Detection of meat and fat quality in pork and beef using X-ray

MASTER THESIS

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Abstract

The aim of this thesis was to study the possibilities of predicting different meat and fat quality parameters using Computerized Tomography (CT) scanning. No studies have previously been published in this field so there is no experience within this subject. Three different data sets were studied and included meat from beef (10 different muscles) and pork (half a pig carcass) along with back fat from pigs only. Meat and back fat samples were scanned with a medical CT scanner, the back fat samples were also scanned with a micro CT scanner. Beef muscles were scanned twice at two different energy levels 80 and 130 kV. Pig carcasses where scanned at 140 kV and back fat samples at 80 kV (micro scanner also 40 kV). Quality parameters related to meat texture and the fatty acid composition of the back fat were used as reference data for the CT scanners.

Images from samples scanned at two different energy levels were analysed by image analysis. The images from the two energy levels were subtracted or divided with each other. Spectra from the CT scanning were found to be asymmetrically distributed therefore they were analysed with spectral analysis (raw and subtracted spectra and by feature extraction) and multivariate data analysis.

The main conclusion of this thesis was: It was not possible to predict meat or fat quality with Computerized Tomography. However, if the methods are improved or modified in future work it might be possible. One of the data sets was scanned at two different energy levels and had better correlations coefficients (R) than the other data sets that were scanned only once. This might be caused by two energy levels providing more information. Several sources of errors were found in both the data sets and in the scanner settings that could affect results from the CT scanning. Using the CT as an analysing tool, experimental design, settings and output should be used with great consideration.

Image analysis did not provide useful results as the program used was not suitable for this method. The multivariate data analysis did not provide useful results for the back fat samples therefore the spaciousness was studied instead. It was found that the outer layer of the back fat had a higher density than the inner layer which might be caused by the collagen and water content. However, this could not be confirmed as these two parameters were not measured.

Results varied depending on the spectral analysis method and the different meat quality parameters, therefore different methods of analysis should be chosen depending on the parameter of interest if CT in the future should be able to predict meat and maybe fat quality. It cannot be concluded which energy level is suitable for which meat quality parameter and neither whether more than one energy level should be used depending on the parameter predicted.

Resumé

Formålet med denne opgave var at undersøge mulighederne for forudsigelse af forskellige kød- og fedtkvalitetsparametre ved hjælp af Computer Tomografi (CT) skanning. Ingen undersøgelser har tidligere været offentliggjort, hvorved der ingen erfaring er indenfor dette område. Tre forskellige datasæt, som inkluderede kød fra oksekød (10 forskellige muskler) og svinekød (halve svinekroppe) samt rygspæk fra grise blev undersøgt. Kød- og fedtprøverne blev skannet med en medicinsk CT-skanner, fedtprøverne blev også skannet med en mikro CT-skanner. Musklerne fra oksekødet blev skannet to gange ved to forskellige energiniveauer (80 og 130 kV). De halve svinekroppe blev skannet ved 140 kV, og fedtprøver ved 80 kV (mikro-skanner også 40 kV). Kvalitetsparametre relateret til kødprøvernes tekstur og fedtsyresammensætningen i rygspækprøverne blev brugt som reference data for CT-skannerne.

Billederne fra prøverne skannet med to forskellige energiniveauer blev analyseret via billedanalyse. Billeder fra de to energi niveauer blev subtraheret eller divideret med hinanden. Spektre fra CT-skanningen viste sig at være asymmetrisk fordelt og blev derfor analyseret med metoden spektralanalyse (rå og subtraherede spektre samt karakteristiske egenskaber/punkter) og multivariat dataanalyse.

Hovedkonklusionen i denne opgave var: Det var ikke muligt at forudsige kød- eller fedtkvalitet med Computer Tomografi. Dog vil dette måske være muligt, hvis metoderne forbedres eller ændres i fremtidige studier. Det ene datasæt blev skannet ved to forskellige energiniveauer og havde bedre korrelations koefficienter (R) end de andre to datasæt der kun blev skannet ved ét energiniveau. Dette kan skyldes, at to energi niveauer giver mere information end en. Hvis CT skal anvendes som et analyseværktøj, skal planlægning af forsøgsopstilling, skannerindstillinger og output ske med stor hensyntagen til disses indflydelse på resultaterne.

Det benyttede program til billedanalysen viste sig ikke at være egnet til denne metode og gav derfor ingen brugbare resultater. Den multivariate dataanalyse gav heller ikke brugbare resultater for rygspækprøverne så rummeligheden af disse blev i stedet undersøgt nærmere. Ved denne undersøgelse blev det konstateret, at det yderste fedtlag i rygspækprøverne havde en højere densitet end det inderste fedtlag. Dette skyldes muligvis indholdet af kollagen og vand, dog kunne dette ikke bekræftes, da disse to parametre ikke er blevet analyseret.

Resultaterne varierede afhængigt af den anvendte spektralanalysemetode og de forskellige kødkvalitetsparametre. Derfor bør forskellige analysemetoder vælges afhængigt af den enkelte kvalitetsparameter, hvis CT i fremtiden skal være i stand til at forudsige kød- og måske fedtkvalitet. Det kan ligeledes heller ikke konkluderes, hvilket energiniveau eller om der eventuelt skal anvendes flere energiniveauer til forudsigelse af kød- og fedtkvalitet.

Preface

This MSc. thesis is the final part of the master program Meat Science and Technology at the Faculty of Life Sciences, University of Copenhagen. The thesis was written during the period of November 2008 to December 2009 with a break of four months during the spring and summer. The thesis consists of three data sets, one was performed by Norfima Food Matforsk in Norway (autumn 2008) the second by The Danish Meat Research Institute (DMRI) (February 2008) and the last data set in a combination of the organisation Danish Pig Production and DMRI performing the practical work except for the CT scanning which was performed by me.

In connection with this project I would like to thank Peder Pedersen from The Danish Technological Institute in Taastrup for lending me the micro CT scanner. Thanks to Claus Borggaard for helping with the multivariate data analysis and always being prepared for a good discussion; as well as I would like to thank Marianne Toft, Eli Vibeke Olsen and laboratory technician Anne-Marie Nielsen. Thanks to Marchen Hviid, Lars Bager Christensen and Anders Karlsson for their professional supervision. Last but not least, I would like to give a special thank to Marchen and Lars who have not only supervised me but also taken me out on a CT adventure. This adventure took me from Roskilde to Hillerød and all the way to Monell in Spain where I had the opportunity of telling about my work. This was just the coolest experience☺

Roskilde, December 11th 2009

Maiken Stubkjær Schubert, FSK07039

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1 Introduction

1.1 Background

When animals are slaughtered, meat quality parameters are used as a tool to classify carcasses. Meat quality can be assessed in several ways, and the methods for doing this vary between people and over time (Warris, 2000). Meat quality is most often defined as *fresh meat eating quality* which describes meat for fresh meat consumption, and *technological quality* which describes meat for further processing (Aaslyng, 2002). The latter is mostly used for classification at the slaughterhouse. The main quality parameters of technological quality are water holding capacity, colour and texture of fat (Warris, 2000). Water holding capacity is an important factor when predicting the yield of processed meat products but is also an indicator for the juiciness after cooking (Warris, 2000; Aaslyng, 2002). Eating quality parameters are mainly characterized by texture, juiciness and flavour/odour (Warris, 2000; Aaslyng, 2002) although for many consumers, fat content is an important factor. All these different meat quality parameters vary between animals, due to feed, sex, breed, housing etc. Breeding of meat production animals is a great contributor to changes in meat quality especially for pigs. Over time, pigs have become leaner. Seen from most aspects this is a positive quality change though it has opened up for another problem of fat containing a higher amount of unsaturated fatty acids. Animal fat containing high amounts of unsaturated fatty acids tends to be softer. This can cause problems in some meat products, further more unsaturated fats have a higher risk of being rancid and are exposed to lipid oxidation (Enser, 1984; Warnants *et al.,* 1996; Warris, 2000). On the other hand, unsaturated fatty acids are healthier for humans than saturated fatty acids. Knowing the fatty acid content of the meat along with other meat quality parameters would make the classification and sorting of carcasses and meat cuts into fresh meat and meat for processing easier for the slaughterhouse.

For the slaughterhouse the most important meat quality parameters are lean meat and the fat percentage. Carcasses from pig and cattle are in Denmark and in several other countries classified after slaughter. In The European Union (EU), special rules are set up for classifying bovine (EUROP grading system) and pig carcasses (Council Regulation (EEC) No. 1208/81; Council Regulation (EEC) No. 3220/84). The objectives of these rules are; for pigs to guarantee producers a uniform and fair payment and to make it possible to compare between countries. For bovine to have a classification system applied for recording prices and for intervention in the beef and veal sector. Classification of pigs should be based on weight and composition of pigs delivered and the settlement for the farmer is most often based on the lean meat content in relation to weight. Bovine classification should be based on carcass conformation along with the degree of fat cover. Council Regulation (EEC) No. 1208/81 also states that information from the bovine classification system should be used as a part of grading carcasses for further dis-

tribution, for pigs this grading is optional and is not a legal requirement (Council Regulation (EEC) No. 3220/84).

Commercially, carcass quality is most often measured on-line during the slaughter process either manually or automatic using different equipment (Hansson, 2003). The equipment used differs between slaughterhouses and/or countries. Fat-o-Meter, Hennessy Grading Probe and Destron are three examples of instruments capable of measuring fat and muscle depth providing estimates for the lean meat content in pig carcasses (Warris, 2000; Olsen *et al.,* 2007). These three probes are all manually handled whereas also automatic systems exist, one is called the Danish carcass classification centre (Swatland, 1995). These four instruments are designated as reflectance probes and use light to find the interface between fat and muscles. Equipment such as Autofom and Ultrafom also for pigs, use reflectance of ultrasound to measure the lean meat percentage and fat thickness. Ultrafom is handled manually where as Autofom are automatic (Olsen *et al.,* 2007). Cattle are classified either manually or automatic using the EUROP grading system. Manually classification is carried out visually by a trained operator at the slaughterhouse whereas different systems are developed for automatic classification, these are all based on Video Image Analysis (VIA) (Allen & Finnerty, 2001).

As mentioned before, most often lean meat percentages and fat depth or a visual grading form the basis of classification for further distribution. This information is only useful to some extent. When carcasses are dissected into different meat cuts for further distribution, the best knowledge of these cuts comes from many years of experience and studies. This knowledge does not provide exact values of the actual cuts and batches dissected on the current day.

In processed meat products the meat is considered as an ingredient along with other food and the quality of these products is highly dependent on the raw meat used in the production. Water, fat and protein content are parameters affecting processed meat products. Different raw meat composition requires different amounts of other ingredients added to the product such as water, fat and salt. For that reason, it is necessary to know for example the fat content of the meat in order to produce a homogeneous product every day.

Raw meat materials are often purchased in large batches with limited knowledge of composition, and the companies rarely analyse these large badges. They just comply with the information they receive from the slaughterhouse. There are numerous methods of measuring meat composition, most of them are both time consuming and expensive. Take for example in large meat batches; the meat is not necessarily homogeneous in composition through the whole batch. Therefore the reliability of chemical analysis depends greatly on the number of samples analysed. At the same time, chemical analysis take time, the meat will not be fresh when results are finished. Some factories have a large instability in the day to day supply of meat raw material, they cannot be sure they receive the same raw meat materials resulting in meat products of varying quality. These factories often use on-line equipment to measure the fat content during processing in order to compensate for fluctuating meat raw materials.

Fresh meat products at for example the supermarkets are sold without any pre-processing besides cutting at different levels, and the options of manipulating with the quality like for processed meat products are limited. Meat quality parameters such as tenderness, water holding capacity and fat content are only known to some extend therefore consumers will occasionally experience low quality meat that does not live up to their expectations. Knowing some meat quality parameters before the product ends at the consumers table would therefore be a great advantage. Measuring fresh meat quality by chemical methods is possible though it is time consuming and therefore not an option due to shelf-life and high cost.

There are other methods to measure meat quality than by chemical analysis. Already mentioned are online methods though other methods are available. Some of these instruments are capable of measuring pH, PSE meat (Pale, Soft and Exudative), DFD meat (Dark, Firm and Dry), tenderness and water holding capacity. However these instruments are often too slow to keep up with the processing speed not to mention that most instruments should be handled manually. Utilization of these instruments will be expensive compared to the output. Therefore, most often these instruments are used for scientific purpose or for sorting out meat for special high cost meat products such as dried ham (In dried ham pH should be 5.6 - 6.2 and pH is therefore measured before production (Beringues, 1999)).

