (Murray *et al.*, 1987)

The level of energy to promote vibrational transitions in the near infrared is in the energy range of 2.65 x 10^{-20} J, which corresponds to the wavelength range of 750 to 2500 nm. This allows for a near universal application to any molecule or structure containing C-H, N-H, S-H or O-H bonds (Pasquini, 2003), such as being applicable to the major constituents of food such as fat, protein, water and carbohydrate all having distinct absorption profiles in the VIS-NIRS region of the spectrum (Geladi, MacDougall & Martens, 1985).

The first studies looking at visible near infrared spectroscopy in the meat industry began in 1994 with Hildrum, Nilsen, Mielnik and Næs (1994) followed by Venel, Mullen, Downey and Troy (2001) and Leroy, Lambotte, Dotreppe, Lecocq, Istasse and Clinquart (2004). More recently, Shackelford, Wheeler and Koohmaraie (2004) showed that measurements in the near infrared can be significantly correlated with beef tenderness measurements, although they are not yet sufficiently accurate for industrial use, with the methodology used only achieving an R^2 <0.38 (McGlone, Devine & Wells, 2005).

There are two main ways of performing measurements in the visible near infrared ranges: transmittance, where light is shone through a sample and a detector receives the light that passed through the sample on the opposite side, and reflectance, where light is shone onto a sample and a detector on the same side as the light source measures the light that interacts with the sample and is deflected back to the detector (Osborne, Fearn & Hindle, 1993). These measurements can be performed as single spectrum or as imaging, where for each pixel in the scanned area a spectrum is

collected. In this current study, the former will be referred to as NIRS or VIS-NIRS, whilst the latter will be referred to as hyperspectral imaging (HSI). Both NIRS and VIS-NIRS refer to the spectroscopy technique that is applied in the spectral ranges covering the visible and near infrared. In general, transmittance NIRS is used for liquids with minimal particulate matter to limit scattering of the light waves, whereas reflectance NIRS is used for solid samples that are opaque in nature and so better reflect the light waves (Osborne & Fearn, 1986). Reflectance is further broken down into specular and diffuse. Specular light is reflected off the surface of the sample back towards the detector, while diffuse reflectance is caused by the light randomly scattering and refracting as it enters the top most layer of the sample and interacts with the molecular and discrete components within it.

These two different reflectance properties can cause detrimental scattering effects of the light source which confuse the relationship between the spectral data and the actual concentrations of the chemical molecules in the sample and need to be taken into account when analysing the results and either removed or mitigated where possible (Esbensen, 2006) (Figure 14).

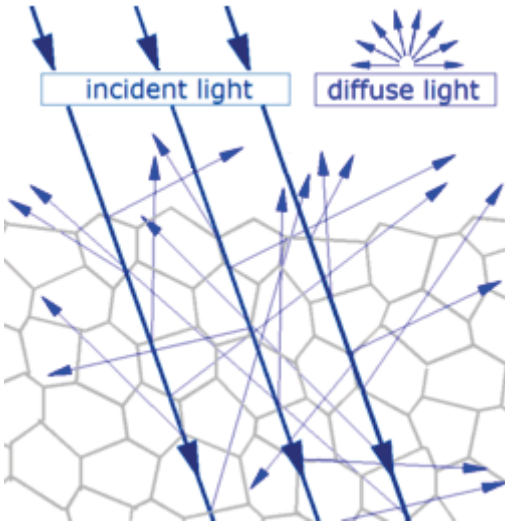


Figure 14: Incident light entering the meat structure and being scattered by its inherent structure creating errors in the responding absorption/reflectance measurements (By GianniG46 [CC BY-SA 3.0] via Wikimedia Commons)

To see if there are any added scatter effects in the data, each spectrum is plotted against the average of the entire spectrum, where additive scatter, when incident light scatters and combines to give a

stronger return reading than actual, will show as a y-axis offset to the average plot and multiplicative scatter, noise created from the incident light being scattered as it hits molecules, will show as increased peak intensity along the spectrum (Geladi *et al.*, 1985) (Figure 15). Depending on the sort of scatter effects present, different pre-treatments are needed before the data can be fully analysed. There are many different sorts of mathematical pre-treatments that can be applied, with the more common spectra-based treatments being normalising, weighing, smoothing using 1st and/or 2nd derivatives, baseline correction, standard normal variate correction (SNV) and multiplicative scatter correction (MSC); while the sample-based treatments include things such as mean centring and variable weighting (Beebe, Pell & Seaholtz, 1998). These are discussed further in the pre-processing section 1.3.5.

