EVALUATION OF PORK QUALITY BY PROTON NUCLEAR MAGNETIC RESONANCE

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Abstract

Consumers today are offered a diversified meat section at their local retail outlets. As the number of meat sources grows, consumers favour meat of higher quality. Competition is coming not only from other meat products but also from meats being imported internationally. There are growing concerns that pork quality is in fact struggling. After decades of research, issues such as pale soft exudative (PSE) pork and dark firm dry (DFD) pork remain prevalent. If such products reach the consumer, the industry risks the consumer no longer choosing pork in the future. To prevent economic losses to the industry, these products need to be detected and withheld. The problem lies in the fact that conventional techniques for meat quality measurement are slow, expensive, and destructive. Effective quality assurance programs that can keep up with market trends require a fast, non-destructive, method for measuring meat quality. The purpose of this study is to investigate low-field time-domain proton nuclear magnetic resonance (NMR) as a tool for rapid, non-destructive, multidimensional meat quality assessment.

In the first section, a review of current literature with regards to meat science, meat quality, and meat quality measurement tools and techniques. This is followed by a review of current literature regarding NMR as a tool for meat quality assessment.

NMR is then used in an experiment to investigate its applicability in measuring cooking loss, drip loss, and thaw loss. This was performed by using a transverse relaxometry experiment using a benchtop NMR at 6 MHZ at 4 °C of samples averaging 539 g – a size and temperature closer to what might be seen in industrial applications. Currently, the literature has exclusively investigated NMR as a tool for measuring meat quality on samples often around 1 x 1 x 5 cm in size and at room temperature. This study seeks to close that knowledge gap. Using multivariate

analysis, a correlation of r = 0.686, 0.573, and 0.452 for cook, drip, and thaw loss, respectively, was obtained.

The second part, NMR was used for the measurement of solid fat content of pork fat at nine separate temperatures in order to predict iodine value. A correlation of r = 0.87 was obtained. This shows potential applicability in industry, though this research raised important questions of what other chemical properties might influence SFC properties; these notions are investigated.

In conclusion, NMR showed promising correlations and revealed that it does, upon further research and development, have the potential to measure meat quality attributes such as cooking loss, thaw loss, drip loss, and fat consistency.

Keywords: nuclear magnetic resonance, time domain, proton, pork, meat, quality, spectroscopy, non-destructive, multi-dimensional, water holding capacity, drip loss, moisture content, fat, lipids, solid fat content

Résume

Les consommateurs font maintenant face à une grande diversité de viandes dans leurs marchers locaux. Cela leur permet de choisir des viandes de plus en plus hautes en qualité. La compétition arrive non seulement de d'autre produits à base de viande, mais aussi de viandes importées de l'international. La qualité de porc n'est pas optimale et les producteurs de porc s'en rendent compte. Après des décennies de recherches, les problèmes de qualité tels que la viande pâle, molle et exsudative en plus de viande foncée, dure et sèche sont encore commun. Quand des produits de basses qualités sont consommés, il se peut que le consommateur décide de ne plus vouloir acheter de porc dans le futur. Une grande partie du problème se trouve dans le fait que les techniques conventionnelles pour mesurer la qualité de viande sont dispendieuses, lentes, et destructives. Pour un contrôle de qualité efficace, il faut des technologies rapides, économiques, et non-destructives. Le but de cette thèse est d'investiguer la relaxométrie par résonance nucléaire magnétique (RMN) comme outil pour mesurer la qualité de viande de manière multidimensionnel.

Dans la première partie de cette thèse, je présenterai une revue de littérature à propos de la science et la qualité de viande, les techniques de mesure de qualité de viande et puis finalement, une revue de l'utilisation de l'RMN pour mesurer la qualité de viande.

L'RMN est ensuite utilisée dans une expérience pour investiguer sa capacité de mesurer la perte en cuisson, perte en eau et en cycle de gèle-dégèle. Cette dernière a été réalisé par relaxométrie transversale par un RMN de 6 MHz sur des échantillons de 539 g, à une température moyenne de 4 °C – des valeurs plus près de ce qui se verraient en industrie. En ce moment, la littérature regarde uniquement des échantillons de viande d'environ 1 cm x 1 cm x 5

cm à température pièce. Cette étude cherche à approfondir nos connaissances dans le domaine de qualité de viande par RMN. Après une analyse multi variable, une corrélation de r = 0.686, 0.573, 0.452 a été obtenue en perte à la cuisson, perte en eau et ainsi qu'en cycle de gèle-dégèle.

Dans la deuxième partie, RMN a été utilisée pour mesurer le pourcentage de gras en état solide à 9 températures différentes pour chaque échantillon dans le but de prédire la valeur d'iode. Une corrélation de r = 0.87 a été obtenue. Ceci démontre l'applicabilité de l'RMN pour l'industrie. Cela aussi nous permet d'investiguer quels autres facteurs pourraient influencer la consistance d'un gras.

En conclusion, l'RMN pourrait devenir applicable dans l'industrie si les recherches et développements de l'RMN ainsi que la viande se poursuivent. Elle démontre le potentiel de mesurer la perte à la cuisson, perte en eau et en cycle de gèle-dégèle, ainsi que la consistance des gras.

Mots clés : résonance nucléal magnétique, porc, viande, qualité, non-destructif, capacité de rétention d'eau, perte en eau, gras, gras solide, lipides

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Contributions of the Authors

The work reported on here was performed by Ian Burelle under the supervision of Dr. Michael Ngadi of the Department of Bioresource Engineering, Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue.

All experiments were performed at the Agriculture and Agri-Food Canada Research and Development Centre laboratory in Saint-Hyacinthe.

Both papers in the following have the following authorship: Ian Burelle, Michael Ngadi, Marie-Rose Van Calsteren, and Claude Gariépy. Both Dr. Van Calsteren and Dr. Gariépy are from the Agriculture and Agri-Food Canada Research and Development Centre laboratory in Saint-Hyacinthe.

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Chapter 1 – Introduction

1.1 Market Trends and Pork Quality

The pork industry is becoming increasingly aware of market segregation and changing consumer habits. With the diversity of meats available, as well as alternative protein sources, consumers are no longer limited in their purchasing decisions. This has increased competition between meat products domestically and internationally. Consumers are now choosing products of higher quality. An unfavorable experience with pork might cause the consumer to discriminate against such products in the future. Some claims have been made that the point may be reached where consumers refuse to eat pork products entirely. Another aspect of concern is that of consumer habits. Consumer often experience confusion between poor quality and a health safety risk; this leads to a tendency for consumers to consider pork unsafe to eat which further re-enforces consumer dissuasion from purchasing pork (Cassens, 2000; Kauffman, Cassens, Scherer, & Meeker, 1993).

As of 1960, when pale, soft, exudative (PSE) pork was first documented – though first recognized as early as the 1950s – scientists having been trying to tackle this problem, and even though some of the conditions causing it have been nearly eliminated, its prevalence continues; after 30 years of progress the occurrences of PSE has only been reduced from 18% to 16%. PSE has undesirable qualities in the form of high levels of drip loss, a pale colour, and is not optimal for further processing due to poor binding abilities inability to maintain its shape. Further processing increases the value of a cut, and the inability to do so is a significant loss in potential profit. It is also unappealing to the customer if over 2-3% of its mass is exuded in the package because of the wet appearance and its soft, poorly defined, dimensions. Levels as low as only 2%

drip loss is required for the consumer to notice it and deem it watery and undesirable. Knowledgeable customers know that meat which losses a lot of water also loses nutrients and proteins, reducing the nutritional value (Cassens, 2000; Correa, Méthot, & Faucitano, 2007).

The culprit preventing an effective solution to the problems of poor quality pork products, despite nearly 40 years of fervent efforts (Cassens, 2000) is the lack of recorded assessment (Kauffman et al., 1993) which in turn is caused by the lack of efficient techniques for measuring meat quality attributes. As Bertram & Andersen, 2007 expressed: “powerful techniques for elucidating the underlying biophysical mechanisms causing formation of drip loss are essential in meat science” (H. C. Bertram & Andersen, 2007).

Low-field time-domain proton nuclear magnetic resonance (NMR) has the potential to solve these issues. NMR measures the entire sample, not just the surface, and is unaffected by solids. A product still within a package, would not affect the results. Considering the measurement time is on the order of seconds rather than minutes or hours, NMR could measure entire products, in their shipping package, at nearly conveyor belt speeds. This form of NMR – further described in the following section – is particularly sensitive to water and the location of water within a sample. Considering that a large portion of pork quality issues arise from water holding capacity problems, NMR is uniquely placed to further investigate the mechanisms and detect issues in this regard (Bertram & Andersen, 2007; Gillies, 1992; Kauffmanet al.,1993).

1.2 Differentiating Nuclear Magnetic Resonance Instruments

There exist several kinds of nuclear magnetic resonance instruments, and it is worth differentiating which one is being investigated for this study as compared to the various other tools available. For this study, low-field time-domain proton nuclear magnetic resonance (NMR)

was used. While most scientists would be familiar with nuclear magnetic resonance as a tool for elucidating chemical structure, or even macromolecular structure such as those of proteins or enzymes, these pieces of equipment are considered high resolution (S. W. Provencher, 1993; Wand, Ehrhardt, & Flynn, 1998), the following work uses low resolution. The reason for using low resolution is that meat contains far too many different compounds and molecules to obtain usable information from high resolution spectra. Low resolution NMR can obtain information from complex, and even *in vivo,* samples such as metabolite concentrations in live animals (S. W. Provencher, 1993). This is not to say that low resolution proton NMR does not have a high resolution equivalent, it does (Mittermaier & Kay, 2006). Low resolution for analyzing meat will provide information about largely prevalent molecules such as water and fat, facilitating the differentiation between the vast array of molecules present and the molecules of interest.

The next important difference to distinguish is that other forms of low resolution time-domain nuclear magnetic resonance exist other than proton NMR. ^{31}P NMR has been used to study mitochondrial metabolism in humans and animals amongst other applications (ARNOLD, MATTHEWS, & RADDA, n.d.). ^{23}Na NMR has also had applications where it studies the transport of sodium across cell walls (Riddell & Hayer, 1985). Other NMR active nuclei can be used as well. A requirement for NMR active nuclei is that the number of protons be different from the number of neutrons so as to prevent the magnetic moments produced from the spinning of these subatomic particles from canceling each other's signal. The first biological experiment by NMR was performed by Bloch in 1952, where he obtained a signal from his finger shortly after sharing a Nobel prize for the discovery and development of NMR (Gillies, 1992).

1.3 General Objectives

This study is composed of three sections. The first is an extensive review of meat quality and science, the second an experiment investigating the applications of NMR to cooking, drip, and thaw loss. The third is an investigation of NMR for measuring solid fat content and comparing it to the conventional of measure: iodine value.

1. The main objective is to investigate the potential of NMR as a tool for elucidating meat quality attributes

2. To explore the applicability of replacing long, expensive, and destructive conventional methods with fast, non-destructive, multi-dimensional methods such as NMR. These conventional methods are:

    a. Using drip loss to measure water holding capacity

    b. Physically cooking or freezing and thawing the meat to measure cook and thaw loss

    c. Using iodine monochloride to measure fat consistency

1.4 References

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Chapter 2 - Literature Review

2.1 Physiology of Meat and Muscle

Meat anatomy and the role of pH post-mortem is important to the understanding of WHC and drip loss. The structure of muscle is quite complex, but also fundamentally important to understanding where water is situated and how those different water populations have different implications on drip loss. This influences how muscle is converted to meat, and ultimately, meat quality.

The tendons are located on each extremity of the muscle and connect muscles to bones. Tendons are continuous with the sheath that envelopes the entire muscle, called the epimysium. Inside the epimysium are several fasciculi, which are a groupings of muscle cells. The fasciculi are also enclosed by a sheath called the perimysium. The muscle cells themselves are encapsulated by a layer called the endomysium. Touching the endomysium is the membrane belonging to the muscle cell called the sarcolemma, and below this is the basement membrane called the sarcoplasmic reticulum. The striations seen on the muscle cells are the myofibrils. Myofibrils contract and transmit force across the entire muscle and finally to the bone to then cause movement in the animal. Within these myofibrils are bands, called the A-bands, I-bands, and Z-bands. The A-bands are protein dense, and the Z-band intersects the less dense I-band. A-bands have thicker filaments and some thin filaments, while the I-band is made up of thin filaments. Thick filaments are composed of myosin, a protein. Thin filaments are made of actin, also a protein (Huff-Lonergan & Lonergan, 2005; Pearce, Rosenvold, Andersen, & Hopkins, 2011).