In general, instruments available only measure a few parameters but most often more quality parameter is of interest. Whether it is meat for processed or fresh products and depending on the company handling the product this could be a combination of both technological and eating quality parameters. Sorting out carcasses according to different demands is only based on a few parameters. If extra quality parameters should be measured, more instruments should be implemented. Also, the extra parameters measured would be different depending on the purchaser. To speed up efficiency in the meat industry, less manually handled instruments or chemical analysis should be used and more automatic equipment measuring quality on-line during the process should be implemented. Already mentioned is Autofom and the Danish carcass classification center. Another equipment commercially available is the MeatMaster from Foss which scans (X-ray) and estimates that fat content of raw meat but also spots metal and bone (Foss, 2009). CFS MultiTrack from CFS is integrated into the grinder and measures fat and lean meat percentage by Near-Infrared Technology (NIR) during mincing (CFS, 2004). However none of them are capable of measuring more than one or two meat quality parameters. Investigating methods to develop new equipment capable of measuring both eating and technological meat quality at the same time would therefore be of great interest for the meat industry. Developing the Danish carcass classification center and Autofom for measuring other meat quality parameters is not possible, since they only measure the interface between muscle and fat. When measuring meat quality parameters the tissues are of interest. Instead it could be interesting to look at the techniques used by the MeatMaster or CFS MultiTrack, X-ray and NIR. The method of NIR has shown to be able to predict meat tenderness (Warner-Bratzler

shear force) (Mitsumoto *et al.*, 1991; Park *et al.,* 1998; Liu *et al.,* 2003), protein, moisture and fat content (Mitsumoto *et al.*, 1991). In different studies Dual Energy X-ray Absorptiometry (DEXA) has demonstrated that the method is able to predict carcass composition (fat and protein content and body weight) in pigs (Mitchell *et al.,* 1996), along with fat content in beef (Brienne *et al.,* 2001), and finely tenderness in beef striploins (Kröger *et al.,* 2006). Another X-ray equipment is Computerized Tomography (CT) which is already used in the Danish slaughter industry to calibrate Autofom. Normally calibration of this equipment (lean meat percentage) is done towards a manual dissection performed by trained butchers. CT scanning has proved to be more precise and reliable predicting carcass composition than manual dissection in both pig and lamb carcasses (Kongsro *et al.,* 2008; Vester-Christensen *et al.,* 2009). CT scanning has been approved by the commission of the European communities as another method of dissection, though using this method, a few carcasses still have to be manually dissected (Commission Regulation (EC) No. 1249/2008). Beside this, CT is able to predict salt gradients in cured pork (Vestergaard *et al.,* 2004), the salt content of dry-cured ham (Vestergaard *et al.,* 2005) and *in vivo* muscle volume of the hind leg and lumbar region of lambs (Navajas *et al.,* 2006).

1.2 Problem

No laws or EU regulations lay down rules for the slaughterhouse to measure, classify or declare fresh meat eating or technological quality to the buyers, though they to some extend have knowledge in this area and uses this to inform buyers. There is no doubt that more accurate measurements of meat quality would be of great advantage for the slaughterhouse, having the possibility to sort out the meat in categories depending on buyer and end product (fresh or processed). However, no methods capable of measuring more than one or two meat quality parameters are available at the moment; therefore studies within this area would be of great interest.

The aim was to study the possibilities of predicting different meat and fat quality parameters using CT scanning.

- Is it possible to detect different meat and fat quality parameters using CT scanning and which method of analysis should be used?
- How many energy levels are needed to obtain the proper information?

1.3 Delimitation

In the paragraph background meat quality was defined as either fresh meat quality or technological quality and different quality parameters were connected to each of these two definitions. As mentioned in the preface, the thesis consists of three different data sets, two were performed on meat (pork and beef) and one on back fat (pork) and most of the experimental work was performed before this thesis was started. The author of this thesis had therefore no influence on which quality parameters should be measured in the meat and back fat samples. Several different parameters have been measured though not all of them will be mentioned in this

thesis. In one data set, 17 different meat quality parameters were measured chemically, sensorial and visually. For the other data set performed on meat, only four quality parameters were measured. In this thesis, only meat quality parameters related to meat texture (sensory variables tenderness and hardness, WB measurements, collagen and protein content as well as pH, fat and moisture content) will be dealt with. In the last data set, the fatty acid composition of back fat was analysed, other fat depots are not included. Samples were analysed for 44 different fatty acids though only seven of them were quantifiable. These seven fatty acids; myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) along with the saturation level, the omega 6/3 ratio and the iodine value were studied. Other meat and fat quality parameters mentioned in the background such as colour, water holding capacity and texture of fat will not be deal with. The meat and fat quality parameters studied are used as reference data for the CT scanning results.

Several different methods which are capable of measuring different quality parameters were mentioned in the paragraph background; for example methods that use reflectance probes, Near-Infrared technology and X-ray. Two X-ray methods were mentioned Dual Energy X-ray Absorptiometry (DEXA) and Computerized Tomography (CT). As the problem points out, only the method of CT will be studied in this thesis though DEXA will be included in the theory.

2 Theory

2.1 Meat and fat quality

2.1.1 Fat quality

The quality of fat, from meat animals, is defined as being firm and white in pigs and firm and creamy-white in cattle and sheep (Wood, 1984). This definition is derived from butchery and cooking manuals, where poor quality fat is described as being oily, soft, wet, gray and floppy. Meat products containing soft fat can show quality defects such as rancidity development due to lipid oxidation, insufficient drying and inferior fat consistency (Hugo & Roodt, 2007). Fat is composed of approximately 84 percent lipids, 14 percent water and 2 percent collagen, where lipids are the major contributor to consistency (Wood *et al.,* 1989).

Whether a fat or oil material is soft or hard is determined by the fatty acids composition (Wood, 1984; Warriss, 2000; Hugo & Roodt, 2007). Plant oils which are liquid at room temperature consist mainly of unsaturated fatty acids whereas fat from animals is often solid at room temperature due to a significant higher amount of saturated fatty acids (Warriss, 2000). Fat quality is therefore most often measured by the level of saturation, either by determining the amount of iodine (iodine number) (Warriss, 2000) or by gas cromatographic analysis (determines the fatty acid composition) (Hugo & Roodt, 2007). Also physical properties such as melting point and colour can be measured (Hugo & Roodt, 2007).

Comparing different kinds of animal fats can be done by using the ratio between saturated and unsaturated fatty acids (S/U). Fat coming from lamb has the highest ratio 1.1 followed by beef (0.8), pork (0.7), chicken (0.6) and salmon (0.3), for comparison corn oil has a fat ratio of 0.2 (Warriss, 2000). Firmness of the different kinds of fats follows the same order, lamb being the hardest and salmon most soft.

Pig adipose tissue consists of several fatty acids, most often mentioned is myristic (C14), palmitic (C16), palmitoleic (C16:1), stearic (C18), oleic, linoleic and linolenic acid (C18:1-3) (Kock *et al.*, 1968a; Kock *et al.*, 1968b; Enser *et al.*, 1984; Wood, 1984; Whittington *et al.,* 1986; Scheeder *et al.*, 2000). Oleic acid is the major component of pig adipose tissue (approximately 40 percent) though it has poor relations to the consistency of the fatty tissue (Hugo & Roodt, 2007). Stearic and linoleic acid each represent approximately 10 percent of pig adipose tissue but is very important for the consistency of fat tissue.

The fatty acid composition of pigs also varies between the different depots, where perirenal fat has the largest amount of saturated fatty acids, followed by subcutaneous fat and with inter and intra muscular fat having the lowest saturation level (Whittington *et al.,* 1986; Wood *et al.,* 1986; Bejerholm *et al.,* 1992). Within the subcutaneous fat depot the fatty acid composition also varies. This depot can be divided into two (Koch *et al.*, 1968a; Koch *et al.,* 1968b; Whittington *et al.,* 1986; Warnants *et al.,* 1998) and sometimes three fat layers (Moody & Zobrisky, 1966; Fortin, 1986; Daza *et al.*, 2007) (Figure 1), the outer layer being more unsaturated than the

inner layers (Koch *et al.*, 1968a; Koch *et al.,* 1968b; Whittington *et al.,* 1986; Warnants *et al.,* 1998; Daza *et al.*, 2007).

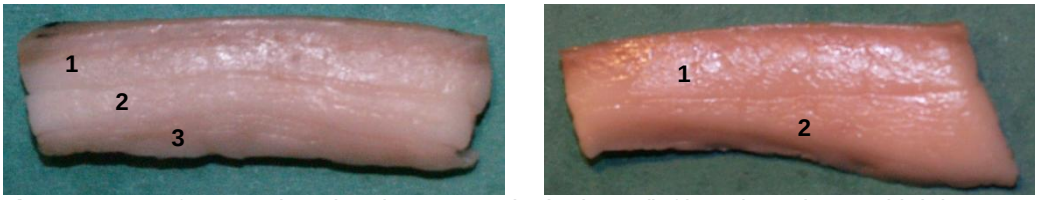


Figure 1. Two fat samples showing respectively three (left) and two layers (right). Layers are divided by connective tissue layers.

The fatty acid composition is among other things dependent on sex and breed (Koch *et al.,* 1968b; Cameron & Enser, 1991; Bejerholm *et al.,* 1992; Warnants *et al.,* 1999), diet (Koch *et al.,* 1968b; Brooks, 1971; Whittington *et al.,* 1986; Cameron & Enser, 1991; Warnants *et al.,* 1998; Scheeder *et al.*, 2000; Hugo & Roodt, 2007) and age (Bragagnolo & Rodriguez-Amaya, 2002; Hugo & Roodt, 2007). The diet has shown to influence the fatty acid composition, whereas some fatty acids in the diet can be recognised directly in the fat depots after slaughter (Warnants *et al.,* 1998; Warriss, 2000). This is especially seen for linoleic acid that increases in the back fat with increasing amount in the diet (Koch *et al.,* 1968b; Whittington *et al.,* 1986; Brooks, 1971; Larick *et al.*, 1992; Warnants *et al.,* 1998).

Depositing of the different fatty acids between the fat layers should also be taken into consideration. Is the depositing uniform or is the uptake in one layer larger than the other? There is no clear pattern for any of the fatty acids, myristic, palmitic, palmitoleic, stearic, oleic, linoleic or linolenic acid (Koch *et al.,* 1968b; Whittington *et al.,* 1986; Warnants *et al.,* 1998). Linoleic acid shows a higher deposit in the outer than the inner layer in two studies by Koch *et al.* (1968b) and Warnants *et al.* (1998) when linoleic acid is increased in the diet, while the opposite is seen in a study by Whittington *et al.* (1986). Stearic acid stays constant in the back fat layers even though it is either in- or decreased in the diet (Koch *et al.,* 1968b; Warnants *et al.,* 1998) and at the same time Whittington *et al.* (1986) show a variation between layers with a higher amount of stearic acid in the inner than outer layer when it decreases in the diet. The same random variation is seen for the rest of the fatty acids (Koch *et al.,* 1968b; Whittington *et al.,* 1986; Warnants *et al.,* 1998).

Fat consistency has not shown to be affected by collagen and water (Whittington *et al.,* 1986; Enser *et al.*, 1984), even though both collagen and water content vary between layers (Whittington *et al.,* 1986; Wood *et al.,* 1989). The outer layer contains more collagen than the inner layer (Whittington *et al.,* 1986).

2.1.2 Fatty acids

Fat, also termed lipids, is a very important energy source having almost the double energy value as that of carbohydrate or protein. Fat is stored in four different depots in the body; subcutaneous, perirenal (around the kidneys), omental (around abdominal organs) and inter and intra muscular (Warriss, 2000).

All lipids principally contain carbon, hydrogen and oxygen (Warriss, 2000). In terms of nutrition, they are recognised as triacylglycerols (triglycerides), phospholipids, sterols and fat soluble vitamins (Brindley, 1984; Purves *et al.*, 2003). Triacylglycerols also refers to oils and fats and the chemical composition of triacylglycerols is three fatty acids and one glycerol (Figure 2). Glycerol is considered the backbone of triacylglycerols whereas the fatty acids can vary depending on for example the location of the fat (depot), age, nutrition, sex and breed.

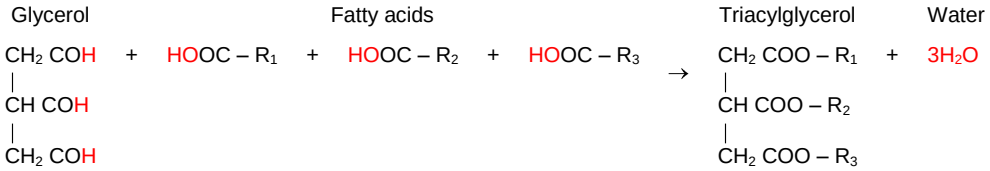


Figure 2. The formation and structure of a triacylglycerol also called condensation (Ockerman, 1996; Purves *et al.*, 2003). The -OH group is removed from the fatty acid along with a –H from the OH group of glycerol, resulting in three water molecules and a triacylglycerol.