Figure 15: Example of a Near Infrared profile exhibiting spectrum scatter before and after correction using various types of pre-processing to eliminate additive and multiplicative scatter creating a tighter, and more uniform band of spectral information (Gallagher, Blake, Gassman, Shaver & Windig, 2006).

1.2.2 Hyperspectral imaging

Hyperspectral imaging is a technique that generates a spatial map of spectral variation. The use of hyperspectral imaging for both automatic target detection and recognising its analytical composition is a relatively new area of research. A hyperspectral imaging system produces a three-dimensional

array which represents the spectrum at each pixel location for a scanned area. The resulting three-dimensional dataset containing the two spatial dimensions and one spectral dimension is known as the datacube or hypercube (Figure 16) (Sun, 2010).

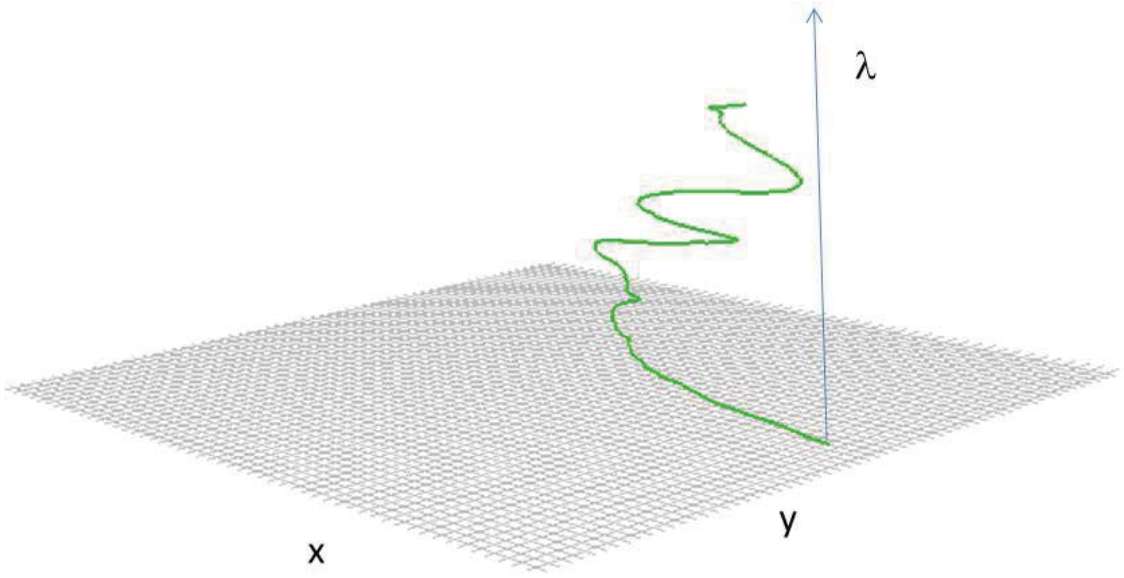


Figure 16: A three dimensional representation of a data cube with x and y representing the spatial elements of a sample and λ representing the spectral dimension. The spectrum shown is for an individual pixel.