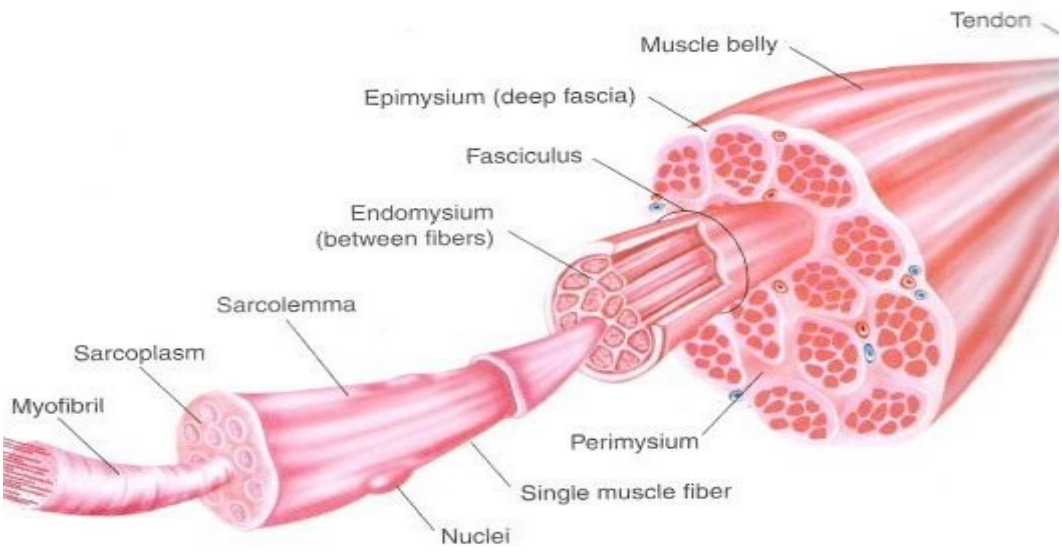

Figure 2.1: General Structure of Skeletal Muscle (Pearce et al., 2011)

Post-rigor, or during contraction, an interaction between actin and myosin generates a complex called actomyosin. When calcium is released from the sarcoplasmic reticulum, the protein tropomyosin, which normally covers the myosin binding site on actin, reveals said site. This allows the globular head on myosin to attach to actin. Myosin has a globular head which hydrolyzes ATP to liberate energy, which is then used to pull the actin inwards, towards the center of the sarcomere, by slightly coiling/turning on itself and in turn shortens the myofibril. This leads to muscle contraction. The myosin is released from the actin binding site when a new ATP molecule appears. When the ATP reserve is depleted, myosin becomes a permanently bound to actin (Huff-Lonergan & Lonergan, 2005). This leaves the muscle in a semi-permanently contracted state, called rigor (Egelandsdal, Martinsen, & Autio, 1995; Huff-Lonergan & Lonergan, 2005). Tenderization will follow due to a disintegration of endomysium and perimysium into fibres and fibrils of collagen (Liu, Nishimura, & Takahashi, 1995).

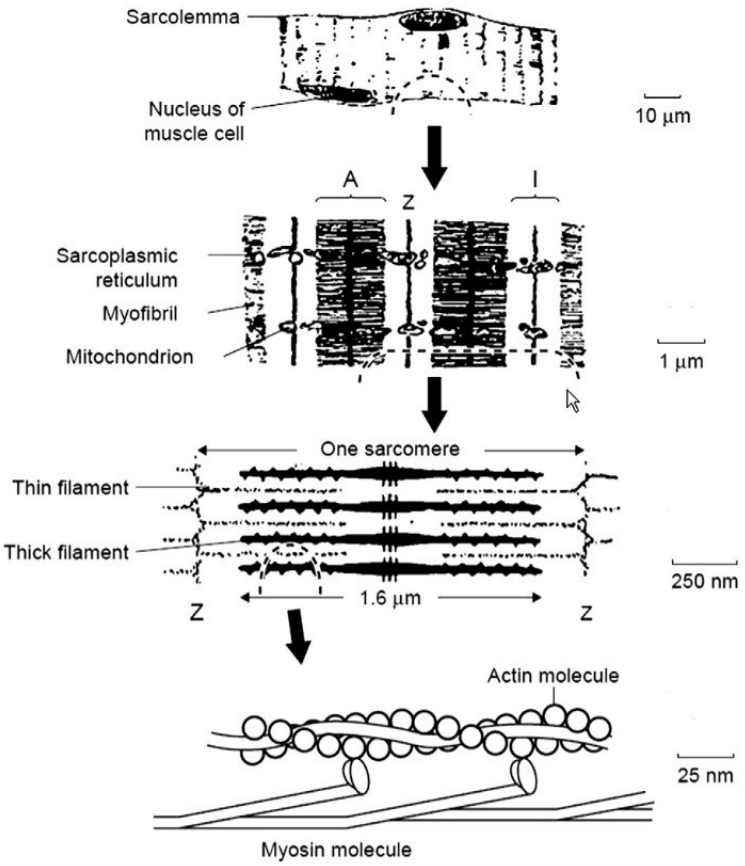

Figure 2.2: Fibrillar and molecular structure as would be seen under light microscope (top two images), electron scanning microscope (third from the top), and a reconstruction indicated from X-ray crystallography (Pearce et al., 2011)

The depletion of glycogen reserves – the source of ATP – after death, in anaerobic environments, will produce lactic acid and cause the drop in pH typically seen post-mortem with the onset of rigor. PH is largely important to meat quality, and a rapid post mortem decline can cause serious issues (Boler et al., 2010).

There is a complicated relationship between meat quality and pH. In fact, the relationship between tenderness and ultimate pH is still disputed between several competing hypotheses, the leading hypothesis is that they have a curvilinear relationship. The reason for this still unknown

(Devine, Graafhuis, Muir, & Chrystall, 1993; Silva, Patarata, & Martins, 1999; Watanabe, Daly, & Devine, 1996). Factors affecting pH include genetics, pre and post mortem treatment, and muscle fibre type. Nonetheless, pH is known to affect colour and water holding capacity, two fundamentally important meat quality traits (Boler et al., 2010; Fletcher, Qiao, & Smith, 2000; Gou, Comaposada, & Arnau, 2002).

The ideal pH range is between 5.4 and 5.6. Values above 5.8 are deemed undesirable for the quality of the pork (Węglarz, 2010). A rapid decline of pH after slaughter causes issues of pale coloured pork, with a low water holding capacity and overly soft texture. This is called pale soft exudative (PSE) pork (Boler et al., 2010). On the opposite end of the spectrum, a high ultimate pH will cause a dark coloured, firm, meat which is more prone to the development of rancidity and will have a less appealing flavour. This is called dark firm dry (DFD) pork (Silva et al., 1999).

2.1.1 Location of Water within Meat Structure

A fraction of the in meat is found inside the myofibrils, this is called intra-myofibrillar water. This is the most abundant water population in most cases. Next is the extra-myofibrillar water which is found outside of the myofibrils but inside the sarcolemma. Water is a charged, dipolar, molecule. It is therefore attracted to other charged species like proteins, which water will binds to tightly. This is referred to as bound water or hydration water. This population is resistant to being removed by any means, whether it be heating, or a freeze thaw cycle (H. Bertram et al., 2001; H. Bertram, Dønstrup, Karlsson, & Andersen, 2002; Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011). Closely related to hydration water, which only accounts for about a tenth of the entire moisture content, is entrapped or immobilized water. This water is close to the protein

though not directly bound to it, being held by its attraction to bound water and steric effects. Entrapped water is held relatively strong in place: it will not move within the tissue but unlike bound water, it can be affected by chemical changes caused by rigor and the associated pH drop, as well as cooking process like drying, and freeze thaw cycles. It can eventually be lost in the form of drip loss. Retaining the entrapped water, which is the water which is most affected and of greater importance in maintaining a high water holding capacity. Finally, there is the inter-fascicular water, which is water trapped between fasciculi and is more easily exuded (Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011).

2.2 Meat Quality

2.2.1 Water Holding Capacity

One of the most important meat quality attributes is water holding capacity, measured as drip loss. Dry meat is not as flavourful, nor does it have a desirable texture. Water content directly affects juiciness (Webb & O'Neill, 2008). Meat must retain the water within it to prevent dry meat. Its ability to retain water is what water holding capacity (WHC) refers to. Precisely, its meats ability to retain liquid under the forces of gravity. Notably, it refers to liquid, rather than simply water, because other products – such as protein – will be exuded during the drip loss (H. Bertram, Dønstrup, et al., 2002; Huff-Lonergan & Lonergan, 2005). This introduces the next term, drip loss, which is simply the amount of liquid lost from the forces of gravity acting on a piece of meat. Both WHC and drip loss are expressed as a percentage of either lost or retained liquid compared to its original mass. Generally, WHC is measured as drip loss (H. Bertram, Dønstrup, et al., 2002; Fischer, 2007).

It has more importance than just juiciness, as many positive attributes associated with consumer acceptance are closely correlated with a high WHC. A high WHC has a reduced cooking loss. Cooking loss is the loss of liquid due to thermal exchange, be it oven, grill, microwave, sous-vide, or other forms of cooking. A low WHC is closely correlated to a high cooking loss (Li et al., 2012). Tenderness is the second most important attribute for consumer palatability and it too, is closely linked to WHC. Hence, WHC is an attribute that provides valuable insight into the consumer experience of eating pork (Barge, Destefanis, Toscano, & Brugiapaglia, 1991; Chen et al., 2015; Kauffman et al., 1993; Węglarz, 2010). There is another reason why this is of large importance to the industry. Poor WHC costs the industry millions of dollars annually, when cuts of meat loses as much as 10% of their mass in the form of exuded liquid. Since this causes the same cut of meat to sell for less, since it is now 10% lighter and sold on a per mass basis (Cassens, 2000; Chen et al., 2015).

The mechanisms behind water holding capacity start with the progression of rigor mortis. This induces a lateral shrinkage of myofibrils causing the compartmentalization of water to change. Water will tend to move from intra-myofibrillar spaces into extra-myofibrillar spaces Once in this space, specifically the sarcoplasm, water is held in place by capillary forces alone. These forces are weak in comparison and therefore this water can be more easily exuded (Huff-Lonergan & Lonergan, 2005). The extent to which water is held in the intra-myofibrillar spaces is dependant on the pH drop immediately post-mortem and ultimate pH of the meat. Water being a dipole molecule, it is attracted to other charged molecules. The isoelectric point is the pH at which the number of positive and negative charges are equal to one another; in meat this around 5.1. At the isoelectric point, water will have few to no charged particles attracting it, making for only a weak force binding it in the intra-myofibrillar space. At high pH there is a greater number

of charged particles attracting the water and holding it strongly in place. In the case of PSE, a pH of 5.2 is reached in roughly two hours. This is close to the isoelectric point, and this is the primary cause for this kind of meat being so exudative. It also is the cause of the pink colour attributed to PSE. In the case of DFD, the pH often remains high, the decline is slow and progressive, stabilizing between 6.6 and 6.9. Being far from the isoelectric point, this make for a higher WHC and consequently a dark colour (Apple & Yancey, 2013). Figure 2.3 demonstrates the differences in WHC and corresponding pH of PSE, regular, and DFD meat.

Figure 2.3 Relationship between pH, colour, and water holding capacity

(http://www.aedilemma.net/images/pH2.jpg**, viewed Aug 10th, 2016)**

There are other causes for poor WHC. Pale, soft, exudative pork (PSE) occurs more frequently in pork containing the halothane gene. The gene was nearly eliminated through selective breeding and yet variability in the WHC of pork products remains high (H. C. Bertram & Andersen, 2007). Other factors such as post-mortem chilling and pre-mortem stress have been studied, where stress was considered as largely important. Animals that were exposed to stressful situations would have a higher incidence of PSE. Stressful situations include the season since

large temperature fluctuations can stress a pig; and breed because some breeds are more excitable when faced by situations that other pigs would not have responded to with quite as strong a reaction (Cassens, 2000). An exact understanding of the mechanism behind WHC is still not completely understood (H. C. Bertram & Andersen, 2007). There are also cases of red, soft, exudative pork (RSE). This meat has the correct colour but the same negative attributes as PSE. The cause of RSE is still unknown (L & Kauffman, R, 1999).

Not all subpar products fall under the category of PSE or DFD. For acceptable palatability, estimates have been made showing that as much as 50% of pork products on the market are considered to have a WHC below acceptable levels (Chen et al., 2015).

The first method for measuring WHC is the Honikel bag method. A sample is placed on netting and suspended in a bag. This bag is inflated to prevent it from touching the sample and affecting the way liquid is exuded. The sample is left in this position for 48 hours. The technique is gravimetric, so the initial mass of the sample is compared to the final mass. This technique is generally accepted, but has inherent pitfalls. From the moment the animal is slaughtered, it can take 72 hours to obtain a result, rendering it impractical for producers, especially those who intend to sell their product to retailers within that time frame (H. C. Bertram, Andersen, & Karlsson, 2001; H. Bertram, Dønstrup, et al., 2002; K. O. Honikel, 1987; K. Honikel, 1998). If the product is sold before obtaining results, and the results indicate poor quality, such as the occurrence of PSE, then the producer would be hard pressed to prevent that sample from making it to the consumer. Furthermore, it is destructive, and requires that samples be taken in a discrete area of a cut of meat which may, or may not, be representative of the whole cut.

A second method is to place a sample of meat on a piece of filter paper, apply a certain amount of compression force, and then study the size of the wetted area of the paper produced by

exudation. Studying the shape of a wet area of filter paper can be difficult since it is rarely a perfect circle, making it less easily calculated. A planimeter is usually used to aid the measurement of such surfaces. A ratio of the surface area of the meat against the surface area of the wetted paper allows for the calculation of the WHC. The main advantage of this method is that it does not require a 24 to 48 hours waiting period to be performed, and can be performed early in the processing of meat, and requires only a few minutes. The disadvantages is the ability to measure the surface area reliably, maintaining even and constant pressure, and some studies have demonstrated that this method can be more inaccurate when compared to other methods of measurement (Barge et al., 1991).

A second technique for measuring WHC uses centrifugation. A protocol with specific centrifugation parameters was developed for this protocol. Then the same gravimetric technique is applied by comparing the mass before and after centrifugation (H. Bertram, Dønstrup, et al., 2002; K. Honikel, 1998).

A method similar to the Honikel bag method, called EZ-DripLoss, was developed. The original method was to simply put the sample in a special tube so that it could exude liquid over the course of 24 hours. It was then weighed in the tube. The technique received criticism when its results were less consistent with the Honikel bag method, though it was easier in terms of manipulation and equipment to simply place a small sample in a small plastic tube. The method was subsequently corrected in two ways: it added a step called dabbing, and it increased the time frame to 48 hours much like the original Honikel bag method. Dabbing is the act of patting the sample dry to remove surface moisture. Both these improvements were deemed successful and it is now a popular method used in several pork production facilities (Correa et al., 2007; Otto, Roehe, Looft, Thoelking, & Kalm, 2004).