If the fatty acids R_1, R_2 and R_3 (Figure 2) are the same, the triacylglycerol is termed a simple triacylglycerol, whereas if one of the three fatty acids is different, the triacylglycerol is said to be mixed (Ockerman, 1996). It is also possible for the glycerol only to have one or two fatty acids attached. Then they are called mono- or diglycerides (Gurr & Harwood, 1991). The most dominant lipids in subcutaneous fat are triacylglycerols and may constitute up to 95 percent of the weight of the tissue (Enser, 1984). For comparison mono- and diglycerides only represents 1 - 2 percent.

The combination of the three fatty acids in the triacylglycerol determines melting point, potential for oxidation and also to some extent nutritional value (Warriss, 2000). The general form can be seen in Figure 2 (R_1, R_2 and R_3). Fatty acids vary in the number of carbon atoms, whereas the simplest fatty acid is acetic acid (**C**H_3**C**OOH) with only two carbon atoms (Gurr & Harwood, 1991; Warriss, 2000). Fatty acids can be divided in to two groups whether they are saturated or unsaturated (Gurr & Harwood, 1991; Ockerman, 1996; Warriss, 2000; Purves *et al.*, 2003). The level of saturation refers to the number of double bonds (C=C) between the carbon atoms in the fatty acid, whereas fatty acids containing only single bonds (C-C) are termed saturated fatty acids. Unsaturated fatty acids are further divided into mono- and polyunsaturated fatty acids (PUFA), mono containing only one double bond and poly two or more (Gurr & Harwood, 1991; Ockerman, 1996; Warriss, 2000). Another characteristic of unsaturated fatty acids is the position of the first double bond from the methyl end of the molecule (Warriss, 2000). In linolenic acid the double bond is three carbon atoms away and is said to be an omega 3 fatty acid or n-3. Besides n-3 there are also n-6 and n-9 fatty acids. The first double bond in linoleic acid is six carbon atoms from the methyl end and is an n-6 fatty acid. Linolenic acids are essential, meaning that the body is not capable of synthesising this fatty acid, therefore it is crucial that linolenic acid is a part of the diet.

Cis or *trans* isomers, are terms used for molecules that have the same molecular formulae, but differ either in structure, or their atoms are arranged differently in space (Ockerman, 1996; Warriss, 2000) (Figure 3). Naturally occurring fatty acids are most often seen as *cis* formation, but can be converted into *trans* fatty acid during fat processing like hydrogenation. The two isomers differ in melting point, whereas *cis* isomers have a lower melting point than *trans* isomers.

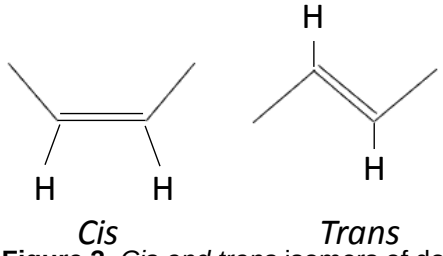


Figure 3. *Cis and trans* isomers of double bonds.

A short way of designating fatty acids is writing the number of carbon atoms followed by a number that refers to the number of double bonds (Warriss, 2000). An example could be linoleic acid which is a polyunsaturated fatty acid containing 18 carbon atoms and two double bonds, C18:2. Stearic acid is a saturated fatty acid also containing 18 carbon atoms without a double bond, C18:0. You could say that stearic acid is the saturated form of linoleic acid, along with linolenic (C18:3) and oleic acid (C18:1), because they all consist of 18 carbon atoms.

2.1.3 Meat quality

Meat quality is a standard term used to describe properties and perceptions of meat (Maltin *et al.*, 2003) and varies between people and over time (Warriss, 2000). Consumers' choice at the supermarket is often based on appearance and on predetermined ideas of the eating quality of particular cuts. However, when the meat has been prepared, cooked and eaten, the eating quality can vary and may not live up to the consumers' predetermined expectations. Asking the average consumer, texture and tenderness are the most important of all the attributes of eating quality followed by juiciness (Lawrie & Ledward, 2006). It is not only the regular consumer who has different demands for meat quality. All meat processing companies have some kind of quality requirements (technological quality). The most important requirement is water holding capacity (WHC) followed by the texture of fat and colour (Warriss, 2000).

There are several methods to measure different meat quality parameters (Warriss, 2000). Some methods are developed to imitate *fresh meat eating quality* such as a trained sensory panel or by different physical methods as for example the popular test of Warner-Bratzler that measures the shear force. Slaughterhouses and processers are often interested in knowing certain quality parameters before they distribute or process the meat. However this is not possible when using a sensory panel or physical measurements because they are performed on cooked meat. Predicting these quality parameters can be done by using different chemical methods though these methods are mostly used for research purposes. For example, analyses

of total collagen and protein content have shown to be good predictors of tenderness (Jurie *et al.*, 1995; Stolowski *et al.*, 2006).

Texture or tenderness are affected by a lot of pre and post slaughter factors. Of pre slaughter factors can be mentioned breed, age, exercise level (free range or conventional rearing), species and handling at the slaughterhouse (Lawrie & Ledward, 2006). Tenderness is heritable though there is a huge variation between studies which show heritabilities from 0.09 up to 0.70 (Burrow *et al.*, 2001). The age of the animal has an impact on tenderness, which is reduced when the animals gets older (Lawrie & Ledward, 2006). There is a natural variation in tenderness between and within muscles (Wulf *et al.,* 2002; Lawrie & Ledward, 2006; Stolowski *et al.*, 2006). *Psoas major* also known as the tenderloin is recognized both by experts and consumers to be the most tender muscle or meat cut. Two muscles that show a variation in tenderness is *longissimus dorsi* and *biceps femoris*. The tenderness of *biceps femoris* increases from insertion to origin in beef, whereas tenderness in *longissimus dorsi* increases from the lateral part to the medial part in pork (Lawrie & Ledward, 2006).

Post mortem tenderness is affected by factors such as post mortem glycolysis, chilling rate, conditioning, processing and cooking (Warriss, 2000; Lawrie & Ledward, 2006). First muscles are converted into meat by a number of metabolic and structural changes. The blood circulation stops, and the supply of oxygen, free fatty acid and glucose to the muscles ceases (Warriss, 2000; Toldrá, 2003). In the living animal these compounds are converted into energy (ATP) which along with Ca^{2+} are involved in the contraction-relaxation process. Early post mortem, while the temperature and pH are still high, the normal level of ATP is maintained preventing actin-myosin cross-bridge formation (Warriss, 2000; Lawrie & Ledward, 2006). When the oxygen supply to the muscles stops, glycolysis continues anaerobically, now only two moles of ATP is produced compared to 12 moles under aerobic conditions (Warriss, 2000; Toldrá, 2003; Lawrie & Ledward, 2006). Among others ATP is produced in the muscle to drive calcium pumps and to provide energy for muscle contraction (Toldrá, 2003). As ATP levels are reduced, Ca^{2+} levels rise and actin-myosin cross-bridges form, resulting in stiffness or rigor mortis (Maltin *et al.*, 2003). These rigor bonds are associated with an increase in toughness.

After slaughter and post rigor the meat is most often conditioned also called the ageing process. During this process or period the meat tenderness increases. The period of ageing before the meat reaches a maximum tenderness depends on species. Chickens have to be conditioned less than a day followed by pigs, needing minimum four days, at last beef has to be conditioned for minimum ten days (Warriss, 2000). Tenderization during conditioning is mainly caused by changes in the myofibrillar only very small changes have been observed in the major connective tissue components such as collagen (Warriss, 2000; Lawrie & Ledward, 2006).

For meat processors the water holding capacity is an important meat quality factor when predicting the yield of processed meat products (Warriss, 2000; Aaslyng, 2002). The water holding capacity is affected by the post mortem changes mentioned above. The anaerobic glycolysis

produces lactic acid via glycogen stores in the muscles (Warriss, 2000; Toldrá, 2003; Lawrie & Ledward, 2006). Lactic acid is normally removed by the blood system, but post mortem this removal stops and lactic acid accumulate in the muscle causing pH to decrease (Warriss, 2000). pH will continue to decline until either the glycogen stores are used or pH is so low that the glycolysis is inhibited (Warriss, 2000). Excessive decreasing of pH post mortem results in decreasing WHC because of pH affecting the electrostatic repulsion between the filaments and thereby the shrinking of myofibrills (Warriss, 2000). WHC and pH are thereby connected.

pH can also be related to tenderness. The rate of pH fall along with ultimate pH (pH_u) has been studied though a precise relationship between tenderness and pH is not fully understood (Van Laack *et al.*, 2001; Maltin *et al.*, 2003). A few studies show that meat with pH below 5.3 also called PSE meat (soft, pale and exudative) has a higher WB shear force compared to DFD meat (Kauffman *et al.*, 1999; Searcy *et al.*, 1969). DFD meat is short for dark, firm and dry and has a pH above 6.0. Other studies have found that peak/shear force was lowest at pH_u 5.4, then increased until pH_u 5.8-6.0 and then decreased again (Watanabe *et al.*, 1996; Purchas *et al.*, 1999). When the ageing time increased the difference between WB peak force between the different pH_u became smaller (Purchas *et al.*, 1999). The same was seen for Watanabe *et al.* (1996) at day five there was no significant difference in shear force between the different pH_u. Wulf *et al.* (2002) compared DFD carcasses with normal pH carcasses for eight different muscles and found a higher WB peak force in the DFD carcasses for three muscles compared with the normal carcasses. The five remaining muscles were not significant different.

The WB shear force deformation curve has two peaks. The first peak (initial yield) is mainly involving the rupture of myofibrillar components and the second also called the peak force correspond to the resistance to rupture both myofibrillar and connective tissue (Harris & Shorthose, 1988).

2.1.4 Proteins

Overall meat consists of approximately 75 percent of water, 19 percent of protein, 3.5 percent of soluble, non-protein substances and 2.5 percent of fat (Lawrie & Ledward, 2006). As already mentioned, the water content is indirectly connected to tenderness through pH, though the biggest connection between meat and meat tenderness is the protein content. Proteins are build up of amino acids chains and consist therefore of carbon, hydrogen, oxygen, nitrogen and sometimes sulphur (Warriss, 2000). Muscle proteins can be divided in to three categories, sarcoplasmic, myofibrillar and connective tissue proteins (Lawrie & Ledward, 2006). Myofibrillar are the largest component of muscle proteins (11.5 percent) followed by the sarcoplasmic proteins (5.5) and at last the connective tissue proteins (2.0 percent).

The structure of muscles is mainly defined by connective tissue sheaths which can be divided in to three subgroups (Warriss, 2000; Lawrie & Ledward, 2006). The epimysium surrounds the whole muscle, perimysium surrounding the fibre bundles and last the endomysium surrounding individual muscle fibres (Warriss, 2000; Lawrie & Ledward, 2006). Connective tissue is non-

soluble at lower temperatures and the major component of this tissue is collagen (Warriss, 2000; Lawrie & Ledward, 2006). There are various types of collagen each having different constituent polypeptide chains (Bailey, 1985; Warriss, 2000; Lawrie & Ledward, 2006). The amount of each collagen type varies between the different sheaths of connective tissue. Type I and III are found in all three sheaths, whereas type I is dominating in the epimysium and type III in the perimysium. In the endomysium type IV dominates and besides type I and III also type V is found in small amounts.

Proteins consist of cross-links, especially collagen (Warriss, 2000; Stryer *et al.*, 2002). Cross-links are seen between polypeptide chains in proteins. Three different kinds of cross-links exist all being covalent. Disulphide bonds (S-S) are formed between a pair of cysteine residues. For collagen this kind of cross-link is only seen in type III and IV, because they alone contain cysteine (Lawrie & Ledward, 2006). Between the α chains of lysine or hydroxylysine aldehydes, divalent bonds are formed. If more than two α chains are joined the bond is more complex. This takes place during ageing and the cross-links become much more resistant to breakdown (Lawrie & Ledward, 2006). Already existing cross-links can form nonreducible links involving three or more chains generating a three dimensional network which causes an increase in tensile strength.

The two remaining categories sarcoplasmic and myofibrillar proteins also have an effect on meat tenderness. The sarcoplasmic proteins are water soluble proteins, which have a globular structure (Sebranek, 2009). They consist of enzymes, pigment and relatively small peptides and they contribute to tenderization post mortem via the enzyme calpain (Sebranek, 2009). Myofibrillar proteins are the structural and contractile apparatus of the living muscle (Sebranek, 2009). The major components of myofibrillar proteins are actin and myosin, and in muscles they form the thin and thick filaments (Warris, 2000; Lawrie & Ledward, 2006; Sebranek, 2009). Myosin is a long filamentous molecule with a head region joined with a neck to the tail. Several hundred myosins aggregate forming the thick filament with heads sticking out. Actin is a globular molecule that polymerizes and forms double helical chains and along with two other proteins forms the thin filament (Warris, 2000). During contraction cross-bridges are formed between the head of myosin and the actin chains of the thin filament (actomyosin). Post mortem the myofibrils weaken resulting in tenderization. This is mainly caused by the breakdown of attachment between actin and the Z discs; however, the muscle does become more extensible as the myosin cross-bridge to the thin filament stays intact.