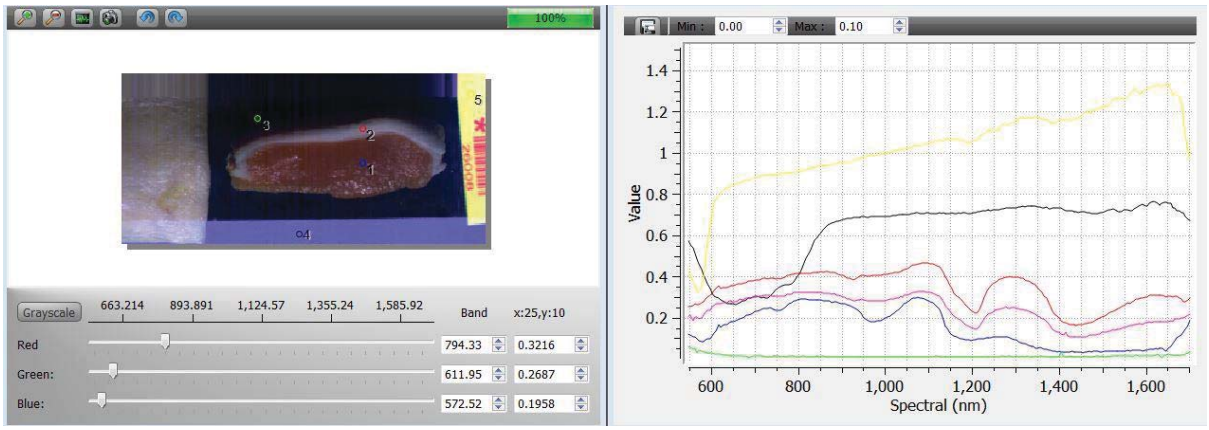


Figure 17: A spatial image of a meat sample on the left with the highlighted pixels showing their corresponding spectrum on the right. Meat in blue, plastic label in yellow, paper tape in black, background in green, intermuscular fat in red, intramuscular fat in pink.

There are four main ways to build a hyperspectral image: area scanning, point scanning, snapshot and line scanning (Elmasry & Sun, 2010). Area scanning involves a stationary sample with one wavelength taken sequentially after another on the same image; it is also known as wavelength scanning. The point scanning, or whiskbroom, method works by taking the entire spectrum at a single point at once and then moving the image to another position to take the next spectrum. This can be very time-consuming and requires either the sample or the camera to be moved in a set side-to-side pattern. Snapshot, or non-scanning, is based on capturing hyperspectral images during a single integration time of a detector array, so that no scanning is involved. The lack of moving parts helps with the avoidance of motion artefacts in the scan but a downside is that a detector with a large amount of pixels is required which limits its cost effectiveness and makes the camera larger and more complex. The line scanning, or pushbroom, method is created by taking the spectrum from a whole line of the image before the image is moved along and another line is captured (Figure 18). Line scanning version works well in situations where the sample moves at a steady and constant rate, such as would be found with a conveyor belt in a meat processing plant.