2.3 Fat

Consumers tend to avoid fat in meat products, considering its presence unhealthy (Wood et al., 2008). High fat diets have been linked to cardiovascular disease, cancer, diabetes, and many more health ailments (Corrêa, Forato, & Colnago, 2009). On the other hand, some fat is necessary, and medical professionals are suggesting to switch the focus from fat quantity to fat quality, choosing products with low levels of saturated fatty acids. As these same professionals have pointed out, saturated fats play a greater role in the prevalence and likely onset of such health issues. Some fatty acids, such as linoleic acid, are valuable for the health of individuals and pork contains linoleic acid (Webb & O'Neill, 2008). Lipids can also contain the fat-soluble vitamins A, D, E, and K (Willian, 2013). As the switch from quantity to quality is beginning to take place in consumers, the need to understand and measure fats grows for the industry.

2.3.1 Intramuscular Fat Content

Fat content has importance beyond being a source of healthy fatty acids, it also plays a significant role in meat quality (Webb & O'Neill, 2008; Wood et al., 2008). Intramuscular fat content is the fat found within muscle fibers, as opposed to the larger fat deposits between muscles called the intermuscular fat or the subcutaneous fat found just underneath the skin, also called the adipose tissue. Marbling score, is a visual property of intramuscular fat (IMF) deposition and spatial distribution in meat, including flecks. Marbling score is measured using trained graders, and the use of humans will tend to incorporate inconsistencies, as well as high labor costs. In response to this issue various techniques using machine image technology are, and have, been developed (Gerrard, Gao, & Tan, 1996).

High quality cuts require a minimum marbling score, and the distribution must be fine. In the case of branded beef products, these characteristics allow for sales at a higher price (Chambaz, Scheeder, Kreuzer, & Dufey, 2003). The reason for selling at a higher cost is associated with the improved quality, and branding the product guarantees to the customer that those quality criteria have been met. The quality provided by a higher IMF can be noticed during cooking where the amount of liquid exuded is reduced which in turn, causes an improved juiciness, flavour, and texture much in the same way a high WHC would. This also applies to a freeze thaw cycle, where less exudate is lost during the thaw (Webb & O'Neill, 2008).

At IMF levels below 2%, it is uncertain whether or not IMF has a positive effect on flavour attributes, with studies showing results for both positive and negative results. Above the 2% range, there is a general consensus that flavour is improved by the presence of IMF. The shear force to cut through a piece of meat has been demonstrated to be reduced by the presence high IMF levels. This translates to improved textural qualities for the consumer (Essén-Gustavsson, Karlsson, Lundström, & Enfält, 1994; Fernandez, Monin, Talmant, Mourot, & Lebret, 1999a, 1999b).

2.3.2 Lipid-Derived Flavours

Flavours, or taste-active compounds, include nucleotides, organic acids, peptides, amino acids, and various lipid components amongst others. This explains why meat is flavourful: it contains many of these taste-active compounds. Flavour, and the concentrations of these taste-active products are related to other factors, of which there are many. Things like gender, diet, and pre-slaughter treatment play a large role followed by the way meat is treated post-slaughter, the way it is cooked. Storage of meat, and freshness will also affect these flavours (Shahidi, 2002).

Studies on meat aroma were performed by extracting the phospholipids with a methanol-chloroform solvent extraction process. Samples were then cooked. These samples had none of the meaty aroma typically associated with cooked meat. Instead, it had an aroma described as roasted and biscuit-like. Phospholipids, upon further investigation, were found to play a role in the complex series of chemical reactions belonging to the family called the Maillard reactions. It is worth pointing out that triacylglycerol (TAG) also played a role, but to a much lesser extent, in Maillard based flavour development. There are a series of acyclic and heterocyclic volatiles that are generated by the presence of phospholipids; or prevented from being generated such as thiols, which are markedly reduced by the presence of phospholipids. The majority of flavour comes from phospholipids rather than TAG. While this is true for adipose tissue is mostly composed of TAGs, IMF is in majority composed of phospholipids (Shahidi, 2002).

Lipids produce positive flavours, but are also responsible for negative ones, through the mechanism of autoxidation. The off-flavours occurring from autoxidation, come from the volatile compounds produced from the degradation of hydroperoxide into hydrocarbons, alcohols, ketones, acids, esters, furans, lactones, epoxy compounds, polymers, and aldehydes. Hydroperoxide is flavourless, while its degradation products are quite flavour-active. The latter, aldehyde, is particularly poignant and can be detected by the consumer at levels as low as a few parts per billion. The flavour has been coined warmed-over flavour (WOF). The presence of such compounds has the undesirable effect of masking the positive flavour attributes. Lipid autoxidation can impact colour and texture, nutritional value and food safety. Prooxidants which can counter autoxidation include transition metal ions, NaBr, and NaCl; while nitrites and similar alternatives also reduce lipid oxidation (Shahidi, 2002).

2.3.3 Fat Consistency

The is a growing demand for meat low in saturated fatty acids. Low levels of saturated fatty acids cause fats to become softer than their unsaturated counterparts. This is conflicting for producers who require fats to be harder so as to facilitate processing (Johnstone & Li, 2011).

Soft fats, in the context of this paper, is defined by the author as fats that are so soft that they are detrimental to product quality. The first issue is that soft fats cannot be properly dimensioned. The meat does not hold its final shape and tends to slice quite poorly; most common is the issue of slicing soft pork belly into bacon. Also, in finer meats, such as hams and prosciutto, the ability to produce thin slices in fundamentally important are require even harder fats. Secondly, soft fats cause meat to separate from the layers of intermuscular fat – not intramuscular fat – and causes layers of subcutaneous fat to separate from one another; a quality which is undesirable, even more so in finer meats or those requiring high speed, thin, slicing (Kauffman et al., 1993; Olsen, Rukke, Flåtten, & Isaksson, 2007).

To further aggravate the problem, soft fats have less oxidative stability, which can cause the accelerated development of rancidity and off flavours such as WOF aromas; as well as reducing the shelf life of the product (Davenel, Riaublanc, Marchal, & Gandemer, 1999; Shahidi, 2002). In the case of pork, separating the short ribs from the carcass is particularly difficult, as is removing the skin from the carcass. If the meat requires drying, insufficient drying is likely to occur. Finally, the unappealing appearance is further amplified by the oily exterior that rises to the surface, especially in the package where it is no longer being manipulated. This could deter the consumer (Davenel et al., 1999; Johnstone & Li, 2011).

Finally, the monetary loss caused by soft fats is of concern to the industry. This occurs when a cut of prime quality is deemed too soft and is down-graded to a lesser quality; receiving less profit. The cost of this can attain 25 USD or more per head. It can also occur that a prime quality cut is down graded unnecessarily, and therefore cause an unnecessary loss in profit (Kauffman et al., 1993).

When it comes to evaluating the consistency of fat in the meat processing industry, the degree of unsaturation and/or the fatty acid profile is typically used. The interpretation of the fatty acid profile can be done by verifying the amount of stearic or linoleic acid, or proportions of certain fatty acids against one another. Another method is the iodine value (IV). The IV is a structural index that indicates the total unsaturation of an oil or fat, originally performed by measuring the milligrams of iodine monochloride absorbed per 100g of sample. It can be also be calculated from the fatty acid profile according to an a recognized protocol (A. O. C. S. AOCS, 2009; Gläser, Wenk, & Scheeder, 2004).

Iodine monochloride is extremely dangerous. According to the material safety data sheet, it is corrosive causing burns, pain, blisters, and coloured stains when in contact with the skin; contact with eyes, even in the form of vapours, could lead to total corneal opacification and loss of the eye, with lesser exposure causing partial clouding of the cornea. If inhaled, the whole respiratory tract (nose, throat, lungs, etc.) will become irritated, with coughing, choking, throat swelling, fluid in lungs, headaches, and dizziness; and this is at 50ppm. If ingested, 150 ml will cause death, and lesser amounts are known to cause delirium, coma, or circulatory collapse. Other issues if ingested: “stomach with edema (severe) of the pharynx, larynx; abdominal spasms, nausea, vomiting, colitis, hypotension.” While chronic exposure can cause “dental erosion, ulceration of jaw, nose, throat; throat and lung disease (bronchitis, pneumonia,

laryngitis), gastrointestinal disturbances, 'iodism,' tissue necrosis, skin blackening, painful joint swelling, conjunctivitis." Lastly, the flash point is dangerously low at only 41.1°C (MSDS, n.d.). Due to the dangers of iodine monochloride, a popular alternative is the American Oil Chemist Society's (AOCS) recommended practice Cd 1c-85 which permits one to calculate IV from the fatty acid profile.

IV in general has been criticized. Although when it was first established in 1884 it allowed for interesting information to be gleaned from its measurements, today the use of gas chromatography to find a fatty acid profile and then calculate the iodine value is considered to be moving backwards: starting with a technique which yields far more information (the fatty acid profile by gas chromatography) only to use a far less informative value, the iodine value (Knothe, 2002). Obtaining iodine value from fatty acid profile has come under criticism itself, for failing to include fatty acids longer than 20 carbons long (Johnstone & Li, 2011). Structural indices, such as the iodine value, are still widely used especially in quality control applications, as a measure of physical and chemical properties. Knothe (2002), with the United States Department of Agriculture published in the AOCS journal that "the IV index is too general to allow the correlation of physical and chemical properties with FA [fatty acid] composition."

There are many other methods of measuring fat consistency ranging from rough estimates, to highly accurate. The durometer can be used, which has an indenter in a frustoconical shape and a spring to force the indenter into the sample. The depth of the indentation is then measured. Some have criticized the fact that the force applied is reduced the deeper the indenter travels into the sample, due to the laws behind spring forces. The Brinell test uses a round hemispherical indenter attached to a rod with a platform on the opposite end of the rod. A weight is placed on this platform assuring an constant force regardless of depth traveled

(Lovegren, Guice, & Feuge, 1957). Similarly, cone penetrometers using different cone shapes have also been used to measure hardness (Haighton, 1959). Though these methods are still used today, new methods are evolving, with the development of new technology such as the Instron compression test where the force required to either penetrate, or the yield strength to rupture the sample, is measured. The variety of possible methods: compression, durometer, hardness meter, penetrometer, are all direct methods (Gläser et al., 2004; Izquierdo et al., 2005; Johnstone & Li, 2011).

A faster, and less accurate method, though still a popular method, used for pork belly is commonly called the belly flop test. It consists of placing a pork belly on a horizontal bar so that the bar is centered on the belly and it remains in balance. The deflection of the belly from horizontal is then measured (Davenel et al., 1999; Johnstone & Li, 2011).

2.3.4 Solid Fat Content

Solid fat content (SFC) is expressed as a percentage and is the ratio of fat in a solid phase over the total amount of fat (solid phase + liquid phase) at a specific temperature (Bruker, 2015; AOCS, 2015). When measured by dilatometer it is typically called solid fat index (SFI) rather than SFC. The specific volume of fat is dependent on temperature, and dilatometry takes advantage of this phenomenon by observing the change in specific volume as a sample is gradually warmed inside a column. Because it is an empirical method, it requires strict adherence to standardized protocols; a tedious and time consuming process. SFC, like SFI, is also rather tedious and time-consuming to measure, though it has demonstrated a lot more accuracy. The step which takes the most time, and is required for both methods, is the tempering of the fat samples, which requires the sample to go through a series of temperature steps, and to rest at

each of those steps for a period of time, ranging from 5 minutes to an hour per step. New methods, such as Fourier Transform Infrared Spectroscopy, are being developed to eliminate the time consuming tempering steps and are capable of measuring the SFI within two minutes (1 AOCS, 2009; van de Voort, Memon, Sedman, & Ismail, 1996).

SFC has good correlations with the other methods. For example, fat hardness measured by the puncture test gives values closely related to SFC (Davenel et al., 1999). Better yet, the hardness of binary blends of fat measured by the puncture test have a correlation coefficient of 0.997 ($p<0.001$) with SFC (Braipson-Danthine & Deroanne, 2004).

2.3.5 Factors Influencing Fat Consistency

It is common knowledge in chemistry that the number of double bonds in a fatty acid or TAG, also called the degree of unsaturation, is a principle contributor in the hardness of a fat. As previously demonstrated, the iodine value, and therefore degree of unsaturation, is no longer entirely accurate for indicating fat consistency. Other factors are important in understanding the phase behavior of fats. Phase behaviour, SFC, and hardness are all the result of combined effects (Braipson-Danthine & Deroanne, 2004; Liang, Li, Xu, & Li, 2004).

TAGs have a glycerol backbone with three fatty acid moieties esterified to it. The fatty acids attached to the glycerol backbone play a large role in the behavior of the fat. Most fatty acids in a TAG have an even number of carbons, between 16 and 20. Some TAGs have three identical fatty acids attached to the backbone, while others have mixed fatty acid lengths on the same backbone. The position of a fatty acid on the mixed TAG will contribute to its hardness by affecting crystallization kinetics, microstructure, and macrostructure of the fat; including the polymorphic forms (covered below). These factors will in turn affect the rheology and

consistency of fats. In fact, even the symmetrical or non-symmetrical placement of the fatty acids on the glycerol backbone can play an important role. For example, a symmetrical 16 carbon - 18 carbon - 16 carbon placement will have different properties than a 16 carbon – 16 carbon – 18 carbon placement, even though the TAG has the exact same composition and number of double bonds (Boodhoo, Kutek, Filip, & Narine, 2008).