As for tenderness, the protein content also varies between muscles, the same is valid for moisture, fat and other constituents of muscles (Lawrie & Ledward, 2006). Strong correlations are found between the total collagen content and toughness (WB shear force, sheared once perpendicular) by Torrescano *et al.* (2003). The bovine muscle *psoas major* showed a collagen level of 2.24 percent of dry weight and had a WB shear force of 2.11 kg. Comparing with *sem-*

itendinosus which had twice this collagen level, 4.75 percent of dry weigth, it had a WB shear force of 4.79 kg.

2.2 Computerized Tomography

Computerized Tomography (CT) is an imaging method used to create three-dimensional images from a series of two-dimensional images (Kalender, 2005). The method is used in health care on hospitals to examine patients but can also be used commercially to for example non-destructive materials testing. There are different models on the market, basically they work as follows.

An X-ray tube rotates 360° around the scanning object, for example the patient or in this case a piece of meat or fat. The object moves through the tube during scanning, creating either single slices or a spiral CT. Every CT slice (rotation) is subdivided into a matrix of elements also called voxels. Intensity of the transmitted radiation is measured by detectors. These intensity data are used to calculate the density or attenuation value (μ) of the tissue at each point in a slice. Specific μ-values are assigned to each individual voxel, and images are now reconstructed as a corresponding matrix of picture elements also called pixels. Each pixel is assigned a numerical value or CT number, which is the average of all μ-values contained within the corresponding voxel. In other words, each voxel might consist of more than one tissue, which will affect the average μ-values and thereby the numerical value or CT number. (Kalender, 2005)

When the scanner is medically or commercially used, there are some small differences. For medical purposes, a so-called medical CT scanner is used. This scanner is dimensioned as for a human to fit in. The spacious resolution in these scanners is low (voxel sizes in mm) compared to micro CT scanners. In the medical scanner the CT number is compared with the μ-value of water (Equation 1) and displayed on the scale of Hounsfield Units (HU) (Figure 4).

Equation 1: $$CT\ number = \frac{\mu_{tissue} - \mu_{H_2O}}{\mu_{H_2O}} * 1000$$

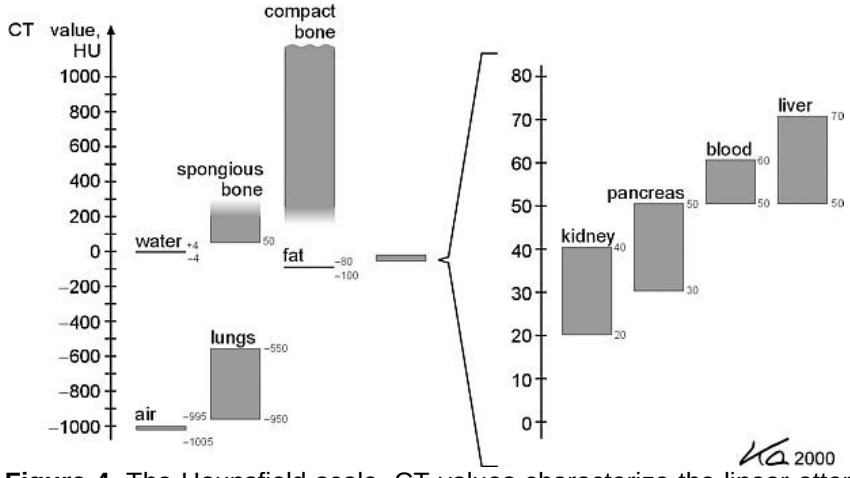


Figure 4. The Hounsfield scale. CT values characterize the linear attenuation coefficient of the tissue in each volume element relative to the μ-value of water. The CT values of different tissues are therefore defined to be relatively stable and to a high degree independent of the x-ray spectrum (Kalender, 2005)

Principally the Hounsfield scale has no upper limit but for medical scanners the scale ranges from -1024 to +3071 HU and states the HU of water to zero and air at -1024 (Kalender, 2005). If the scanner has 12 bits per pixel, consequently 4096 (2^{12}) different values are available. These values are visualized by gray shades in the tomograms or 3D images where it is possible to distinguish between different materials or tissues. In pigs and cattle the three major tissues meat, fat and bone are found in the range of +60 HU for meat tissue, -60 HU for fat tissue and above 150 HU for bone tissue, a tomogram of half a pig carcass is shown in Figure 5.A. As seen, the white pixels are bone whereas meat and fat are seen as gray shapes. It is a bit difficult to see the difference between meat and fat.

From the CT scanning, a histogram can be extracted, showing the distribution of voxels. Scanning half pig carcasses typically results in a histogram or spectra as shown in Figure 5.B. The first peak or distribution corresponds to fat whereas the second peak or distribution corresponds to meat. Bone is out of the range shown in the figure.

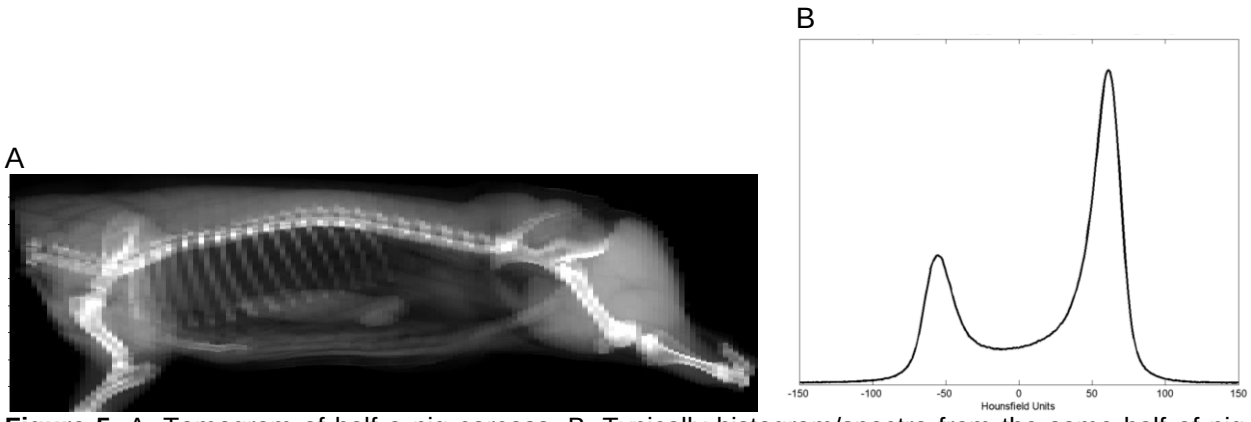


Figure 5. A. Tomogram of half a pig carcass. B. Typically histogram/spectra from the same half of pig carcass as in A.

Commercially purposes are often very different from the medicals, often very small objects are scanned, and therefore a higher spacious resolution is needed. For these purposes a micro CT scanner is used. In the beginning micro CT scanners were developed for research in small animals. It has a much higher spacious resolution than the medical scanner, talking sizes of µm. This scanner deviates from the medical scanner in the setup. In the medical scanner the X-ray source is rotated around the object while in the micro CT scanner the X-ray source stands still while the object rotates. The size of a micro CT scanner deviates, some being small enough to stand on a table others almost as big as a minibus (Figure 6). In the medical scanner the CT number is compared with the µ-value of water and displayed on the HU scale, but the micro CT scanner is not preset for this. Materials scanned might be above +3071 which is the normal maximum range of the HU scale (medical materials above this range are not of interest). Extended scales are therefore used having higher bits 16 or 32 giving a scale range of either approximately 65500 (2^{16}) or 4.29×10^9 (2^{32}). If the micro CT is calibrated it is not calibrated to fit on the HU scale but towards materials suitable for the objects scanned.

Figure 6. Left: Micro CT scanner (Zeiss) about the size of a small minibus. Right: small minibus VW T2a (http://www.film-autos.com/).

2.3 Measuring quality by X-ray

2.3.1 Density of fatty acids

The density of fatty acids varies depending on the number of carbon atoms and the level of saturation, where increasing number of carbon atoms increases the density. As mentioned before saturated fatty acids contain only single bonds whereas the unsaturated fatty acids include double bonds. It is these bonds that contribute to some of the variation in density. Looking at Figure 7, a fatty acid containing only single bonds is able to rotate around these bonds and when a fatty acid contains a double bond this bond is fixed and the molecule cannot rotate where the double bond is located and a kink in the chain is seen (Gurr & Harwood, 1991, Purves *et al.,* 2003 and CDD, 2008). The kink is different whether there is a *cis* or *trans* configuration in the molecule, where *cis* formations create a sharp kink (Figure 7.b) while the *trans* formation is closer to that of an saturated fatty acid (Figure 7.c). This means that the straight chain of saturated fatty acids or unsaturated fatty acids containing double bonds with *trans* formations allows the molecule to pack tighter among other similar molecules compared with unsaturated fatty acids containing *cis* formations (Helmholdt *et al.,* 1972; Gurr & Harwood, 1991; Purves *et al.*, 2003). This means that saturated and unsaturated fatty acids with *trans* formation have a higher density than unsaturated fatty acids with *cis* formations. It is possible for an unsaturated fatty acid to have both *cis* and *trans* isomers that is if they have more than one double bond.

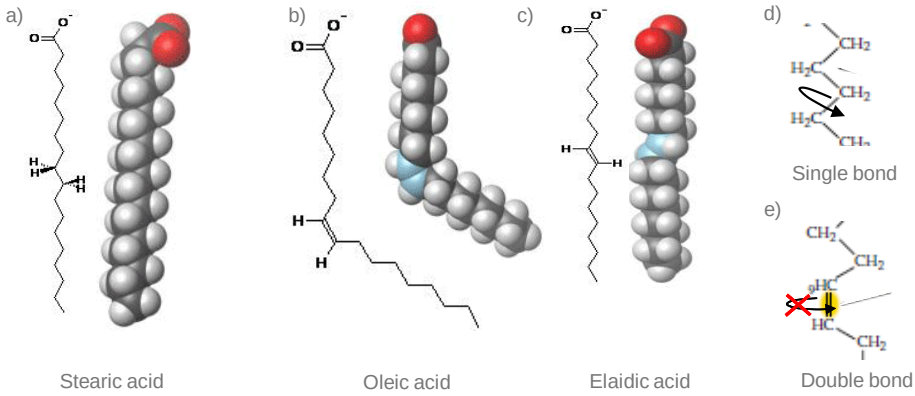


Figure 7. Saturated and unsaturated fatty acids. a) All bonds between carbon atoms are single, in a saturated fatty acid, the carbon chain is straight. b) A double bond, in *cis* formation, between two carbon atoms makes an unsaturated fatty acid, the carbon chain has kinks. c) Also an unsaturated fatty acid, containing a *trans* isomer instead of *cis*. d) Rotation around a single bond. e) Rotation not possible around double bond, the bond is fixed. (mod.a. Gurr & Harwood, 1991, Purves *et al.,* 2003 and CDD, 2008)

As mentioned before, the most dominant lipid of subcutaneous fat is triacylglycerides. Triacylglycerides consist of three fatty acids, whereas the combination of these fatty acids vary. This variation will influence how the molecules pack together both in each individual triacylglycerol but also between triacylglycerides and thereby the density.

2.3.2 Density of proteins

As already mentioned, meat consists of collagen, myosin, actin and calpaine except these are only a small number of all meat proteins. Proteins cannot be divided into for example saturated, and unsaturated proteins as the fatty acids. In meat they are divided in to the three groups described previously, some being soluble in water others contributing to the contractile apparatus of the living muscle or the structure of meat. The density of all these different proteins will naturally vary which means it is a bit more difficult to split them up as did for the fatty acids. Also when the texture and tenderness of meat is in focus, this is not necessary as it is possible to narrow the protein down to collagen, contributing most to the structure.

The density of collagen is not well documented in the literature though a few studies in CT determine the density to be higher than both muscle and fat tissue (Goodsitt *et al.*, 1988; Nordal *et al.*, 1988; Soldevilla *et al.*, 2005). Cross-links in collagens could be one of the reasons for the higher density in collagen compared with muscle and fat tissue even though cross-links are not entirely related to collagen but are also seen in other proteins, but not to the same extend.