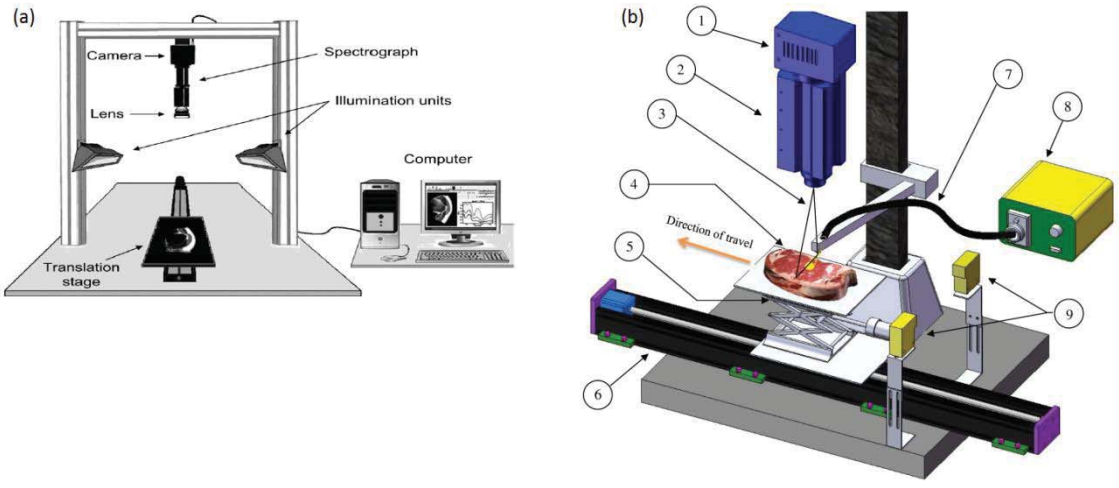


Figure 18: (a) Configuration of a typical hyperspectral imaging system (b) Schematic of line scan hyperspectral imaging system used to collect hyperspectral optical scattering images: 1) camera; 2) spectrograph; 3) Field Of View of line scan camera offset 5 mm from centre of incident beam of light; 4) steak sample; 5) automated

vertical stage; 6) linear slide, moved the sample under the line scan in the direction of travel; 7) incident fibre optic cable; 8) light source tungsten halogen lamp; 9) photoelectric switch.

Line scanning or pushbroom imaging

Line scanning cameras record a whole spatial line and its corresponding spectrum of an image rather than single pixels at a time. It does this using a two-dimensional dispersing element or prism and a two-dimensional detector array. A narrow line of the spectrum is imaged on a row of pixels on the sensor chip and the spectrograph creates a spectrum for each point along said line. This is called a wavelength dispersive system that includes a diffraction prism. There is a slit aperture at the base of the camera that creates the line imaged and it continues to take lines, or slices, of the sample as the sample moves in front of the camera's field of view, building a two dimensional spatial image with a third spectral dimension within each pixel (Figure 19) (NovusLight, 2015).

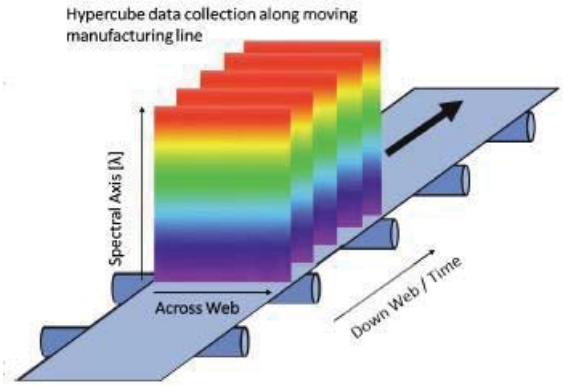


Figure 19: Graphical representation of hypercube data collection along a moving conveyor belt (Kemeny & Stuessy, 2012)

1.2.3 Univariate and Multivariate model types

The spectra collected in the visible and near infrared spectral ranges present a series of overlapped bands which are difficult, if not impossible, to interpret individually. Thus the use of these spectral ranges to predict attributes from the samples requires the using of a mathematical model. The two main types of model can be split into univariate and multivariate regressions. Univariate, as the name implies, is useful for when there is the assumption that the Y variable (the response variable) is

influenced by only one factor e.g. as temperature rises, the amount of sweat a person produces goes up. This implies that in the hypothesis the only thing that is affecting someone's production of sweat is the temperature. Multivariate models, on the other hand, means that there are multiple variables or even combinations of factors that can affect the hypothesis. In the example above it may mean that the sex, age or weight of the person may also affect the amount of sweat a person produces and so must also be taken into account when trying to build a robust and accurate model of sweat production over varying temperatures as they are all interacting with one another. In other words, multivariate regression is for correlating the information in one data matrix (X) to the information in another matrix (Y) (Sun, 2010).

1.2.4 Current spectral imaging considerations

Weeranantanaphan, Downey, Allen and Sun (2011), Prieto, Roehe, Lavín, Batten and Andrés (2009a) and Prevolnik, Čandek-Potokar and Škorjanc (2004) have mentioned that one of the shortcomings of current VIS-NIRS spectroscopy trials was the lack of comparisons and models from high numbers of animals from multiple slaughter plants. This is not a problem of NIRS itself but in the design of current experimental design, possibly due to resource limitation. Any technique that depends on a model based approach will need good, representative information at the very start to build its models from.