In terms of microstructure, the TAG has a few factors of interest. The placement of fatty acids on the glycerol, as well as their angle of tilt coming off the basal plane, and the spacing between layers of TAGs – called the d-spacing – are all part of the microstructure. This affect temperature at which the crystals will melt and the way they pack together. A single TAG can have multiple melting temperatures and this phenomenon is called polymorphism. The temperature at which it melts depends on which form the crystal took, and these forms are called the polymorphic forms. Among the polymorphic forms, the three most important are, in increasing order of stability, and consequently increasing melting temperature: alpha (α), beta prime (β'), and beta (β). Most fats have an α form, though it is often unstable. Not all fats have all three forms, with some only having either the β or the β' form. The form a fat takes depends largely on the heating or cooling rate and its thermal history. For example, cooling the TAG tristearin quickly will likely place it in the α form. Interestingly, under further slow heating, it will melt again and then solidify into the β' form even though the temperature has only increased. It will do this a third time upon further slow heating to enter the β form. Fast heating, or slow cooling will change which forms the TAG takes, and therefore temperature history and process will seriously affect consistency (Timms, 1984). This also serves to explain why SFC measurements must undergo such a long and complicated tempering process before an accurate SFC value can be obtained.

The development of crystal structure in fat is called crystallization kinetics and it seeks to understand and predict crystal growth as a function of composition, structure, form, and temperature. Nucleation mechanisms based on the temperature changes and time require complex modeling to properly predict (Zhang et al., 2013). Even so, most papers have only been able to approach binary blends of TAGs with their models. Given the complex composition of animal fat, accurate methods for predicting composition are still unlikely.

The polymorphic form a fat crystal is found in, as previously mentioned, depends on temperature and thermal history. Going one step further into the understanding of fat hardness is understanding the thermodynamics behind the polymorphs and the proportion of solid to liquid. The equilibrium point, for the fractions of solids and liquids in a sample, is called the solid-liquid equilibrium. A sample can only take one polymorphic crystalline state at a time. Once that state is known, the other two polymorphic states are set to zero and the thermodynamic approach then looks to find the solid-liquid equilibrium which minimizes the Gibb's free energy (Costa et al., 2011; Teles dos Santos, Gerbaud, & Le Roux, 2014).

Final considerations include considering the compatibility of different fat combinations and the fractal nature of fat crystals. Though fat mixtures tend to have monotectic relationships, strong incompatibility between fats can occur, causing eutectic relationships to form. These relationships can occur in complex systems but also simple binary blends. When eutectic relationships form, crystallization effects can change (Braipson-Danthine & Deroanne, 2006). Less investigated is the fractal nature of fat crystals. This has been used to understand fat rheology. The model is based on the mass fractal dimensions, shear elastic modulus, and volume fraction of solid fat (Narine & Marangoni, 1999; Ro & Rousseau, 1996).

2.4 NMR

2.4.1 Mechanisms

Protons, like other subatomic particles, spin on themselves. When the sample is placed inside the magnetic field, the axis of rotation of the subatomic particles will align themselves parallel to the direction of the magnetic field, called B_0, when at thermal equilibrium. In order to understand the movement of the proton, it is often placed on a 3-dimensional plane, where B_0 is considered the z-axis. The alignment of all axes causes the buildup of a macroscopic magnetic moment equal to the resultant of all individual contributions of the nuclear magnetic moments. A coil surrounding the sample emits oscillating radiofrequency pulses which will align the axis of rotation in a direction different from B_0 if the pulses are emitted at resonance frequencies. The resonance frequency depends on the nuclei of interest, such as the proton in hydrogen, and magnet strength. As the sample is released from the radiofrequency pulses, it will return to its thermal equilibrium state, aligned with B_0. This moving magnetic moment, from its new orientation back to B_0 causes a change in magnetic flux and induces a current in the surrounding coil; the same coil which was used to emit the oscillating radiofrequency pulses. The time required for the return to thermal equilibrium is called the relaxation time, and the signal strength is proportional to the total number of NMR active nuclei simultaneously returning to B_0. If the radiofrequency pulses are used to place the axis of rotation on the X-Y plane, it is called transverse relaxation, or T_2. If the axis of rotation is flipped 180° to remain on the Z plane, then it is called longitudinal relaxation, T_1, or the spin-lattice relaxation in the laboratory frame (Jankowski, 2003; Sørland, Larsen, Lundby, Rudi, & Guiheneuf, 2004).

To be NMR active, the nucleus must have an uneven number of protons and neutrons as an even number will cancel out the magnetic moments of the other. NMR active nuclei include H, ^{2}H, ^{13}C, ^{17}O, 31P, ^{23}Na, though the most commonly used in food are H and ^{13}C, sometimes even in combination called 2D NMR where a ^{13}C spectrum is on one axis and the H spectrum on the other (ARNOLD et al., 1983; Belton, 2011; Marcone et al., 2013; Riddell & Hayer, 1985).

2.4.2 NMR for Meat Quality

Organic chemists began adopting NMR in the 1940s for its ability to elucidate the structure of molecules. Food science and engineering applications did not begin until the 1980s, in large part due to the lack of specialized equipment designed for food products, high cost of the equipment, and lack of trained professionals knowledgeable in the field and capable of applying it to products as complex as food. Today, applications of NMR in food are broad and far reaching, where it has seen success in fields such as food microbiology and food authenticity verification (Marcone et al., 2013).

Water content is one of the fundamental capabilities of NMR, as water produces a signal. The stronger the signal, the more water present. With a simple calibration curve, the moisture content of a food product can easily be obtained. Comparative studies demonstrated that moisture content of beef measured by NMR was in close agreement with values obtained by AOAC recognized protocols (H. Bertram & Andersen, 2006; H. Bertram, Purslow, & Andersen, 2002; Bianchi et al., 2004; Todt, Guthausen, Burk, Schmalbein, & Kamlowski, 2006).. Studies continued down this path by observing how water in different environments has a different relaxation speed (time to return to thermal equilibrium parallel to B_0). It was found that the faster the return to equilibrium, the more tightly bound the water population would be, and vice-versa:

the slower the return the weaker the bond (H. Bertram & Andersen, 2006; H. Bertram, Purslow, et al., 2002; Bianchi et al., 2004; Todt et al., 2006).

Early on it was agreed upon that the T_2 relaxation curve in meat was not mono-exponential. Questions remained as to whether it should be described as bi- or tri- exponential. After many studies, it was found that most correlations were possible using only a bi-exponential analysis given how the bi-exponential time constants and populations represented the water which could be lost as drip loss, and explained >90% of the relaxation signal (Bertram et al., 2001). The bi-exponential equation has the form:

$$g = P_{21}e^{-\frac{t}{T_{21}}} + P_{22}e^{-\frac{t}{T_{22}}}$$

Where g is the magnetisation amplitude, T_{21} and T_{22} are the transverse relaxation time constants, and P_{21} and P_{22} are the fractions of the signal dedicated to each water population. A tri-exponential fit would simply add a third term $P_{2b}e^{-t/T2b}$. However, it was later found that a continuous approach, as opposed to a discrete approach, provided even more accurate results. The continuous distribution of exponentials, for transverse relaxation experiment using a CPMG sequence - the sequence used to analyze meat for drip loss and water distribution - can be described as follows (Bertram & Andersen, 2006; Bertram et al., 2002):

$$g_i = \sum_{J=1}^{m} P_j e^{-\frac{t_i}{T_j}}$$

Where the variables are the same as described previously. This equation is solved by minimising the following equation:

$$\left(g_i - \sum_{x=1}^{m} P_x e^{-\frac{t_i}{T_x}}\right)^2 + \lambda \sum_{x=1}^{m} P_x^2$$

Where $\lambda \sum_{x=1}^{m} P_x^2$ is a linear combination of functions. This is added as a zeroth order regularisation because the minimisation of such an equation is ill-conditioned. The choice of λ, the weight of the regularisation, is up to the user of the algorithm and must be chosen carefully. Too small a value will cause false peaks to appear. If the peaks are too broad some may disappear entirely (Bertram et al., 2006; Bertram et al., 2002).

A method for direct analysis of continuous relaxation spectra which could overcome the ill-posed problem, the sensitivity to even small experimental errors, and the unreliable techniques used for numerical differentiation at time zero. This revolutionary approach requires no *a priori* knowledge of a functions form, and no initial estimates (SProvencher & Dovi, 1979; Provencher, 1993; Provencher, 1982). Though the mathematics are extremely advanced, the general idea is that by adding a positive constraint, and seeking the smoothest solution, one can obtain a more objective solution. Three years later, in 1982, the algorithm called Contin© was developed to make the process accessible and automatic. Though these mathematical techniques were not built expressly for NMR relaxation, they were built for other forms of spectroscopic relaxation experiments, in 1993 Provencher published a paper applying it to NMR (Provencher & Dovi, 1979; Provencher, 1993; Provencher, 1982).

When applied to meat, several competing theories arose about what those various water populations could represent. The were four competing theories. The first claimed that the fastest component, T_{21}, and corresponding water population, P_{21}, represented intracellular water, while the other component was the extracellular water (Bertram et al., 2001). The second theory

suggested that these represent different levels of structure within the cell, changing how tightly bound the water is depending on which structure the water is found in, such as water found in varying degrees of contraction of the actomyosin complex (Bertram et al., 2001). The third theory is based on spatial heterogeneity where the distances between water and macromolecules or other substrates play an important role. A study which compared the signal of fresh meat against minced and homogenized meat, found that the water populations remained intact. The meat was then soaked in dimethyl sulfoxide, which is known to disrupt cell membranes and render them permeable, but the bi-exponential populations still remained intact. These two experiments managed to disprove the three theories (Bertram et al., 2001). The fourth theory was proven by soaking meat in urea. Urea unfolds proteins, causing changes to the tertiary and quaternary structures and thus strongly affecting the myofibrillar system. Upon performing the transverse relaxation experiment, it was found that the two components usually seen merge into one single component, demonstrating that the compartmentalization of the water populations was due to the myofibrillar systems (Bertram et al., 2001).

Thus, P_{21} is now considered to represent intra-myofibrillar water. This water is found inside the myofibrils between thick and thin filaments. P_{22} is considered to be extra-myofibrillar water and is found in several locations. This water can be found between myofibrils, inside the sarcoplasm, and is called the inter-myofibrillar space; it can be found between muscle fibers and the fasciculi which is called the inter-fascicular space; and finally, it can be found around fasciculi, called extra-fascicular space. The water found in all three of these spaces are considered extra-myofibrillar water. Finally, in the case where a third exponential is used, or in continuous distribution where a third component is likely, a faster component appears, often faster than 10ms. This population, P_{2b}, and corresponding time constant T_{2b}, represent water

closely bound to macromolecules such as proteins. Water being a dipole molecule, and proteins tending to be charged molecules, make them bind quite tightly together and have been shown to be unaffected by cooking or freeze-thawing. Though the water molecules will continuously exchange with their surrounding immobilised water populations in the intra-myofibrillar space (Belton, 2011; Bertram & Andersen, 2007; Bertram, Dønstrup, et al., 2002; H. Bertram et al., 2001).

As for the time constants T_{2b}, T_{21}, and T_{22}, they represent how tightly bound each water population is to its environment. The faster the time constant, the more tightly bound the water and the less likely it is to move into a different space, or more simply put, the less likely it is to be lost in the form of drip loss (Bertram et al., 2001; Pereira et al., 2013).

2.4.3 NMR for Fat Content

Fat content measurement in meat by NMR is difficult since the spectra produced by fat is nearly indistinguishable from water. In regular fresh meat, the water content is too high to differentiate the fat content from the water content, even in fattier cuts (Todt et al., 2006). Several approaches have attempted to overcome this challenge. The easiest is to simply dry the meat, and measure the signal which will now come entirely from fat (Todt et al., 2006; Toussaint et al., 2002). In cases where drying is used, the process is both long, destructive, and expensive. Pertaining to the simultaneous measurement of both fat and water, one technique is the use of a saturation pulse, whereby a long low energy pulse is emitted to saturate water resonance and then perform the transverse relaxation experiment where only the non-saturated nuclei will produce a signal; in this case fat. This technique is called presaturation (Marcone et al., 2013). The second method is the use of a gradient magnetic field, which requires a special probe attached to the

NMR. This field is position dependant, and in a sequence of specialized pulses in a finely tuned magnetic field gradient, the water signal can be suppressed after the first pulse and increase the difference between water and fat singals (Petrov et al., 2008; Sørland et al., 2004; Todt et al., 2006). There remains a final technique, where a combination of transverse and longitudinal relaxations occur in sequence, and a large mathematical/chemometric process involving an extensive calibration can then be used to find both water and fat content simultaneously (Pereira et al., 2013; Todt et al., 2006). Though this requires more initial work, it does not require expensive or specialized equipment, providing a significant advantage.

2.4.4 NMR for Solid Fat Content

Rather than being a mass ratio, SFC measured by NMR is a ratio of proton signal originating from the solid phase over the proton signal originating from the sum of the solid phase and the liquid phase, expressed as a percentage (AOCS, 2009). When a sample is measured a decaying curve such the one below is obtained (Jehle, 2015).