2.3.3 The mass attenuation coefficient

The mass attenuation coefficient depends among other things on the energy level and the material scanned (Hubbell & Seltzer, 1996). With an increasing energy level the attenuation decreases which is why we see different behaviour at different energies. This is illustrated in Figure 8 where the mass attenuation coefficient (μ/ρ) is plotted against the energy level for human adipose tissue and skeletal muscle tissue. ρ is the specific density of the material in g/cm^3. As

can be seen, the μ/ρ decreases when the energy level increases. At approximately 0.1 MeV (100 kV) two things can be observed. First: The two curves flatten out, still decreasing but at a slower rate. Second: Comparing the two tissues the skeletal muscle tissue curve has higher μ/ρ until this point, afterwards the difference between the two curves becomes smaller.

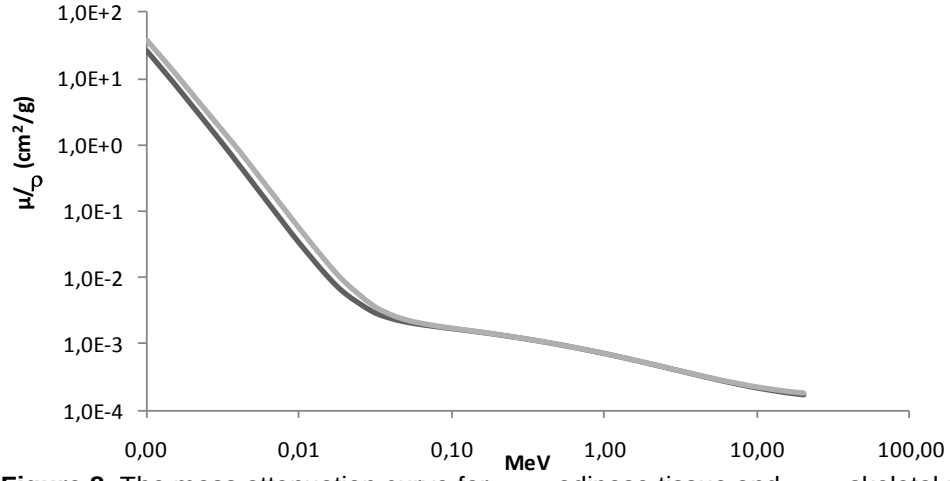
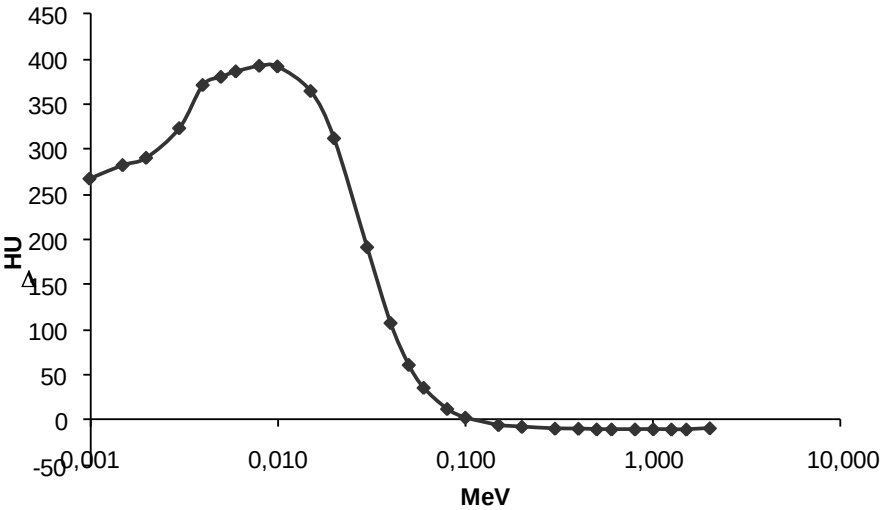


Figure 8. The mass attenuation curve for ▬ adipose tissue and ▬ skeletal muscle tissue (Hubbell & Seltzer, 1996). Both axes are on a logarithmic scale.

In Figure 8 it is very difficult to see to which extent the difference in the μ/ρ is between the two tissues. Instead μ/ρ for each energy level and tissue type can be converted into HU using Equation 1, and ΔHU can be calculated subtracting the adipose tissue from the skeletal muscle tissue. Results of this are shown in Figure 9. Here the difference is even clearer. ΔHU has its maximum peak around 0.008 and 0.010 MeV (8 and10 kV) after this it decreases until 100 kV. As seen for the μ/ρ, ΔHU now flattens out and then increases slightly.



MeV	ΔHU	MeV	ΔHU
0.001	*268*	0.06	*36*
0.002	*283*	0.08	*13*
0.002	*291*	0.10	*3*
0.003	*323*	0.15	*-5*
0.004	*371*	0.20	*-7*
0.005	*380*	0.30	*-9*
0.006	*386*	0.40	*-9*
0.008	*392*	0.50	*-10*
0.010	*392*	0.60	*-10*
0.015	*365*	0.80	*-10*
0.020	*312*	1.00	*-10*
0.030	*192*	1.25	*-10*
0.040	*108*	1.50	*-10*
0.050	*61*	2.00	*-9*

Figure 9. Difference in Hounsfield Units between muscle and adipose tissue at different energies (Hubbell & Seltzer, 1996). MeV and ΔHU is listed in the table. The X-axis is on a logarithmic scale.

The mass attenuation coefficient (μ/ρ) of different fatty acids has been measured by Dual-Energy X-ray Absorptiometry (DEXA) in a review by Pietrobelli *et al.* (1996). Pietrobelli *et al.* (1996) have listed the μ/ρ for different saturated and unsaturated fatty acids at two different energy levels (40 and 70 kV). The author's do not state whether these fatty acids are in the

same physical state (liquid or solid), measured at the same temperature or whether they have measured the μ/ρ by DXA scanning or only calculated it. When individual saturated fatty acids are compared with individual unsaturated fatty acids it is only possible to compare fatty acids with the same number of carbon atoms and they have to be in the same physical state. For example, linoleic and linolenic acid both contain 18 carbon atoms and both are the unsaturated form whereas stearic acid also has 18 carbon atoms but is the saturated form. It is not possible to compare these three fatty acids with for example palmitic acid which contain only 16 carbon atoms. The physical state of a fatty acid is very important for the density, which is changing depending on whether it is gas, liquid or solid.

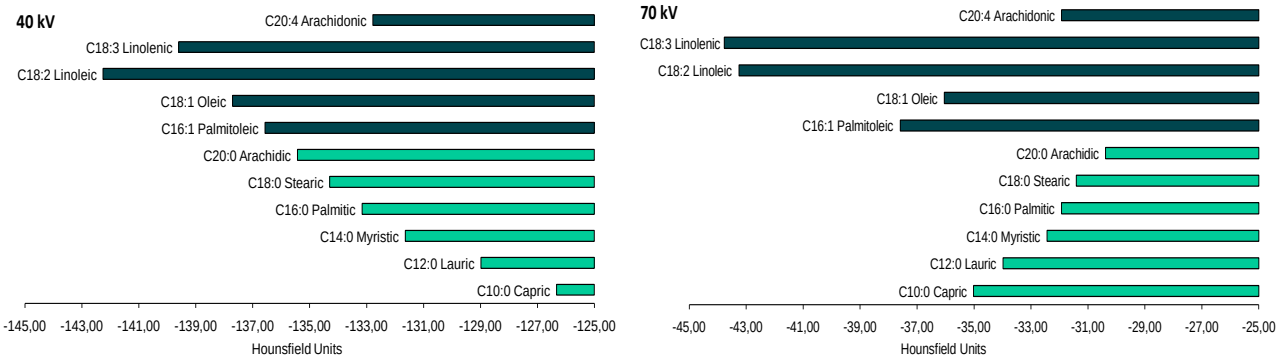


Figure 10. Hounsfield Units (HU) for different saturated and unsaturated fatty acids. HU is calculated using the mass attenuation coefficient for each fatty acid and water from Pietrobelli *et al.* (1996). Saturated fatty acids Unsaturated fatty acids.

In Figure 10, the μ/ρ for the fatty acids from Pietrobelli *et al.* (1996) has been recalculated into HU using Equation 1. At 40 kV the density of the saturated fatty acids decreases with an increasing number of carbon atoms. The opposite is seen at 70 kV where the density increases with increasing carbon atoms. At both energies linoleic (C18:2) and linolenic (C18:3) acid show the lowest density. The variation in the unsaturated fatty acids is most likely caused by the variation in the number of double bonds and perhaps *cis* and *trans* formations are also important. Therefore it is best to compare fatty acids with same number of carbon atoms as mentioned before.

In general, the unsaturated fatty acids have a lower density compared with the saturated fatty acids at both energies (Figure 10). If the individual C16 or C18 fatty acids are compared, the saturated fatty acids also here show a higher density than the unsaturated fatty acids. Comparing the unsaturated C20 arachidonic acid with the saturated C20 arachidic acid at 40 kV, the roles have changed. Now the saturated fatty acid has a lower density than the unsaturated fatty acid. At 70 kV C20 has the same pattern as C16 and C18 at both energies. There is also a difference in HU between the individual saturated and unsaturated fatty acids which is smaller at 40 kV than 70 kV. For example, the difference between stearic and linoleic acid in HU is 12 HU at 70 kV and 8 HU at 40 kV. For palmitic and palmitoleic acid the difference decreases from 6 to 3 HU.

Another source of information related to the density of fatty acids was found in a report made by the International Commission on Radiation Units and measurements (ICRU, 1992). The report does not, like Pietrobelli *et al.* (1996) list the mass attenuation coefficient of specific fatty acids but for a large number of human body tissues. The tissue closest related to the animal fat is the human adipose tissue. This means that the mass attenuation coefficient is a mixture of triacylglycerols, phospholipids, sterols and fat soluble vitamins, water and other compounds of the human adipose tissue. The mass attenuation coefficient is listed for human adipose tissue for different energy levels and ages. In Figure 11, the mass attenuation coefficient has been recalculated into Hounsfield using Equation 1. The HU varies between both age and energy level. It can be seen that the density or HU decreases with age. The biggest decrease is seen at an energy level of 60 kV going from -14 HU for a new born baby down to -38 HU for an adult (>18 years) resulting in a difference in HU of 24. By comparison the difference is 11, 6 and 0 HU for 80, 100 and 150 kV, respectively. Translating this into fatty acids would mean that the human adipose tissue will increase in unsaturated fatty acids with an increasing age and thereby be more saturated in early life. This can be confirmed by two studies performed on humans both finding an increasing level of unsaturated fatty acids with an increasing age (Insull *et al.*, 1967; Baker, 1969).

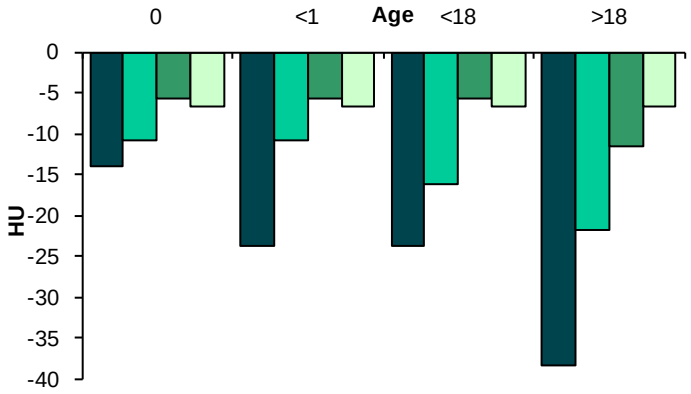


Figure 11. Hounsfield Units for adipose tissue in humans at different ages and at four different energy levels. HU is calculated using the mass attenuation coefficient from ICRU (1992). 0 = new born, <1 = infant 2-10 months, <18 child 1-18 years, >18 = adult. 60 kV 80 kV 100 kV 150 kV

2.4 Energy level

The literature clearly states that the energy level chosen for the scanning plays a large role in the output and thereby in the results. Therefore the energy level should be adjusted to the individual tissue(s) scanned and the kind of information which is expected to be obtained. The tissue scanned in this thesis is beef and pork and back fat from pigs.

In the back fat, it is the variation in fatty acid composition that is of interest. Here it is only necessary to use one energy. At energy levels above 100 kV no difference was seen between humans at different ages (Figure 11). At an energy level of 70 kV the information of interest was shown, matching the density theory best (Figure 10). Using an energy level of 40 kV a different density pattern was seen though the unsaturated fatty acids still had a lower density than the

saturated fatty acids. The medical CT scanner is not capable of scanning below 70 kV. Therefore an energy level in the range of 70 - 80 kV is ideal.

Energy levels for scanning meat tissue are different as for the fatty acids. In meat, two tissues are of interest the muscle and adipose tissue, mostly the former. The fat content of meat is usually very small (±2.5 percent) (Lawrie & Ledward, 2006) thereby making muscle tissue clearly the most dominant tissue of meat. It would therefore not be incorrect to interpret the meat as only one tissue type and as seen there was no difference in one tissue using only one energy level (Figure 8). If only one energy level is used the optimal energy level would be expected to be at 10 kV where ΔHU is the highest (Figure 9).