This is important as abattoirs, even within the same company, all have differences in the way they slaughter, cut and chill the carcasses which all affect meat quality. Both the physical NIRS and HSI machines will be affected by each abattoirs unique environment factors (temperature, humidity, etc.) which need to be taken into consideration when developing models. In addition, the meat is affected by factors such blooming, changes post slaughter, meat composition etcetera. All these factors too must be taken into consideration by the development of models that have these different sources of variation built into the calibration to try and capture as much of the variation as possible.

It is suggested that if the model is fitted on samples from more than one plant it helps take into account these differences and creates a more robust model, as was demonstrated by Shackelford, Wheeler and Koohmaraie (2005) , who made their model from two different slaughter plants.

The major issues with using NIRS and HSI arise from the heterogeneous nature of the meat itself. Meat is solid, fibrous and can consist of many layers of different biological material such as fat, meat and connective tissue, making it very difficult to get a sample free from variations caused by light scattering. Traditionally methodologies to use NIRS are based on the use of meat samples that have been collected and removed to a laboratory and homogenised, freeze dried or made from liquid extracts, giving a much more uniform product to work with and allowing for better fitting models to be built.

The advantages of spectral imaging over traditional meat quality measurement techniques include, but are not limited to:

- minimal sample preparation
- the non-destructive nature of the imaging allows for measurements to be taken on meat and co-products as they are moving along the traditional conveyor belt systems of meat processors without any need of removal or alteration of the samples, creating the ability to guide to the processing that they require in real time
- once the calibration model is built and validated, it becomes a fast and simple analysis method
- it saves time, labour, reagent costs and has no waste to manage when compared to traditional meat quality testing situations

While the advantages of hyperspectral imaging in particular are:

- visualisation of spatial distributions of numerous chemical compositions simultaneously
- allows the ability to see how the sample varies over its spatial elements to potentially assess defects (e.g. bruising, marbling or blood spotting)

Downsides to the use of spectral imaging include:

- standardised calibration models are needed to be produced first, possibly for each plant or type of implementation within a single plant, which increases initial set up and implementation of the hardware

And with HSI in particular:

- large amounts of data collected causes problems for computing power and storage
- to fully analyse an entire hypercube can be a lengthy and time consuming process

(Sun, 2010)

1.3 Modelling

The spectral methods described previously generate a large quantity of complex and related variables, particularly the HSI data sets. As a result, complex mathematical modelling approaches are required that are capable of separating and managing the multitude of interacting and confounding variables. Such approaches include principle component analysis, partial least squares, and neural networks.

1.3.1 Principal Component Analysis (PCA)

Principle component analysis is a method used to reduce, or compress, the available data. When applied to a data matrix of samples and variables it creates new variables which are known as principal components (PCs). These new variables are linear combinations of the original data with the weight vectors that define the combinations being composed of a unit length and are orthogonal (at right angles) to one another. This is useful as the original data points which were once all dependent and related to each other are now shifted into uncorrelated variables. The first new variable attempts to capture as much of the variability in the original data as possible with each successive new variable attempting to account for the remaining variability. The main purpose of this analysis is to try to describe a large, highly multivariate data set with as few variables as possible which greatly helps to

bring out strong patterns in the dataset as well as helps with visualisation of the results, which is normally in the form of scatterplots (Figure 20 taken from Scholz (2006)).

Figure 20: Conversion of a three dimensional matrix with multiple variables into a two dimensional component space showing the relation between principal components (Scholz, 2006)

1.3.2 Partial Least Squares (PLS)

In a very simplified sense, PLS is combining a PCA on X and a PCA on Y, but in a more realistic sense it is a projection of predicted (X) and response (y) variables into a set of orthogonal factors called latent variables (LV), minimising the dimensionality of the data while maximising the covariance between the X and the y variables (Wold, Sjöström & Eriksson, 2001). These LVs are statistically independent, meaning they are uncorrelated with each other, and ideally preserve all relevant information leading to consistent predictions.

1.3.3 Support Vector Machine (SVM)

Support vector machine, or SVM, is a non-linear type of mathematical modelling that attempts to find the same answer as PLS by using given labelled training data (unsupervised learning) and outputting an optimal hyperplane