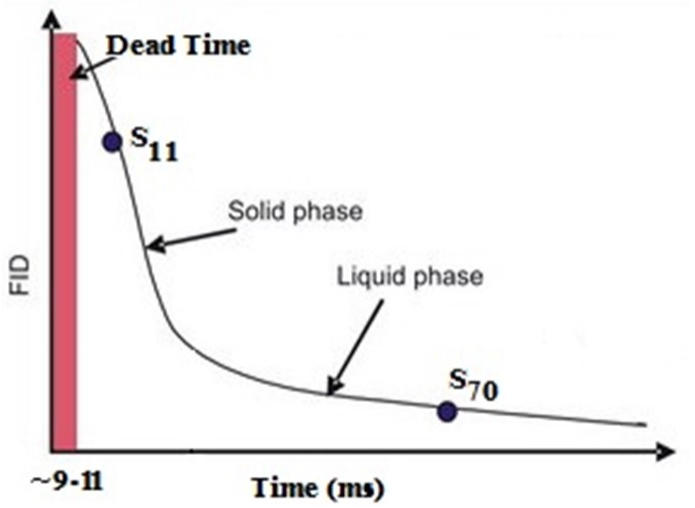

Figure 2.3 SFC signal produced by NMR measurement (Bruker© TD-NMR Presentation)

At the start of the curve, S_0, the signal is the total contribution from the sample which includes solids and liquids but not gases. Therefore, S_0 = solid + liquid. After about 70ms, the only signal remaining is that of the liquid phase, S_{70}. Therefore, the equation for SFC based on the NMR signal is as follows (Gribnau, 1992; Jehle, 2015):

$$SFC = \frac{Solids}{Total} = \frac{Total - Liquids}{Total} = \frac{S_0 - S_{70}}{S_0}$$

When a sample is first released from the barrage of radiofrequency pulses, it remains "stunned" for a few milliseconds. This period of time is called the dead time, and measurements cannot be taken during the dead time (Gribnau, 1992; Jehle, 2015). As can be seen in the figure above, the point where measurements can begin to be taken with certainty, after 11ms (S_{11}), is further on the curve and therefore does not represent the entire signal. A fraction of the total signal is lost. Therefore, a correction factor, *f*, is applied to the signal describing solids (Gribnau, 1992). The equation for solids is therefore:

$$Solid = S_0 - S_{70} = f(S_{11} - S_{70})$$

S_0 also needs to be replaced in the denominator. Since the correction factor for the solids is not applicable to the total, the new total is now solids + liquids, or (Gribnau, 1992; Jehle, 2015):

$$Total = f(S_{11} - S_{70}) + S_{70}$$

The following equation combines the equation for solids as the numerator with the equation for total as the denominator (Gribnau, 1992; Jehle, 2015).

$$SFC = \frac{f(S_{11} - S_{70})}{f(S_{11} - S_{70}) + S_{70}}$$

A correction factor, *k*, also needs to multiply the liquid signal since it is not always the case that after 70ms the signal represents 100% of the liquid phase, it could be pre-mature or too long a time period. Now liquids = kS_{70}. Replacing S_{70} by kS_{70} in the equation, and adding a digital offset, Φ, to the denominator yields the final equation for SFC calculation. The digital offset is usually provided by the manufacturer and dependent on the equipment (Gribnau, 1992; Jehle, 2015).

$$SFC = \frac{f(S_{11} - kS_{70})}{f(S_{11} - kS_{70}) + kS_{70} + \Phi}$$

Only two unknowns remain, and can be found through a simple calibration. The calibration requires that two different samples with known SFC be measured, allowing for both variables to be solved with simple algebra (Jehle, 2015).

The protocol for measuring SFC by NMR, involves stabilizing and tempering. In the case of fats which do not require additional polymorphic stabilization, such as frying and baking oils, animal fats, margarine, and other similar oils is much shorter than the procedure used for confectionary and specialty fats containing significant quantities of 2-oleo-disaturated glycerides. Even so, the procedure requires that the sample be fully melted at 100°C, then maintained at 100°C for 15 minutes, followed by 5 minutes at 60°C, followed by an hour at 0°C – complete crystallization must be able to take place during this time at 0°C. Then the sample can be placed at the measurement temperature where it must rest for 30-35 minutes. All following temperature measurements require another 30-35 minutes and no more than 5°C increase over the previous measurement temperature. One must start the measurements at the coldest temperature and work up when measuring SFC. As many as 20 samples can be done simultaneously, and if multiple water baths are used, the procedure can be done in parallel, significantly increasing the speed at

which the measurements can be done. Samples are roughly 3ml in size, and can be taken by biopsy from the carcass (AOCS, 2009). If several small samples are used, and this procedure is performed in parallel at only a small number of temperatures, SFC by NMR could function at impressive speeds, potentially reaching the speed necessary for industrial applications.

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CONNECTING TEXT

Water holding capacity, measured as drip loss, is important to meat quality and consumer acceptance. It has financial implications in terms of reducing potential profit when the meat loses mass in the form of drip. Since meat is sold on a per unit mass basis, potential profit is lost when selling the same cut at a lower weight. A poor WHC can have an unattractive appearance, that of watery meat, in the package, and an unappealing experience when consuming it. High drip loss meat will lose more water when cooking or freezing and thawing it. In turn, the consumer will consume a drier product which impacts flavour and texture. This could encourage the consumer to discriminate against pork in the future, and hurt the reputation of pork amongst other consumers.

The aim of the following chapter is to investigate the application of NMR as a tool for elucidating all three of those attributes: drip loss, cooking loss, and thaw loss.

Chapter 3
Cooking and Drip Loss Determination by Low-Field NMR Relaxometry on Large Pork Loin Samples at Refrigerated Temperatures

Abstract

In order to find a rapid, multidimensional, and non-destructive tool for measuring meat quality attributes, low-field time-domain nuclear magnetic resonance (NMR) relaxometry was employed on thawed pork loins averaging 539 g at 4°C. These loins were then cooked and the cooking loss was measured. This is then followed by multivariate data analysis. The implications of the NMR signal against cooking loss, drip loss, and thaw loss are then discussed. The analysis revealed correlations of r = 0.686, 0.573, and 0.452 for cook, drip, and thaw loss, respectively. Several studies in the past have correlated drip loss to transverse relaxation, but few have done so at 4°C, and even fewer have done so on such large pieces. This is valuable for bringing this technology to industrial applications

Keywords: NMR; cooking; drip loss; multivariate data analysis; pork; water holding capacity

3.1 Introduction

Though many successful papers have been published on the between drip loss and NMR signals, these were mostly performed on small pieces (often 1 cm x 1 cm x 5 cm) at room

temperature. The next logical step in bringing this technology to industry is to investigate the potential on larger loins at refrigerated temperatures.

3.1.1 The Water Holding Capacity Issue

Water holding capacity (WHC) is defined as meat's ability to retain water even against the forces of gravity (K. O. Honikel, 1987). WHC determines the closely related term, drip loss (Bertram et al., 2002); which is the amount of water exuded due to the forces of gravity (Fischer, 2007). Water holding capacity (WHC) is the most important quality trait in meat. It affects juiciness, texture, and tenderness. Once cooked, a large portion of the water is lost and yields a drier, less juicy, product. Given how important those characteristics are to the consumer's experience, the mechanisms behind WHC and the measurements thereof have become an area of interest. It is estimated that as much as 50% of pork products have unacceptably high drip loss, where unacceptably is defined as causing a detrimental effect on palatability (Barge et al., 1991; Chen et al., 2015; Kauffman et al., 1993).

Today, drip loss costs the industry millions of dollars due to the % weight exuded (Fischer, 2007; Huff-Lonergan & Lonergan, 2005). In other words, 1 kg of meat with 10% drip loss may only weigh 900g when it comes time to sell it, and therefore 10% of potential revenue of that cut is lost.

Poor WHC became of large interest in 1960s when pale soft exudative (PSE) pork became a more widespread problem. Typically, PSE pork had drip loss values above 10%. It was found that PSE was tightly linked to the halothane gene, and was subsequently bred out of many of the pork breeds. Nonetheless, large variations in WHC are still seen today (Bertram & Andersen, 2007).

As Bertram et al. said in 2007, “efficient techniques for measuring WHC are still needed in pork production, and powerful techniques for elucidating the underlying biophysical mechanisms causing formation of drip loss are essential in meat science.” (Bertram & Andersen, 2007) A lack of recorded assessment (Kauffman et al., 1993), and the difficulty of measuring a large number of products which are being sold on the market, have made finding the cause of unacceptable drip loss challenging.

3.1.2 Common Methods of Measurement

A common gravimetric method exists whereby a sample of known mass is suspended on netting in a bag for 24 hours before being weighed again. The bag is inflated during this period so as to prevent the bag from touching the sample and influencing the results. The difference in mass is then divided by the original mass to provide a ratio of mass lost due to drip against original mass. The result is typically expressed as a percentage. Rather than be suspended, the sample can be centrifuged under specific, controlled, parameters for a pre-determined amount of time. This sample loses water during this time, and the difference in mass can once again be described as a ratio of mass lost over original mass, as a percentage (Honikel, 1987; Honikel, 1998). Yet another method is to press a certain amount of meat against a filter paper. The surface of the meat in contact with the filter paper is compared to the surface area of the wetted filter paper. As the meat is pressed against the paper, the more water is lost, the larger the wetted area as it diffuses through the paper. These areas are often measured with the aid of a planimeter (Barge et al., 1991). In this study, the gravimetric method, EZ drip loss is used, which uses a specifically proportioned sampling method and container to minimize variations and improve accuracy but is otherwise quite similar to other gravimetric methods. The sample is left in a tube where the it can drip for 48 hrs, and is then patted dry. The difference between original and final

mass is divided by the original mass and expressed as a percentage (Christensen, 2003; Correa et al., 2007; Otto et al., 2004).

3.1.3 Time Domain Nuclear Magnetic Resonance

The above methods of measuring WHC are destructive and time consuming (Bertram, Dønstrup, et al., 2002) preventing the post-analysis sale of the product (or section of product) tested. This entails that the products on the shelves do not have specific, precise, meat quality information but rather an extrapolated estimate from a specified sample taken from the piece. The cost in terms of time and wastefulness of the analysis, could make adequate and extensive data collection financially non-viable. This adds to the issue of deteriorating meat quality described by Kauffman et al. (1993). It has been suggested that since the gravimetric methods have poor repeatability, low-field time-domain nuclear magnetic resonance (NMR) could become a superior method of measurement (Bertram & Andersen, 2006).

NMR has several applications in the food industry (Marcone et al., 2013) and is rapidly developing due to advances in equipment and techniques (Jankowski, 2003) as a non-destructive, rapid, assessment of WHC, and even other meat quality parameters such as fatty acid content, fat content, and moisture content (Marcone et al., 2013). Studies have demonstrated good correlations between WHC and NMR signal (Bertram et al., 2001; Bertram & Andersen, 2007; Bertram et al., 2002; Bertram et al., 2001; Bianchi et al., 2004; Pearce et al., 2011; Straadt et al., 2011).

The most common NMR active nuclei for the analysis of food is that of water. The phenomenon behind NMR relaxometry involves aligning the axis of rotation of the protons of the water molecule's nuclei with that of a magnetic field and then perturb them using

radiofrequency pulses to tilt the axis of rotation onto the transverse plane. Upon cessation of the pulses, the axes will return to the original direction – that of the magnetic field it was first placed in – a process called relaxation. This produces a decaying signal which can then be described as bi- or tri- exponential. This T_2 experiment produces two (in the case of biexponential) or three (in the case of triexponential) populations: P_{2b}, P_{21}, and P_{22} where P_{2b} exists only in the case of triexponential models. Similarly, time constants T_{2b}, T_{21}, and T_{22} are produced (Pearce et al., 2011). These values have been associated with:

- P_{21} is associated with the number of protons found in the myofibriliar space. i.e. an increase of this value demonstrates an increase in the amount of water.
- Likewise, P_{22} is associated with the number of protons in the extra-myofibrillar space.
- T_{21} is associated with the spacing between myofibrils whereby a tighter spacing makes for faster time constant.
- Similarly, an increase in T_{22} is associated with an increase in extra-myofibrillar space meaning the molecules are less tightly bound
- P_{2b} is associated with the number of protons belonging to water found in a macromolecular complex such as hydration water in protein.
- Finally, T_{2b} is associated with how strongly bound the protons are bound within the P_{2b} population (Bertram et al., 2001; Bertram, Dønstrup, et al., 2002; Bertram et al., 2001; Pearce et al., 2011).

The time constants T_{21} and T_{22} tend to be those which correlate most strongly with WHC (Bertram & Andersen, 2007; Bertram et al., 2002; Bertram et al., 2001). The mechanisms which explain the correlation between T_{21}, T_{22}, and WHC is as follows: rigor, and shrinkage of the muscle cell, pushes water out of the myofibrils into the extra-myofibrillar spaces. Water in this

area is exuded as drip loss before any of the other water populations (Huff-Lonergan & Lonergan, 2005).

These studies have been performed on small samples, often only a couple centimeters in each direction, weighing less than 10g. They were then measured at 25°C (Bertram et al., 2001; Bertram et al., 2002; Bertram et al., 2002; Bertram et al., 2001; Straadt et al., 2011). For this technology to be applied in industry, studies on larger pieces at refrigerated temperatures are needed – which is the purpose of this study.

3.2 Materials and Methods

3.2.1 Sampling

A total of 39 samples were obtained from pigs of a variety of weights (from 32 kg to 52 kg), castrated, and uncastrated males, and females. The samples demonstrated a wide range of meat quality based on colour, levels of androstenol, fat and moisture content. These quality attribute measurements were provided by the producers. The loin was taken from the first rib to the forth rib, counting from rib closest to the animals tail towards its head.

3.2.2 Determination of Drip Loss

The drip loss was measured using the modified EZ-driploss method, as described by Correa et al. 2007. This modified method stores the samples for 48 hours. The sample is weighed before hand and after the storage period, the samples were dabbed and then weighed. This method provides a higher level of accuracy (Correa et al., 2007). It was performed on fresh samples, before being frozen.