The medical CT scanner is not able to scan at energy levels below 70 kV and also, along with the micro CT scanner they are both not capable of scanning with two energies at the same time. For one energy scanning 70 kV would be the second most optimal choice to 10 kV and a solution to the two energy problems is to scan the tissue, in this case the meat or fat, twice but at two different energies. Scanning with two energy levels, energy number two should be higher than 10 kV but lower than 100 kV where the μ/ρ curve flattens out.

2.5 Interpretation of CT

Studies have helped documenting a difference in the density of fatty acids and the difference in the attenuation curves and ΔHU between tissues and energy levels. These studies bring us further to the interpretation of the CT scanning results. It is these variations that the CT scanning shall try and find, thereby showing that it might be capable of predicting meat and fat quality of pork and beef.

Changes in the density of adipose and muscle tissue is seen in the Hounsfield Units and in the histogram or spectra of the tissue(s) scanned (Gjerde, 1987; Nordal *et al.*, 1988; Rye, 1991; Goodpaster *et al.*, 1999; Goodpaster *et al.,* 2000; Soldevilla *et al.,* 2005; Larson-Meyer *et al.,* 2006). Two studies show that the spectra from CT scanned fish (Atlantic salmon and Rainbow trout) were different depending on the fat content (Gjerde, 1987; Rye, 1991). The dispersion of the spectrum in both studies increased with an increasing fat content. It should be mentioned that these spectra deviated from the one showed in Figure 5.B having only one distribution compared with two in the pig carcass. The dispersion was seen in the left side of the spectra also in the direction of fat on the HU scale. Soldevilla *et al.* (2005) reported that density (HU) of the forehead muscle in Cuvier beaked whales decreases with increasing lipid content. Going from fish and whales over to humans the same pattern is seen. Some diseases like at infiltration, atrophy and hypertrophy (breakdown or increase in an organ or tissue) or health conditions (obesity) can be studied using CT. If the fat content of the muscle tissue increases, a density reduction was observed (Nordal *et al.*, 1988). If the density showed to increase they would have expected fibrosis (development of connective tissue in organs and tissues) though this was not the case. A number of obese women during a weight loss have also been studies (Goodpaster *et al.*, 1999). The woman where CT scanned before and after their weight loss

and were compared with a control group, also women of normal weight. The study showed the same pattern as for the two fish studies. The dispersion of the spectrum increased with an increasing fat content and the dispersion was seen in the left side of the spectra (low HU). Larson-Meyer *et al.* (2006) investigated CT scanned normal and overweight men and women and found that changes by 1 HU represented a lipid concentration change of 1 g/100 ml. The same result was seen in a study by Goodpaster *et al.* (2000). They used lipid emulsion phantoms with different lipid emulsion content and saw that increasing the lipid content with 1 g/100 ml increased HU by one unit.

No studies have been made on collagen or connective tissue. It would be expected that an increased collagen content in meat or fat would affect the spectra in the opposite direction than an increased fat content, towards the right on the HU scale. This is caused by the density of collagen being higher than both adipose and muscle tissue as mentioned earlier. Whether the dispersion of the spectra will in- or decrease is difficult to say with the information available, therefore at first no expectations of this is set before results are obtained.

Another method to interpret the CT scanning could be made by image analysis. Maybe it is possible to analyse images by subtracting or dividing these and thereby extract the difference or information found in the theory, more of this in the method chapter.

3 Materials and Method

3.1 ProSafeBeef

The data set described in the following chapter is from a project called ProSafeBeef. ProSafeBeef is a five year project supported by the EU and includes 18 countries. The purpose of this project is to look at beef safety and quality through research and innovation. The project is divided into seven pillars, and this data set was generated in a project from pillar four. Pillar four is focusing on innovation in beef processing and products. The end result of this specific project was to produce a database containing spectroscopic information and CT images along with different muscle quality parameters, all measured on the same meat samples in order to compare reference data and spectroscopic data/CT data. Materials have been selected and chemical methods along with the CT scanning have been performed by Nofima Food Matforsk in the Norway in autumn of 2008.

3.1.1 Animals and muscles

10 bull carcasses of Norwegian Red cattle were randomly collected at a commercial slaughterhouse during 2 days. Age and weight were recorded to be 19 – 30 months (average 22 months) and 250 – 439 kg (average 333 kg). The carcasses were chilled conventionally for 48 hours at 4ºC. Subsequently, chuck and round from the right side were removed for further dissection of individual muscles. Three muscles were dissected from the chuck; *supraspinatus* (C2), *infraspinatus* (C3) and *triceps brachii* (C6), and six muscles were dissected from the round: *rectus femoris* (R2), *vastus lateralis* (R4), *biceps femoris* (R5), *semitendinosus* (R6), *semimembranosus* (R7) and *adductor* (R8). The caudal part of the *longissimus dorsi* (Cont) was collected as a reference muscle. 10 muscles from each carcass gave a total of 100 samples. All 10 muscles from each carcass were vacuum packed in polyethylene bags and aged at 4ºC until 9 days post mortem.

3.1.2 Warner-Bratzler and sensory

Each muscle was prepared by heat treatment for Warner-Bratzler and sensory measurements. 9 days post mortem the muscles were cut across the longitudinal direction of the muscle into 3.5 cm thick slices and were again vacuum packed in polyethylene bags. The vacuum packed slices were heated in a water bath at 70ºC for 50 minutes. The samples were chilled in running ice water for 45 minutes, and stored at -1.5ºC for as much as seven days. Before measurements were performed, samples were conditioned at ambient temperature for at least one hour and thereafter cuts were made with the fibre direction to make pieces of 1cm x 1cm x 3.5 cm from all muscles. Structures of visible fat and sinew were avoided.

Warner-Bratzler (WB) shear force measurements were performed on 10 pieces of each sample with the triangular Warner-Bratzler device of the Instron Materials Testing Machine (Model 4202, Instron Engineering Corporation, High Wycombe, UK). The samples were sheared once,

vertical to the muscle fibre direction. It is not stated whether they measured peak for initial yield or both. Therefore from now on the WB value from the ProSafeBeef set is called the WB (force unit (fu)).

The sensory analysis was performed by a trained panel with 10 assessors, using a flavour profile method (modified ISO 6564, 1985). Samples from each muscle were evaluated by all assessors. Sensory variables assessed were: hardness (first bite, across the fibre direction) and tenderness (whole chewing process).

3.1.3 Chemical measurements

Collagen content. A 3.5 cm thick slice of each muscle was homogenized to make samples for chemical analyses. The amount of hydroxyproline in the meat samples was determined according to ISO 13903, 2005. Total collagen concentration was calculated by multiplying the amount of hydroxyproline by 7.52, as described by (Stolowski *et al.*, 2006). The concentration of collagen is expressed as mg collagen per gram wet tissue.

Protein, fat content moisture and pH. Two 2.5 cm slices of each muscle were homogenized individually and divided into subsamples for further analyses. All analyses were performed in duplicates. The protein content of the samples was determined according to EU DIR 93/28, while the fat content was determined by EU DIR 98/64. Moisture was assessed by the NMKL 23 (1991) method. pH was measured at 48 hours post mortem before each muscle was packed in plastic bags.

3.1.4 Data

Results from WB, sensory and chemical analysis are shown in Table 1. The variation within each muscle group shows different variation between the meat quality parameters. For the parameters WB, collagen and fat the standard deviation are high compared to the mean values. For one muscle the standard deviation within the same muscle is 36.6 WB (fu) compared to a mean value of 86.9 WB (fu). In general muscles from the chuck are not significantly different from each other though few exceptions for the parameter tenderness and hardness are seen. When muscles from the round are compared also here they do not differ significantly from each other with the exception of tenderness and collagen. Comparing muscles from the round and chuck muscles from the round are significantly tenderer and harder in sensory score also pH are significantly smaller in the round whereas the protein content are significantly higher.

Table 1. Average WB shear force, sensory tenderness and hardness score, collagen, protein, moisture and fat content and pH for all ten muscles. [a,b,c,d,e] Means within a column not sharing a common superscript differ ($p<0.001$). * The two chuck and round mean value differs significantly from each other ($p<0.001$). *Supraspinatus* (C2), *infraspinatus* (C3), *triceps brachii* (C6), *rectus femoris* (R2), *vastus lateralis* (R4), *biceps femoris* (R5), *semitendinosus* (R6), *semimembranosus* (R7), *adductor* (R8) and *longissimus dorsi* (Control).

	Meat quality parameters															
	Hardness Score		**Tenderness** Score		**WB** Force unit		**Collagen** %		**Protein** %		**Moisture** %		**Fat** %		**pH**	
	Mean	std	Mean	std	Mean	std	Mean	std	Mean	std	Mean	std	Mean	std	Mean	std
Chuck																
C2	5.2[a,c,d]	0.6	4.5[a,c,d]	0.8	62.8[a,b,c]	11.7	10.9[a]	2.1	22.0[a,c]	1.0	75.6[a]	0.9	1.68	0.72	5.65[a]	0.1
C3	3.6[b]	0.5	6.5[b]	0.7	44.4[a]	8.9	11.9[a,c]	5.1	21.4[a]	1.1	73.9[a,c]	2.2	3.63	1.65	5.66[a]	0.1
C6	4.5[a,b,c,d]	0.8	5.6[a,b,c]	0.9	57.5[a,b]	8.8	8.1[a,c]	2.2	22.8[a,c,e]	1.3	74.5[a,c]	1.3	2.16	1.33	5.59[a,b]	0.1
Mean	4.4*	0.9	5.5*	1.2	54.9	12.4	10.3	3.7	22.1*	1.1	74.6	1.7	2.49	1.50	5.63*	0.1
Round																
R2	4.7[a,b,c,d]	0.8	5.2[a,b,c]	0.8	61.6[a,b]	13.3	8.7[a,c]	5.1	23.0[a,c,d]	1.1	74.0[a,c]	1.6	2.03	1.09	5.56[b]	0.0
R4	5.8[a,c,d]	0.8	3.9[a,c,d]	0.9	65.6[b,c]	14.1	7.6[a,c]	2.1	23.5[a,c,d]	1.6	74.2[a,c]	1.3	2.03	1.04	5.54[b]	0.0
R5	6.0[a,c,d]	1.0	3.8[a,c,d]	1.1	86.9[a,b]	36.6	12.7[a,d]	5.3	22.9[a,c,d]	1.3	73.6[b,c]	1.1	2.3	0.96	5.51[b]	0.0
R6	4.9[a,c,d]	0.5	5.1[a,c,d]	0.6	60.0[b,c]	7.6	9.1[a,d]	2.0	23.5[c,d]	0.9	74.3[a,c]	1.0	1.44	0.72	5.56[a,b]	0.0
R7	5.8[c]	0.8	3.9[c]	0.7	69.4[b,c]	8.4	5.9[b,c]	1.4	23.9[b,d,e]	0.8	73.6[b,c]	1.0	1.80	1.28	5.53[b]	0.1
R8	4.6[d]	0.4	5.3[b,d]	0.7	57.2[a,b]	12.6	5.6[b,c,d]	2.5	23.7[c,d]	1.4	73.0[b,c]	1.3	1.86	0.84	5.55[b]	0.0
Mean	5.3*	0.9	4.5*	1.0	66.9	20.1	8.2	4.1	23.4*	1.2	73.8	1.3	1.91	1.0	5.54*	0.0
Control	4.9[a,b,c,d]	1.4	5.0[a,b,c]	1.5	70.3[a,b]	19.9	5.9[b,c]	1.2	24.2[b,d]	0.6	72.9[b,c]	1.5	2.38	1.07	5.52[b]	0.0

3.1.5 Computerized Tomography

The scanner belonged to The Danish Meat Research Institute in Roskilde Denmark. The CT scanner used was a GE HiSpeed CT/I, 12 bit single slice scanner and the scanning protocol parameters were set up as follows; two energy levels were used 80 kV and 130 kV (106 mA) with a voxel size of 0.5 x 0.5 x 1 mm and 49 mm between slice centers. For these muscles a whole body scan was not performed due to a slice thickness of 1 mm and the distance between slice centers is 49 mm. For that reason, only three pictures/slices are recorded with a width of 1 mm. The three slices were recorded at position 0 mm, 50 mm and 100 mm.

3.1.6 Image analysis

Comparing the mass attenuation coefficient and ΔHU of muscle and adipose tissue llustrated that they differed between energies and within the tissues. The connection between this information and the meat quality parameters measured by sensory and chemical analysis using image analysis was tested. Images from the two energies were both subtracted and divided by each other, trying to extract the differences in the tissue discovered in the theory. As described in the paragraph concerning the CT method, three Dicom pictures were recorded at each energy level, at the same three positions (0, -50 and -100 mm). This resulted in two Dicom pictures from each position each coming from separate energies (Figure 12). These two Dicom pictures were then subtracted or divided from each other in the programme ImageJ as follows. Dicom picture 80 kV, 0 mm was subtracted/divided from 130 kV, 0 mm. Dicom picture 80 kV, -50 mm were subtracted/divided from 130 kV, -50 mm and 80 kV, -100 mm were subtracted/divided from 130 kV, -100 mm.