3.2.3 Determination of Thaw Loss

Sample mass was measured precisely before being frozen. Samples were then thawed in a fridge at 4.0°C until the center of the cut had a temperature of 4.0°C. The sample was then patted dry and weighed again. The thaw loss is calculated as the difference in mass before freezing (M_0) and after freezing (Mf) divided by the original mass, all multiplied by a hundred for a percentage.

$$\%\ thaw\ loss = 100 * \frac{M_0 - M_f}{M_0}$$

3.2.4 NMR Measurements

Inside a fridge at 4.0°C, where the samples internal temperature is stabilized at 4.0°C after thawing, they were prepared for measurement. The thaw loss is calculated base on the mass of the sample at this point once it is patted dry. If the samples were longer than 18cm, they were cut to that length. Then the samples were trimmed if they were not able to fit into an 11 cm tube, though most did not require trimming as they were smaller than 18 cm by 11 cm. The adipose tissue on the surface of the meat was removed, paying close attention not to remove, or damage, the surface of the meat. The sample is then patted dry and placed in a plastic bag and measured immediately after. The plastic bag does not produce a signal; this was tested beforehand.

Measurements were performed on a Minispec LF110® NMR Analyzer (Bruker Corporation, Milton, ON, Canada) which operates at 6 Mhz – the resonance frequency for protons given the magnet strength. The probe was a fixed temperature probe, operating at 37 °C with an active measurement area of 18 cm long by 11 cm in diameter. The transverse relaxation (T_2) was measured using the Carr-Purcell-Meiboom-Gill sequence, with a τ-value of 340 μs measuring every even numbered echo to reduce the amount of error produced from inexact instrument settings. A total of 2000 points per scan were collected and 16 scan repetitions with 2 s between scans was performed.

3.2.5 Determination of Cooking Loss

The same samples used for the drip loss, thaw loss, and NMR measurement was then cut to a precise size of 10 cm long by 8 cm wide by 3.5 cm in thickness. It is then patted dry and weighed precisely. The loin is then placed in a bag with a special adapter going through the bag allowing a thermocouple to be inserted into the centre of the meat while maintaining impermeability and the vacuum under which it will be placed. The bag was then vacuum sealed. The vacuum sealed bags are then placed in a controlled, agitated water bath held at a precise 72.0 °C until the internal temperature reaches 68.0 °C. Once the internal temperature is attained, the sample is plunged into an ice bath for 15 minutes before being placed in a fridge at 4.0 °C overnight. The next morning the samples are patted dry and weighed precisely. The equation for calculating cooking loss is the same as the one for calculating thaw loss.

3.2.6 Data Analysis

As suggested by Bertram et al. 2002, the continuous distribution analysis provides better results over bi- or tri- exponential fitting. For that reason, as described by Provencher et al. 1979, the continuous analysis of relaxation spectra was used. The CONTIN algorithm, also developed by Provencher et al. 1982 was used (Bertram et al., 2002; Provencher & Dovi, 1979; Provencher, 1982).

Data analysis was performed on SPSS (IBM Corporation, Armonk, New York, US) using the linear multivariate regression, descriptive statistics, and bivariate correlation. Outliers were checked using the Mahalanobis distance.

3.3 Results and Discussion

Table 3.1 shows the mean, range, and variation of dependent and independent variables. Of the independent variables P_{21}, P_{22}, and T_{22} showed a normal distribution according to the

Shapiro-Wilk test. The remaining independent variables P_{2b}, $T_{2b,}$ and T_{21} showed a non normal distribution. Of the independent variables, cooking loss and thaw loss showed a normal distribution while drip loss did not. The average mass of the thawed samples after trimming was 539 g, ranging from 407 to 729g.

The results of the Contin algorithm produced up to five components. The first two components occurred too quickly (<2ms) to be measured and were thus considered a mathematical artifact and discarded. The remaining values were similar to those found in the literature, with the first component occurring between 2-10ms (mean=8.88ms), the second component occurring between 10-100ms (mean=36.53ms) and the final component occurred slightly faster than anticipated. It would normally occur between 100-1000ms, the third component in this study occurred at a mean of 88.23ms. These findings, along with the variations and range can be seen in Table 3.1.

Table 3.1: Mean, range, and variation of parameters

	Mean	Standard Deviation	Coefficient of Variation (%)	Range
Drip loss (%)	2.73	1.54	57.41	0.57 - 9.30
P_{2b}	1.53	1.18	77.12	0.07 - 7.41
T_{2b} (ms)	9.22	5.14	55.74	3.00 - 22.40
P_{21}	52.02	9.84	18.92	24.39 - 76.47
T_{21} (ms)	38.52	3.50	9.09	30.00 - 40.00
P_{22}	13.53	2.97	21.95	7.14 - 21.69
T_{22} (ms)	88.41	5.12	5.79	81.40 - 103.00
Thaw Loss (%)	7.05	1.74	24.68	3.90 - 11.60
Cook Loss (%)	15.78	2.14	13.58	12.23 - 22.56

A total of 39 samples were measured, one sample gave invalid results, and another one was deemed an outlier given the Mahalanobis distance with $X^2_{0.05}$ (df=6) leaving 37 samples remaining. The level of variation on the samples is quite high, as the coefficient of variation in Table 3.1 demonstrates.

The two-tailed Pearson's correlation coefficient for each combination of independent variable against dependant variable was produced followed by a comparison of dependant variable correlations between one another. These findings can be seen in Table 3.2.

Table 3.2: Pearson's correlation coefficient between variables

	P_{2b}	T_{2b}	P_{21}	T_{21}	P_{22}	T_{22}	Thaw	Cook
Drip	-.216	-.053	-.193	.043	.198	.103	.663*	.505*
Thaw	.055	-.070	-.074	.132	.114	.147		.260
Cook	-.128	.020	-.130	-.286	.427*	.290		

* Correlation is significant at the 0.01 level (2-tailed).

The strongest correlations occurred between dip loss and thaw loss, followed by drip loss and cooking loss. The existence of significant correlations was expected between the dependent variables. Next, between independent and dependent variables, it can be noticed that T_{21} has proportionately less of a correlation than the other variables, which is unexpected and difficult to explain. P_{2b} has a much weaker correlation with thaw loss, than it does with drip or cook loss. Likely the water associated with macromolecules is not affected by the freeze thaw cycle to a great degree, while cooking and storage has a larger effect.

According to the literature, during cooking the T_{21} peak will shift to a slightly faster time frame, but the peak's amplitude is reduced while the width of the curve is increased. In contrast, the T_{22} peak increases in both width and amplitude, with a slight shift towards a slower time (Bertram et al.,2006; Li et al., 2012). The strongest correlation between independent and dependent variables was between P_{22} and cooking loss. Interestingly, the P_{22} value is measured before cooking, not during or after, and still provides significant insight into its performance during cooking ($p<0.01$).

The last interesting piece of information Table 2 provides, is how T_{21} has a positive correlation in drip loss and thaw loss, yet a negative correlation with cooking loss. A positive correlation can be explained: a greater value for T_{21} means a greater drip loss since the myowater is more weakly bound. Curiously, a greater value for T_{21} for cooking indicates a reduction in cooking loss. The correlation is in fact stronger between T_{21} and cooking loss is stronger than the correlations of T_{21} and drip loss or thaw loss.

3.3.1 Cooking Loss

Performing a multiple regression using the independent variables P_{2b}, T_{2b}, P_{21}, T_{21}, P_{22}, and T_{22} with the dependent variable being cooking loss, a correlation of r = 0.686 was obtained. The results were significant, passing the F-test in the analysis of variance ($p<0.05$). Figure 1 shows the histogram of standardized residual with a normal curve superimposed on it for cooking loss.

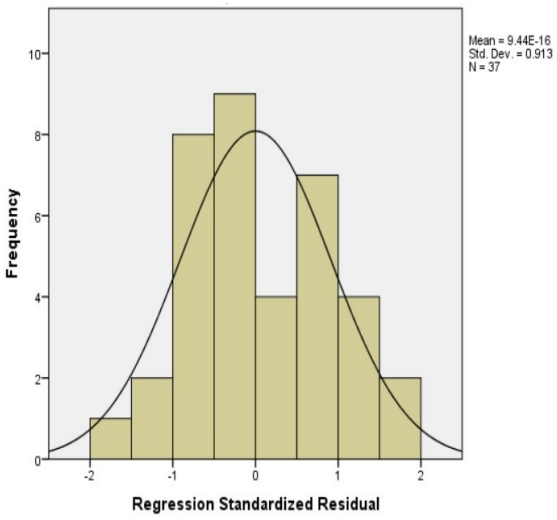

Figure 3.1: Histogram of cooking loss standardized regression residuals with superimposed normal curve

The regression equation for a multivariate regression is as follows:

$$f(x) = constant + X_1B_1 + X_2B_2 + \cdots X_nB_n$$

Table 3.3 contains the coefficients B, and the standardized coefficients β. Both P_{21}, P_{22}, and T_{22} were significant, passing the t-test ($p<0.05$). Logically, these same values also had the highest contribution when considering their standardized regression coefficients. The significance and contribution of these components coincides with the theory that movement of water moves from the intra-myofibrillar space to the extra-myofibrillar space where it can be lost in the form of exudate during cooking. Sensibly, the water population representing the intra-myofibrillar water (P_{21}) and extra-myofibrillar water (P_{22}) would have the greatest contribution to cooking loss predictions. P_{21} and P_{22} are equal and opposite contributors to cooking loss, having similar absolute standardized regression coefficients, with P_{21} being negative while P_{22} is positive. The negative correlation is easily explained, where the more water found in the intra-myofibrillar space, the less will lost during the cooking. The more found in the extra-myofibrillar space, the more will be lost during cooking. Also interesting is that the remaining components were still large contributors as their standardized regression coefficients demonstrate, with the exception of T_{21}, which also was not a significant result. As will be seen below, T_{21} is typically an important in understanding drip loss. This could be because the location of the center of the peak (how tightly bound it is) does not affect how much water is lost while as much as the total amount of water in that myofibrillar space does. A previous study noticed that the T_{21} peak broadens during cooking, and only a slight shift is seen (Bertram et al., 2006). This too seems to indicate that the water population takes precedence over the location of the peak's center. This could also possibly be explained by the greater forces placed on water within tissue during cooking as compared to simply resting at standard pressure under the forces of gravity.

Table 3.3: Transverse relaxation regression coefficients for cooking loss

Component	B	β
Constant	6.502	n/a
P_{2b}	-0.633	-0.195
T_{2b}	-0.112	-0.243
P_{21}	-0.142	-0.649
T_{21}	0.150	0.024
P_{22}	0.489	0.676
T_{22}	0.129	0.300

3.3.2 Drip Loss

Performing a multiple regression using the independent variables P_{2b}, T_{2b}, P_{21}, T_{21}, P_{22}, and T_{22} with the dependent variable being drip loss, a correlation of r = 0.573 was obtained. The results were deemed significant according to the F-test ($p<0.05$). As previously mentioned, drip loss did not have a normal distribution. Figure 3.2 demonstrates the distribution. In comparison to cooking loss, drip loss had a vast majority of the residuals within a tight range (-1 to 0.5) and then residuals falling well outside of the normal curve, around 3.5 - 4.

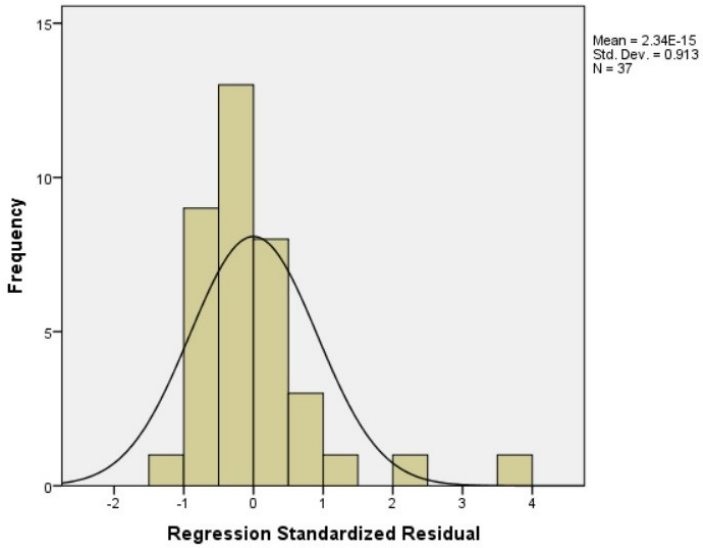

Figure 3.2: Histogram of drip loss standardized regression residuals with superimposed normal curve

This distribution could, in part, explain why the correlation between drip loss and transverse relaxation was not as high as between transverse relaxation and cooking loss.

The regression coefficients and the standardized regression coefficients can be seen in Table 3.4. P_{21}, T_{21}, and P_{22} were all significant based on the t-test ($p<0.05$). The most important differences between cooking loss and drip loss occur at P_{2b} and T_{21}. P_{2b} was a relatively important contributor in the cooking loss regression (β=-0.195) while it is a full order of magnitude lower in the drip loss regression (β=0.031). The denaturation of proteins in cooking explain the cooking loss correlation to P_{2b}. As a previous study confirms, little change is seen in P_{2b} populations (Bertram et al., 2006).

Table 3.4: Transverse relaxation regression coefficients for drip loss

Component	B	β
Constant	-6.109	n/a
P_{2b}	0.073	0.031
T_{2b}	-0.094	-0.282
P_{21}	-0.114	-0.725
T_{21}	0.168	0.381
P_{22}	0.393	0.755
T_{22}	0.042	0.136

3.3.3 Thaw Loss

Performing a multiple regression using the independent variables P_{2b}, T_{2b}, P_{21}, T_{21}, P_{22}, and T_{22} with the dependent variable being cooking loss, a correlation of r = 0.452 was obtained. As previously mentioned, the thaw loss followed a normal distribution and the residuals were much better than those seen in drip loss, as Figure 3.3. The results were insignificant, not passing

the F-test in the analysis of variance. However, the variables P_{21}, T_{21}, and P_{22} all passed the t-test as being significant in the regression, though all near a p-value of 0.05.