A B

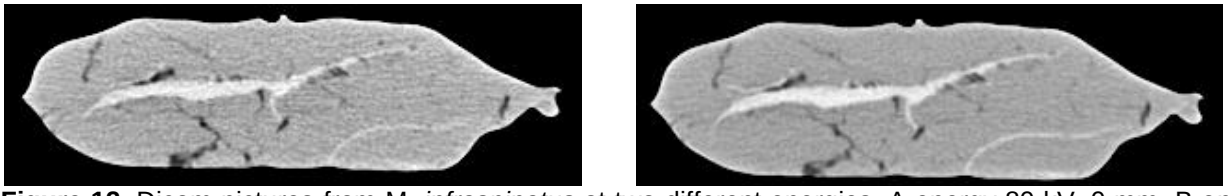


Figure 12. Dicom pictures from M. *infraspinatus* at two different energies. A energy 80 kV, 0 mm. B energy 130 kV, 0 mm. Same slice position and animal in both pictures.

3.2 Calibration study

The following data set is from a calibrating study performed by the Danish Meat Research Institute during a six week period which started in February 2008. Half carcasses were CT scanned followed by a total manual dissection. The study was supplemented with chemical measurements of protein, fat, water and collagen content. The aim of this study was to compare chemical measurements with the CT scanning. Collecting samples, chemical analysis and CT scanning were performed by the Danish Meat Research Institute.

3.2.1 Animals

27 pigs were chosen based on slaughter weight and fat depth between second and third thoracic vertebra so they would cover the heterogeneity of the Danish pig population. All the pigs chosen came from conventional herds. The pigs were slaughtered at a Danish commercial slaughterhouse. 24 hours post mortem one half of each carcass was CT scanned followed by a manual dissection. After dissection two muscles *longissimus dorsi* and *biceps femoris* was vacuum packed and frozen for further analysis of fat, moisture, protein and collagen content.

3.2.2 Chemical analysis

Before analysis, the two muscles *longissimus dorsi* and *biceps femoris* from each carcass was thawed and minced, and samples of 200 g were collected from each muscle. The protein content was analyzed by the Kjeltec 1035/1038 ANF 008. Moisture was assessed by the ANF 002 NMKL 23 (1974, 2rd Ed.) method. The amount of hydroxyproline (measure for collagen content) in the meat samples was determined according to ANF 015, Mod.e. AOAC official method 990.26 (31.1.27 of 1997) while the fat content was determined by ANF 004, Mod.e. NMKL 131 (1989). All methods were performed by the Danish Meat Research Institute (DMRI) in Roskilde and are accredited by ISO/IEC 17025 (DANAK test reg. No. 392).

3.2.3 Data

The protein, water, fat and collagen content of the two muscles are shown in Figure 13. *Biceps femoris* has a significantly lower protein level than *logissimus dorsi*. For the water content the opposite is seen as *Biceps femoris* has a significantly higher water content than *logissimus dorsi*. There is no difference in the fat and collagen content between the two muscles. The standard deviation for the different muscles in this data set is small compared to the mean values and also in comparison to the ProSafeBeef data set.

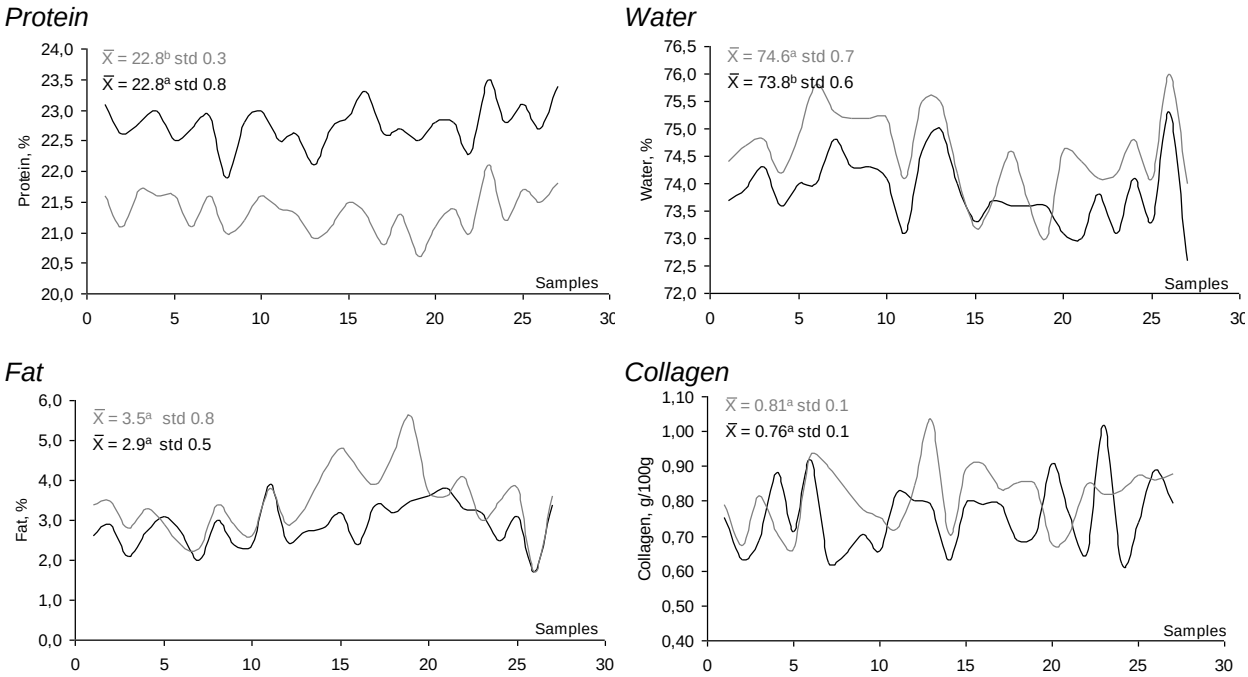


Figure 13. Protein, water, fat and collagen content for all 27 samples of *longissimus dorsi* and *biceps femoris*. *— Longissimus dorsi, — Biceps femoris.* Average values are stated for each muscle in each variable, ^{a,b} means within a variable, not sharing a common superscript differ ($p<0.001$).

3.2.4 Computerized Tomography

The CT scanner used was the same as for the ProSafeBeef data set and the scanning protocol parameters were set up as follows; One energy level was used 140 kV (80 mA) with a voxel size of 0.9 x 0.9 x 10 mm and 10 mm between slice centers. For these carcasses a whole body scan was performed due to a slice thickness of 10 mm and a distance between slice centers of 10 mm.

3.3 Back fat

The organisation Danish Pig Production (DSP) has performed an experiment where pigs were fed with different levels of maize. The aim of the experiment was to reassess the maximum limit for using maize in feed for growing-finishing pigs without lowering the fat quality (colour). At the same time the basis of data could be expanded so as prediction of the iodine value in fat would be possible. All work done until slaughter including collecting samples was performed by DSP. After slaughter, samples of back fat were taken from the loin to determine fatty acid content. In the present study, a piece of this back fat was cut off before analysis for fatty acid content and used for CT scanning. Analysing the raw data by multivariate data analysis ended up showing no positive results (details in the next chapter) therefore another method of analysis was tried, studying the spaciousness of the data set.

3.3.1 Animals and feed

Hundred and forty nine female growing-finishing pigs of mixed breed Danish landrace, Yorkshire and Duroc (LYD), having a starting weight of 30 kg were used for this experiment. Pigs were fed with five different levels of maize corresponding to five different diets with thirty pigs on each diet. The level of maize in the diets varied between 0 and 60 percent (Table 2). For each diet, the maize was grounded and mixed with the remaining ingredients, followed by pelleting. When the pigs reached a live weight of approximately 110 kg (mean 108.1 kg), they were slaughtered at a commercial Danish slaughter house, and carcasses were chilled for 24 hours at 5 °C, the average slaughter weight was recorded to be 82.5 kg. After chilling, 10 cm loin, taken between 3rd and 4th rib counting from last rib, was removed from each carcass (Figure 14). The samples were vacuum-packed and stored at minus 18 - 20 °C until further analysis.

Table 2. Description of experimental diet for each group. [a] FEsv = Danish feed unit for growing pigs. All diets are optimized to comply with nutrition norm for growing-finishing pigs (30-110 kg) (no safety margin).

	Diet treatment				
	I	**II**	**III**	**VI**	**V**
Number of pigs	30	30	30	29	30
Soybean meal, peeled	17,92	20,23	22,64	25,01	23,12
Barley	8	8	8	8	8
Wheat	68,74	46,40	24,06	1,7	3,7
Maize (grounded)	0	20	40	60	60
Fat (vegetable)	1,5	1,5	1,5	1,5	0
Molasses	1	1	1	1	2,5
FEsv[a]/kg	1,13	1,15	1,16	1,17	1,13
Digestible protein/FEsv	130	130	130	130	130
Crude fat/FEsv	3,23	3,5	3,77	4,03	2,85

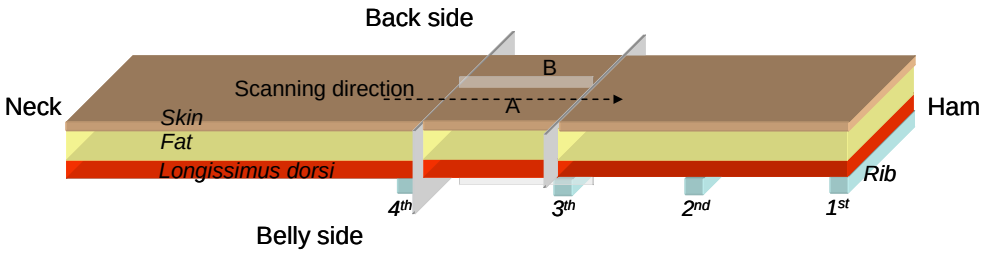


Figure 14. Collecting of back fat samples for fatty acid analysis and CT scanning. A and B are described below. A. Back fat sample for fatty acid analysis. B. Back fat sample for the CT scanning.

3.3.2 Fatty acid composition

The method (ANF 029) used for determination of fatty acid composition of fat and tallow is developed by Danish Meat Research Institute (DMRI), Maglegårdsvej 2, 4000 Roskilde, Denmark. Overall the fat is melted in the microwave, and interesterified into methylesters and analyzed by gas chromatography. All laboratory work for determination of fatty acid composition has been performed by the laboratory at the Danish meat research Institute (DMRI) in Roskilde.

Preparation of back fat samples

For determination of fatty acid composition and CT scanning, only back fat from the loins was needed. 24 hours before starting the fatty acid analysis, the frozen samples were placed in a refrigerator at 5 °C for thawing in order to separate back fat (with skin) from loin. Subsequently, the back fat was cut into two pieces, one was used for fatty acid analysis and the other half for the medical CT scanning (Figure 14 above). Five samples were prepared for the micro CT scanning. The samples were taken from the same samples used for the medical CT scanner using a circular knife (Ø 25mm).

Render of fat

Filter paper was folded and placed in a plastic container. Back fat samples without the skin were cut in smaller pieces and placed on the filter paper along with one teaspoon sodiumsulphate. Next the fat was melted in the microwave at 450 W for 3 min. and thereafter containers were placed in the refrigerator ready for further analysis.

Production of methylesters

0.15 g of melted fat was weighed out into a conical flask along with 3.5 ml sodiummethanolat and pumice. The mixture was boiled in a water bath (75 - 80 °C) with a reflux condenser for 5 - 10 min. until the fat was dissolved. 4 ml borontrifluoride-methanol-complex was added and the dissolution was boiled for additional 2 min. Then 6 ml heptan was added, and the dissolution was boiled for 1 min. The flask was now transferred to an ice bath. After cooling, the conical flask was filled with saturated salt water, and the dissolution was left for 10 min before the heptanphase was transferred to a vial.

Gas chromatography

Methylesters were analysed on a gas chromatograph with flame ionization detector (Hewlett Packard 6890, GC System). Program settings; Injection temperature 250 °C, detector temperature 300 °C, column program 120 °C for start temperature, increase of temperature 8 °C/min. until 180 °C, increase in temperature 5 °C/min. until 240 °C, following 240 °C for 10 min., flow 1.5 ml/min.

Calculating fatty acid composition

A standard sample containing all fatty acids of interest was run through the gas chromatograph, and chromatograms from the fat samples were inspected and compared with the standard chromatogram and fatty acid composition was now calculated in a preset Excel programme.