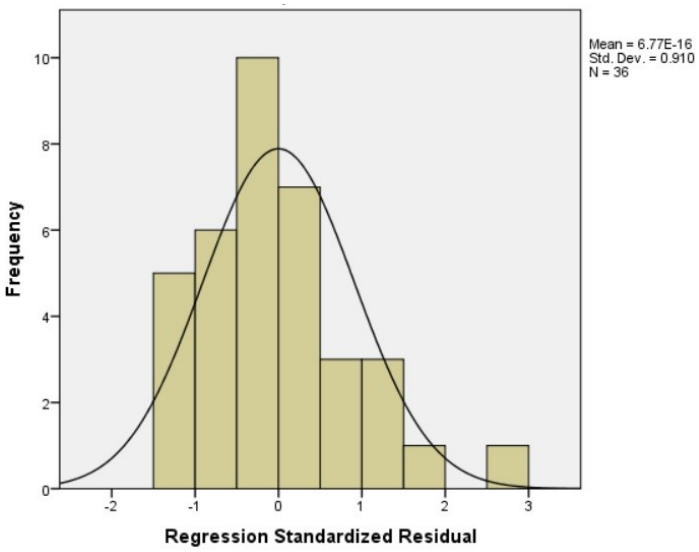

Figure 3.3: Histogram of thaw loss standardized regression residuals with superimposed normal curve

It should be noted that the transverse relaxation experiment was performed on thawed meat, and not before the freeze thaw cycle. This could explain why the correlation is weaker than other studies performed on fresh meat. Table 3.2 gave the impression that P_{2B} would not be a large contributor to the regression for thaw loss. Table 3.4 demonstrates that it does play an important role in the regression, even more so than T_{22}. The strongest contributor is P_{22}, which is logical given how it would be the first myowater to be exuded during the thaw, and the greater the population after the thaw the more that was likely lost and transferred from P_{21} to P_{22}. The second largest contributor, at nearly the same scale though as a negative correlation, is P_{21}. The more water remaining in the P_{21} population the less that was lost during the thaw, since it would have to move to the extra-myofibrillar space beforehand.

Table 3.5: Transverse relaxation regression coefficients for thaw loss

n	Component	B	β
0	Constant	-4.992	n/a
1	P_{2b}	0.802	0.204
2	T_{2b}	-0.064	-0.164
3	P_{21}	-0.093	-0.530
4	T_{21}	0.207	0.423
5	P_{22}	0.349	0.593
6	T_{22}	0.043	0.124

3.4 Conclusion

This study found a significant correlation (r=0.686) between cooking loss and T_2 relaxation data. Furthermore, this was obtained on large pieces of 539 g on average, rather than 1 cm x 1 cm x 5 cm such as is seen in the literature, as well as being at fridge temperatures, an important step forward to application of this technology to industrial settings. Interesting correlations were developed and potential explanations between NMR results and physical phenomenon were discussed.

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Connecting Text

Fat consistency in pork is important for the consumer as well as the producer. When meat containing soft fats are processed, several problems arise can cause a plethora of issues such poor dimensioning, trouble butchering, and reduced shelf life. The consumer will find tat soft fats make the meat appear oily and give the impression that the meat is fattier than it truly is. The texture during chewing will also be oily, which further reinforces the idea that the meat is more fatty than it truly is. Together, these fat consistency problems can cause serious economic losses for pork producers.

The following section investigates NMR as a tool for measuring solid fat content, and whether it can replace iodine value as an indicator of fat consistency. Iodine value measurements can be long, expensive, and even involve dangerous chemicals. Some even believe that iodine value is not an accurate predictor of fat consistency. This will be addressed below.

Chapter 4

Relationship Between Solid Fat Content, Fatty Acid Composition and Iodine Value of Pork Adipose Tissue

Abstract

Soft fats in pork products present difficulties with processing and dimensioning such as: removing the skin from the carcass, meat separating from fat layers, fat layers separating from one another, poor slicing, accelerated development of rancidity, diminished cooking and flavour quality, and finally loss of revenue from the downgrading of prime cuts. Typically, the hardness of fats is measured with the iodine value. The goal of this paper is to examine the relationship between fatty acid content, iodine value, and solid fat content for rapid, non-destructive, detection of soft fats.

Solid fat content (SFC), which is an indicator of fat consistency, was measured by pulsed low-field proton nuclear magnetic resonance at the following temperatures: 0, 5, 10, 15, 20, 25, 30, 35, 40°C. The nine SFC measurements were then compared to iodine value, and fatty acid composition. Backfat samples from 46 pigs were used. Using a multivariate regression with SFC measurements at each of the temperatures as the independent variables, a correlation with iodine value was obtained ($r = 0.874$) with over 76% of the variation in iodine value accounted for by the SFC measurements. The regression also correlated with composition, yielding $r = 0.883$ for palmitic acid, $r = 0.866$ for linoleic acid, and $r = 0.825$ for alpha-linoleic acid. The validity of iodine value as an indicator of fat consistency was discussed.

Keywords: solid fat content, fat consistency, iodine value, pork fat, fatty acid composition, nuclear magnetic resonance

4.1 Introduction

Iodine value and fatty acid composition are perceived as indicators of fat consistency in the meat processing industry (Gläser et al., 2004). Soft fats are fats that are too soft for the intended processing and maybe even too soft for consumer appreciation. The consistency of fat can be measured, and thus described, in several different ways. One of which is solid fat content; a ratio of fat in a solid state to fat in a liquid state expressed as a percentage (AOCS, 2009). Soft fat in a pork belly intended for production of bacon would prevent it from being properly dimensioned into slices in the typical fashion. It will be down-graded and receive a monetary return that lower than what it could have had if the adipose tissue had been sufficiently hard. In the case of finer meats, such as prosciutto, the need for hard fats is intrinsically important, and consequently the potential losses are much higher. The increasing occurrence of soft fats does have some inherent pitfalls beyond just the loss of value. Some have even suggested that "the point may be reached where consumers object seriously or even reject pork, and manufacturers experience difficulties in acquiring suitable pork for further processing" (Kauffman et al., 1993). This could be due to undesirable appearance, poor quality after cooking, and less appealing flavour. Other issues for processors and/or consumers include: reduced oxidative stability, leading to faster development of rancidity and reduced shelf-life; difficulty removing skin from the carcass; difficulty removing short ribs in the case of pork; difficulty dimensioning and slicing during processing; and the development of off-flavours (Olsen et al., 2007); and insufficient drying and an oily appearance within the product's package as the oil rises to the surface when the product is no longer being manipulated (Davenel et al., 1999; Johnstone & Li, 2011).

Soft fats in pork adipose tissue occur more regularly than they did in the past. It is theorized that this is due to two reasons. The first maybe attributed to increasing use of large amount of inexpensive feed in the form of dried distiller's grains with solubles mostly from the ethanol manufacturing industry. It has been demonstrated that feeding dried distiller's grains with solubles to pork makes for softer fats (Johnstone & Li, 2011; Testroet et al., 2015) . The second reason is due to selective animal breeding, in order to obtain leaner meat, over the last century (Gläser et al., 2004; Kauffman et al., 1993). There has been conflicting demands on the pork industry. Consumers desire leaner meat, but also want to reduce their consumption of saturated fat due to health concerns. Unsaturated fat is softer and thus more difficult to process (Johnstone & Li, 2011). The ideal solution would be having as much unsaturated fat as possible whilst maintaining processing ability.

The key to solving this issue is to find a method by which the processor can accurately identify soft fats. This prevents soft fats from being processed only to be downgraded later, or sufficiently hard fats from being unnecessarily downgraded. This would in turn benefit both the processor and the producer by minimizing losses and maximizing profits.

Several methods have been proposed for measuring and characterising fats, some of which rely on identifying fatty acid composition, such as stearic and linoleic acid. Also, the iodine value which measures the degree of unsaturation has been suggested as a suitable predictor of consistency amongst other methods (Gläser et al., 2004). The idea behind this is rather basic: the number of double bonds is directly proportional to the consistency of the fat. For obtaining information on degree of unsaturation and oxidative resistance, iodine value is a good indicator as it measures the number of double bonds directly. Iodine monochloride binds to the double bonds, and one can measure the amount of iodine monochloride absorbed by the sample

to determine the degree of unsaturation. However, since iodine monochloride is a dangerous chemical, an indirect method can be used by using gas chromatography to find the fatty acid profile and then calculating the iodine value according to AOCS 1c-85 protocol (2009). This protocol has recently come under question for failing to include fatty acids with a chain length above 20 carbons (Johnstone & Li, 2011). In the American Oil Chemist Society Journal, a publication by Knothe (2002) titled *Structure indices in FA chemistry. How relevant is the iodine value?* describes the process of using the iodine value – originally developed as tool to better understand fats and oil in 1884 – calculated from a fatty acid profile as backwards given today's technology. It uses the fatty acid profile which yields far more information to obtain an iodine value which provides far less information; claiming that it is "too general to allow the correlation of physical and chemical properties with FA [fatty acid] composition." (Knothe, 2002)

Another method for estimating firmness of the pork belly, so as to allow it to maintain its rigidity during the high speed slicing of bacon, is to place the pork belly on a rod at the centre of the piece, and then measure the deflection from horizontal – commonly referred to as the belly flop test. This is a simple method for estimating firmness and therefore has inherent inaccuracies (Davenel et al., 1999; Johnstone & Li, 2011).

Direct methods involve the use of Instron compression, durometer, hardness meter, and penetrometer. These tests are difficult to perform in the slaughterhouse. (Gläser et al., 2004; Johnstone & Li, 2011).

One fast method that is showing promise is the use of H-Nuclear Magnetic Resonance spectroscopy (NMR) to measure solid fat content (SFC). SFC is a measure of the fraction of a sample that is in solid state. When measured by H-NMR, it is not a mass fraction but rather a ratio of NMR response from hydrogen nuclei in a solid phase against and NMR response of

hydrogen nuclei in a liquid phase, expressed as a percentage (AOCS, 2009). H-NMR is growing in popularity for its speed and precision. It has been successfully demonstrated that SFC at 20°C provides useful information with regard to fat consistency, with results similar to those obtained by the puncture test (Davenel et al., 1999). The technique has become an interesting method for the industry since it can be measured at a pace necessary for industrial processing. In both pure fat samples and in backfat samples obtained by biopsy the SFC values corresponded with one another, allowing for an even faster sample processing prior to measurement (Davenel et al., 1999; Gläser et al., 2004). Contrary to iodine value, SFC has a correlation coefficient of 0.997 ($p=0.001$) with hardness measured by penetration force in binary lipid blends (Braipson-Danthine & Deroanne, 2004) suggesting that SFC is a macroscopic interpretation of fat consistency and has the ability to detect soft fats.

The objective of this paper is to investigate the relationships between fatty acid composition, iodine value and solid fat content of pork adipose tissue.

4.2 Materials and Methods

4.2.1 SFC

A total of 46 pork backfat samples were obtained from pigs of different ages, castrated males, uncastrated males, and females to increase variability in the samples. The samples were cut with a knife to as small a dimension as possible by hand, roughly 1 - 2 mm cubes. They were then submerged in liquid nitrogen and ground to fine powder with a pestle. The slurry was then transferred to an aluminium tray and placed in an oven at 100 ℃ for 30 minutes with a slight vacuum of 1-2 kPa so as provide a flow of air to remove condensation and humidity from the

oven. The sample was quickly transferred to a centrifuge tube - leaving the coagulated connective tissue which tends to stick to the tray behind - and placed in a centrifuge at 10 000 rcf for 10 min at 30℃. The liquid, transparent, fat was then removed from the centrifuge tube and frozen until the day of the measurement. SFC measurements were performed according to the AOCS Official Method Cd 16b-93 for temperatures of 0 to 40℃, at 5℃ intervals using a td-NMR (Minispec mq20, Bruker, Billerica, United States). The temperature control of the baths had a precision of ±0.5℃. All samples were measured in duplicates.

4.2.2 Fatty Acid Composition

Fatty acid composition was measured using a Gas Chromatography (Agilent 6890 Series, model 7683, Santa Clara, United States) with flame ionization detector with a split-splitless detector. The capillary column is made of fused silica of 30 m x 0.25 mm i.d. filled with DB-FFAP (d_f = 0.25 µm). Carrying gas was hydrogen at a flow rate of 1.2 ml/min at the exit of the column. The makeup gas was nitrogen at a flow rate of 25 ml/min. At the detector the flow rate for hydrogen and compressed air was 35 ml/min and 350 ml/min. The split ratio of the injection port was 25:1. A standard curve was made using an internal standard stock solution of methylated fatty acids (C4, C6, C7, C8, C10, C12, C14, C15, C16, C18, C18:1, C18:2, C18:3) in hexane. Fat samples were methylated using potassium methylate in methanol before being analysed.

4.2.3 Iodine Value

The iodine value was calculated from the fatty acid composition using the AOCS Recommended Practice, Protocol Cd 1c-85. This is not considered a fast method, but rather a

method for obtaining two results from one analysis and a way by which to avoid the use of the highly dangerous chemical iodine monochloride. The protocol can be performed on triglycerides as well. The equation for calculating the iodine value from free fatty acids is a sum of the concentration of each unsaturated and poly-unsaturated fatty acid, each of which is multiplied by a factor specified in the table provided by the protocol. If the saturated fatty acid has a *trans* conformation it will receive a different multiplication factor than its *cis* counterpart. An example of a sample containing six fatty acids multiplied by their respective factors can be seen below (1 AOCS, 2009).

$$Iodine\ value\ = 0.9976 * (\%\ hexadecenoic\ acid) + 0.8986 * (\%\ octadecenoic\ acid) + 1.810 * (\%\ octadecadienoic\ acid) + 2.735 * (\%\ octadecatrienoic\ acid) + 0.8175 * (\%\ eicosenoic\ acid) + 0.7497 * (\%\ docosenoic\ acid)$$

4.2.4 Statistical Analysis

Statistical analysis was performed on SPSS from IBM using the multivariate linear regression. The Mahalanobis distance was used to check for outliers.

4.3 Results and Discussion

One sample was removed since it was deemed an outlier according to the Mahalanobis distance using chi-squared value $X_{0.025}$ (df=9), leaving 45 samples used for the analysis. A high consistency between duplicates was observed, often less than 0.1% difference between sample measurements. The coefficient of variation for each measurement can be seen below in Table 4.1. The fatty acid profile demonstrates an average coefficient of variation of 10.24%. Further

confirming the good variability of the samples are the SFC measurements, hitting coefficient of variations as high as 66%, with an average of 29.27%. The high variability translated to a high variability in the iodine value, with a coefficient of variation of 12.43%.. This level of variation is deemed acceptable as it can also be seen in other studies which obtained 10.4% as a coefficient of variation (Olsen et al., 2007).

Table 4.1 Fatty Acid Composition and SFC Values

	Parameter	Average (%)	Range (%)	Standard Deviation	Coefficient of Variation (%)
Fatty Acid Profile	Palmitic	42.02	38.27 - 44.86	1.51	3.59
	Stearic	27.49	23.07 - 32.70	2.50	9.09
	Linoleic	23.03	16.77 - 30.03	2.92	12.68
	Oleic	4.07	3.13 - 5.12	0.44	10.81
	Myristic	1.92	1.58 - 2.23	0.17	8.85
	Alpha-linolenic	1.16	0.84 - 1.69	0.19	16.38
SFC Measurements	SFC 0	39.51	28.07 - 55.33	5.86	14.83
	SFC 5	38.16	26.88 - 54.04	5.72	14.99
	SFC 10	34.72	24.94 - 48.58	4.95	14.26
	SFC 15	30.45	21.24 - 43.33	4.74	15.57
	SFC 20	25.16	16.88 - 37.94	4.44	17.65
	SFC 25	17.78	10.47 - 29.71	4.08	22.95
	SFC 30	5.08	1.49 - 16.98	3.36	66.14
	SFC 35	2.28	0.72 - 5.12	0.90	39.47
	SFC 40	1.06	0.06 - 3.00	0.61	57.55
	Iodine Value (g/100g)	46.41	33.92 - 60.04	5.77	12.43

* Remaining fatty acids <1%

Regarding normality, the fatty acid distribution, as well as the iodine value, passed the Shapiro-Wilk test ($p<0.05$). All SFC measurements, except at 30°C, also passed the Shapiro-Wilk test.

Below in Figure 4.1 is the melting profile of a sample. The shape of the curve was highly similar for most samples, all of whom contain what appears to be a point of inflection either at, or near, 30°C. This could explain why there exists a non-normal distribution for samples measured at the temperature.

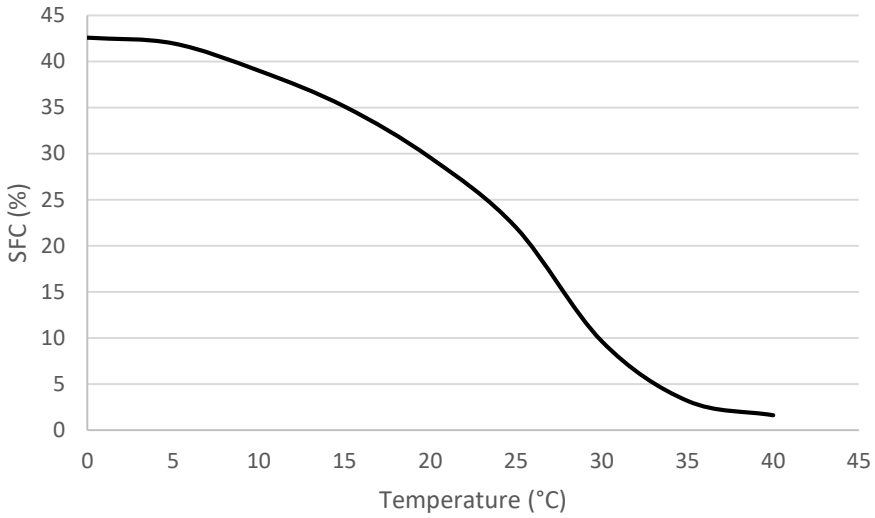

Figure 4.1: Example melting curve

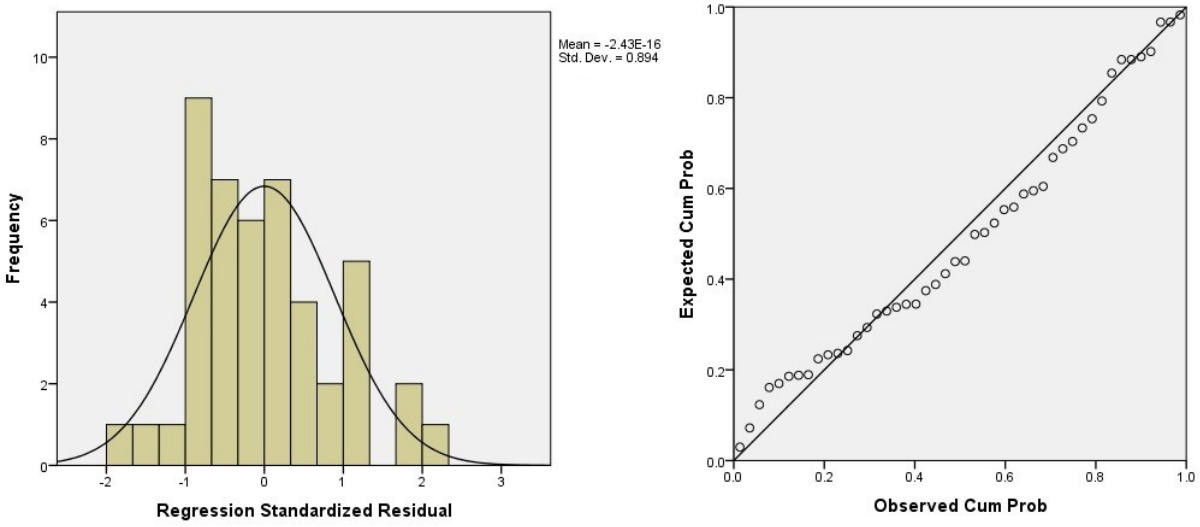

Figure 4.2: Histogram with superimposed normal curve (left) and Normal P-P Plot of Regression Standardized Residual (right)

Using a multivariate regression with all nine SFC measurements at different temperatures as the independent variables and iodine value as the dependant variable, an R^2 value of 0.76 was obtained. Compared to the literature, the coefficient of variation between SFC at 20 °C and iodine was found to be 0.80 (Davenel et al., 1999), confirming similar results, though it should be

mentioned that fat composition and consistency was drastically different. The palmitic acid content was double that of Davenel et al. and the SFC content was lower.

The regression equation takes the form:

$$f(x) = constant + X_1B_1 + X_2B_2 + \cdots X_nB_n$$

The values obtained for the following regression can be seen in Table 4.2. Accompanying the regression coefficients B is the standardized regression coefficient β'.

Table 4.2 Regression coefficients

n	X	B	β
0	Constant	65.519	n/a
1	SFC 0°C	2.933	3.012
2	SFC 5°C	-5.673	-5.711
3	SFC 10°C	-1.039	-0.902
4	SFC 15°C	2.021	1.680
5	SFC 20°C	6.681	5.201
6	SFC 25°C	-6.828	-4.890
7	SFC 30°C	0.164	0.097
8	SFC 35°C	2.120	0.335
9	SFC 40°C	3.493	0.372

The standardized regression coefficients demonstrated that SFC measurements at 0, 5, 20, and 25 °C provided the largest contribution to the regression. In the interest of predicting iodine value with a minimum number of measurements, the regression was performed using only those four temperatures and an R^2 value of 0.74 was obtained. This could mean that fewer

measurements would be required to obtain good results in industry, allowing for faster and easier analysis of samples.

From Table 4.2, again looking at the standardized regression coefficients, the smallest contribution comes from the measurement obtained at 30°C. The contribution is more than three times smaller than the second smallest contributor, and more than fifty times smaller than the largest contributor. This too, can likely be attributed to the point of inflection seen near, or at, that temperature.

By performing the same multivariate regression with all nine SFC measurements as the independent variable and various fatty acids as the dependant variable, the correlation with fatty acid content and SFC can be obtained. The coefficient of determination for palmitic, linoleic, stearic, and α-linolenic acid are 0.78, 0.75, 0.69, and 0.68, respectively. The literature found the coefficient of determination between stearic acid and SFC at 0 °C to be 0.69, and 0.92 at 20 °C (Gläser et al., 2004). For linoleic acid, Davenel et al. found the coefficient of determination to be 0.48 at 20 °C (Davenel et al., 1999).

The strongest correlation between the SFC and composition was for palmitic acid present in a sample ($R^2 = 0.78$). This is likely because palmitic acid was the most prevalent, on average doubling the amount of stearic acid – the second most prevalent. Interestingly, the correlation with stearic acid ($R^2 = 0.69$) was weaker than with the less prevalent linoleic acid ($R^2 = 0.75$). Studies have typically seen oleic acid as the most prominent fatty acid, with half the amount of palmitic acid present, though their correlations were higher when combining polyunsaturated acids together to predict SFC (Davenel et al., 1999; Glaser et al. 2004). The correlations obtained were not improved by combing polyunsaturated fatty acids together (results not shown). This

indicates, both from the literature and this experiment, that the poly-unsaturation of linoleic acid has a greater influence on SFC than saturated stearic acid.

measured at various temperatures and fatty acid (FA) content

Referring to Figure 4.3, it can be seen that all but oleic acid had better correlations at colder temperatures, many of the best correlations occurring at 0-5°C, and getting progressively worse as the samples warmed up. Oleic acid on the other hand had stronger correlations at 20-25°C. Yet again an odd phenomenon occurs at 30°C: the worst correlations across the board occurred at that measurement temperature. This leads to the conclusion that measurements near a point of inflection will provide inaccurate results and should be avoided when attempting to build a predictive model for fat composition or fat consistency.

4.4 Conclusion

SFC has good correlations with iodine value and certain fatty acids, which coincides with the literature. However, it does not provide a complete correlation with 24% of the variation in iodine value unexplained by the solid fat content values. Iodine value provides accurate information in terms of degree of unsaturation, but this information alone cannot explain macroscopic textural properties with accuracy. This is likely due to the fact that iodine value only takes one microscope property into consideration in attempting to understand a larger macroscopic property such as consistency or hardness. Next, points of inflection in a melting curve indicate areas where measurements would be inaccurate, or at the least, uninformative. Finally, this study suggests that the use of iodine value by meat processing facilities would not provide accurate information for processing meats.

4.5 References

Chapter 5
Conclusions and Recommendations

5.1 General Conclusions

In this study, NMR transverse relaxation data and solid fat content were measured. Both measurements were compared to conventional techniques in order to investigate the applicability of NMR as a tool for elucidating such attributes in a cost-effective, rapid, multi-dimensional manner. Based on the experiments performed, and the literature review, the following conclusions can be made:

I. Solid fat content itself may be a better predictor of fat consistency than iodine value.

II. The correlation with iodine value is good, and further investigation may allow NMR to replace it entirely.

III. NMR transverse relaxation signals correlated best with cooking loss, and given that pork is always cooked before consumption, this has the potential to provide tremendous insight into the consumer experience of pork consumption.

IV. NMR has the potential to correlate strongly with thaw loss, as of this writing it has not been investigated previously. Further research could be interesting and valuable for processed pork products which are frozen before being sold, such as frozen sausages or ready made meals.

V. NMR transverse relaxation signals correlate with drip loss.

VI. NMR can be scaled up to larger samples. This study demonstrated that correlations continued to be prevalent even when sample sized was increased over a hundred times.

VII. NMR can still provide valuable insight at fridge temperatures.

VIII. Multiple meat quality attributes, such as drip loss, cooking loss, and thaw loss, can be correlated to only one signal/measurement.

5.2 Recommendations

WHC remains one of the most important attributes, and only a minor success was obtained in correlating drip loss to NMR signal. This is likely due to the fact that drip loss was measured, then the sample was frozen, then the sample thawed, and then the NMR data was obtained. Because the NMR signal represents the new, post freeze-thaw, rearrangement of water within the meat structure, it is likely not representative of how the water was compartmentalized when it was being lost as drip. It is recommended that the drip loss be measured after NMR signals are obtained; as soon as possible, with minimal alteration on water distribution in the form of temperature fluctuations or excessive manipulation.

It is the opinion of several authors in several publications, including some in the American Society of Oil Chemists that the iodine value as a measure of fat consistency is dated and inaccurate. It is recommended that new standards be developed for the industry so that they may accurately classify fat consistency. Correlating solid fat content, as measured by NMR, to other measures of fat consistency would be beneficial. Today, the maximum iodine value for processing a pork is used. Above that threshold, the cut is rejected as too soft. New limits, measured by solid fat content, or penetrometer, should be established. This new threshold should

be compared to how well the product undergoes processing, and then compared to consumer experience. New standards based on new measurements would be tremendously beneficial to consumers and producers alike.