3.3.3 Data

The fatty acid composition of the back fat samples between the five diet treatments are listed in Table 3. Overall it is seen that an increasing level of maize in the diet has different effect on the fatty acids. Some stays unaffected others either increase or decrease. The amount of saturated fatty acids decreases along with an increasing amount of unsaturated fatty acids. Within the unsaturated fatty acids, the biggest change is seen for the polyunsaturated fatty acids, increasing from 14.41 to 19.68 percent. The standard deviation of the different fatty acids does not vary much compared to the mean values in any of the diet treatments.

Table 3. Fatty acid composition in back fat samples listed as an average of each diet treatment. [a,b,c] Means within a row not sharing a common superscript differ ($p<0.001$). Fatty acids are given as percentage of total fatty acids.

	Diet treatment									
	I		II		III		VI		V	
	Mean	std	Mean	std	Mean	std	Mean	std	Mean	std
C14:0 Myristic	1.17	0.09	1.19	0.10	1.16	0.08	1.13	0.08	1.15	0.09
C16:0 Palmitic	23.77[a]	0.77	23.64[a,b]	0.93	22.98[b,c]	0.80	22.26[c]	1.01	22.35[c]	0.94
C18:0 Stearic	13.20[a]	0.79	12.33[b]	1.02	11.54[b,c]	1.03	11.35[c]	0.98	11.61[b,c]	1.06
Total saturated FA	38.58[a]	1.25	37.62[a,b]	1.70	36.15[b,c]	1.63	35.20[c]	1.86	35.66[c]	1.87
C16:1 Palmitoleic	1.91[a]	0.26	1.94[a]	0.26	1.85[a]	0.25	1.64[b,c]	0.22	1.81[a,c]	0.20
C18:1 Oleic	40.66[a]	1.57	40.22[a]	1.20	40.01[a,b]	1.31	39.41[a,b]	1.16	38.83[b]	1.40
C18:2 linoleic	12.76[a]	1.88	14.22[a]	1.34	16.07[b]	1.71	18.02[c]	1.94	17.79[c]	1.81
C18:3 Linolenic	0.94	0.12	0.96	0.11	0.91	0.12	0.91	0.11	0.87	0.12
Total unsaturated FA	60.36[a]	1.26	61.35[a]	1.70	62.84[b]	1.59	63.85[b]	1.81	63.43[b]	1.82
Total monounsaturated FA	45.95[a]	1.74	45.41[a]	1.26	44.97[a,b]	1.50	43.94[b]	1.36	43.75[b]	1.56
Total polyunsaturated FA	14.41[a]	2.15	15.94[a]	1.54	17.87[b]	1.93	19.91[c]	2.13	19.68[c]	2.00

3.3.4 Computerized Tomography

For the back fat samples both the medical CT scanner and a micro CT scanner were used. The medical CT scanner was the same as for the two other studies GE HiSpeed CT/I single slice scanner and the scanning protocol parameters were set up as follows; 80 kV – 130 mA, 0.9 x 0.9 x 5 mm voxel size and 5 mm between slice centers. Surface and core temperatures of fat samples were measured before and after scanning. The instruments used were; for core temperature a Testo 926 (Testo AG, Lenzkirch, Germany) and for surface temperature a Ebro TFI100 (Ebro, Ingolstadt, Germany). Back fat was taken directly from the refrigerator (5.0 °C) and the starting temperatures were recorded as being; surface 4.2 - 6.6 °C and core 5.4 - 8.0 °C, the end temperature; surface 8.0 - 10.6 °C and core 9.2 - 11.6 °C. The back fat samples were placed in the medical scanner as shown in Figure 15.

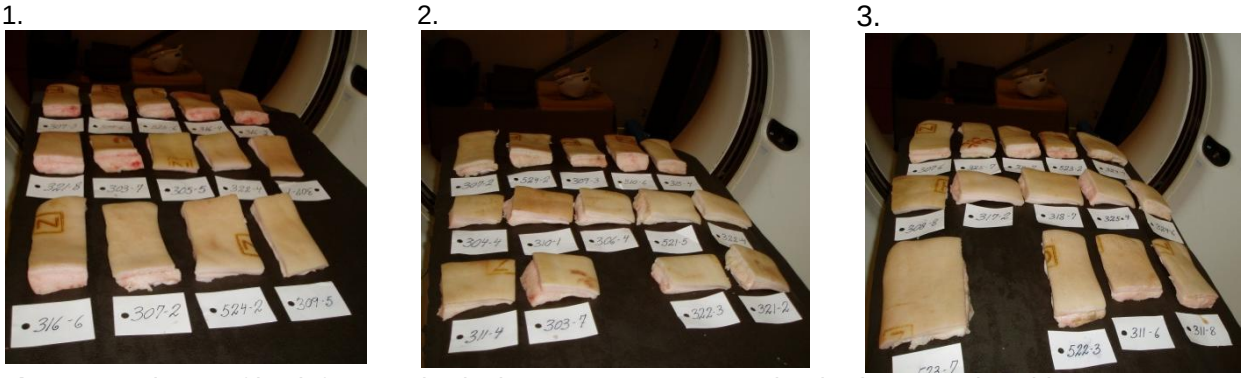


Figure 15. Picture of back fat samples in the CT scanner. ID number is shown on the white tags. 1. Bottom layer. 2. Middle layer. 3. Top layer.

The micro CT scanner is a Zeiss, Metrotom 1500, 32 bit. This scanner deviates from the medical scanner in being able to use lower energy levels. Due to this option it was decided on the scanning day that the back fat samples should be scanned at two energy levels. 80 kV corresponding to the energy level used in the medical scanner and 40 kV corresponding to the article of Pietrobelli *et al.* (1996) that used 40 (and 70 kV). The voxel size was respectively 45 x 55 x 50 µm (45 kV) and 20 x 55 x 50 µm (80 kV). Each scan took 20 minutes therefore only five samples were scanned. Each sample was scanned twice, one at each energy level. The five samples were sorted out in the following way. Spectra from all back fat samples were sorted according to their spectra shape and coloured in order to see the spaciousness of the samples (see below for spaciousness). The five samples were collected to represent the different spectra shapes (not spectra containing $>$ 2 peaks) and colour pattern. As mentioned the micro CT scanner had 32 bit resulted in 2^{32} different gray tone values compared to 4096 values in the medical scanner. The medical CT scanner was compared with water whereas the micro CT scanner was not calibrated at all. When the back fat samples were scanned they were placed on Styrofoam. Styrofoam is very close to air on the spectra of a CT scanning therefore the peak arised from this material was defined as air (-1000). The 2^{32} different gray tone values were

then projected down to only 1300 values in all, having -1000 as air and 0 as water, as so the scale range from -1000 to 300.

3.3.5 Spaciousness

The raw spectra from the CT scanning have shown to vary more than first expected. This variation was seen in the number of peaks. It was therefore decided to look at the special distribution of the spectra. Each peak is interpreted as a separate distribution (distributions are not calculated) as shown in (Figure 16).

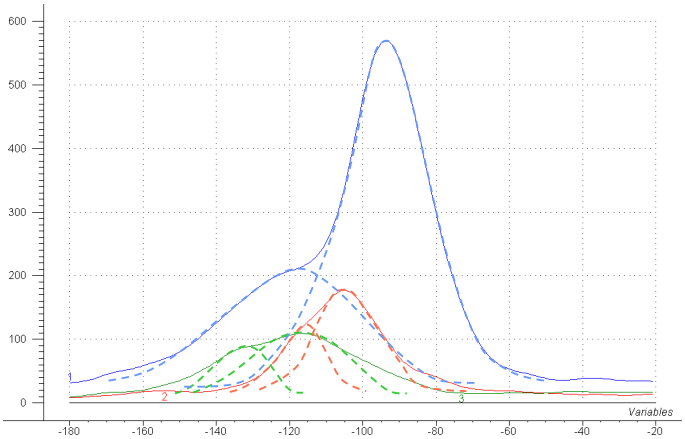


Figure 16. Three spectra containing two peaks from three different back fat samples each spectrum has been added two drawn distributions. The distributions are not calculated and only an outline on how it might look like if the spectra consist of more than one distribution.

The spectra were coloured according to the number of peaks/distributions in order to see what the colour pattern would look like in the back fat samples. Spectra containing one peak have been divided into two, at the top point (peak point) by a vertical line and each side of this line was assigned a colour (green and red) (Figure 17). Dividing of spectra containing two peaks was done by drawing a line where the two distributions meet each other, and each distribution was given a colour (green and red) (Figure 17). The same was applied for spectra containing three peaks though there were three colours according to three distributions (green, blue and reed) (Figure 17).

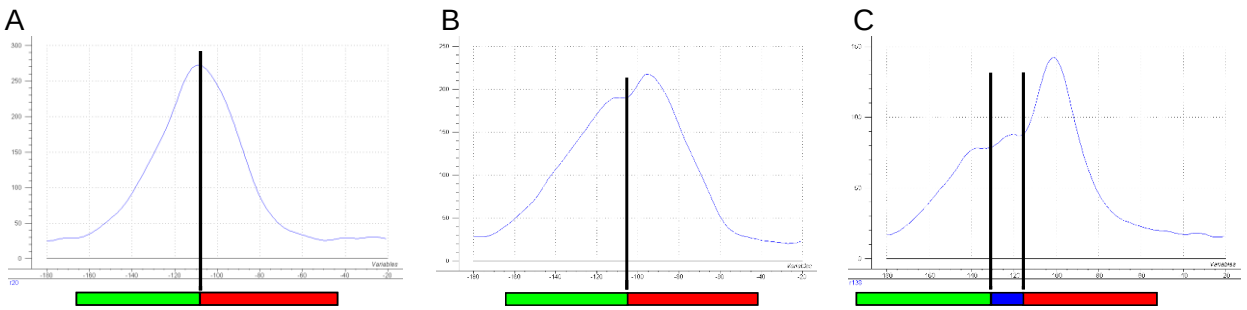


Figure 17. Colouring of spectrum according to number of distributions. A. One distribution B. Two distributions. C. Three distributions.

3.4 Statistics

3.4.1 Spectral analysis

In the theory it hypothesized that the interpretation of the CT result could be done in the spectra. Therefore a spectral analysis was chosen for the ProSafeBeef and Calibration study data sets. As mentioned above, the raw spectra from the back fat samples showed to vary more than first expected and it was found that spectral analysis was not suitable for this data set. The spectra of ProSafeBeef and Calibration had an asymmetrical distribution which can be termed as a Poisson type distribution. The spectral analysis is divided into two. A statistical analysis of the raw spectra and an asymmetric statistical analysis. An asymmetrical distribution can interpret the possibilities for required information in analysis to lie in the top point/peak or towards right or left in the spectrum depending on the material dominating. Six points on the spectra was chosen for the asymmetric statistical analysis (Figure 18) with special reference to the theory of the spectrum moving towards left or right on the Hounsfield scale depending on the composition of the muscle/back fat scanned. The six asymmetrical points chosen are illustrated as the peak point of the spectra (t) both X and Y values and the points 50 and 95 percent projection of the peak point (only Y value). The projection values 50 and 95 percent was randomly chosen as it is possible for the essential information to be position in other projection points of the spectrum.

Samples from the ProSafeBeef project was scanned twice at two different energies therefore 12 asymmetrical points were chosen. Instead of one spectrum in Figure 18, there would be two spectra, end to end, each illustrating the same sample but at two different energy levels.

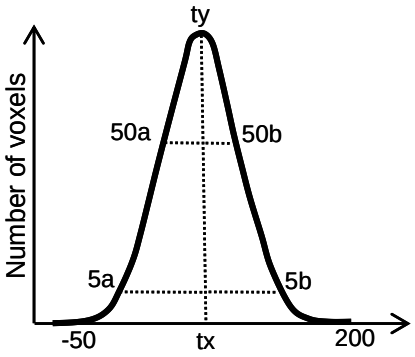


Figure 18. Selected asymmetrical points on a Hounsfield spectrum illustrating one muscle scanned at one energy level.

Before a spectral analysis can be performed on the Calibration data set, the spectra have to match the reference data, meaning that raw spectra cover the whole half carcass whereas the reference data are derived from only the two muscles *longissimus dorsi* and *biceps femoris*. Therefore we are only interested in the spectra from these two muscles. It was not possible to completely isolate the two muscles and extract the spectra, instead areas in the muscles have been selected and an average spectrum was calculated for each muscle.

In the method of image analysis the Dicom pictures were subtracted from each other, the same principle is tried for the raw spectra. In the ProSafeBeef data set and the back fat samples scanned with the micro CT scanner there are two spectra for each sample corresponding to each energy level. The low energy spectra (80 kV) are subtracted from the high energy spectra (130 kV). This is done subtracting the values at the same HU.

3.4.2 Multivariate data analysis

For analysing the raw data, spectra and reference data, multivariate data analysis was chosen, and the program The UnscramblerV92 (version 6.5.20.0) was used. All spectra were normalized by area to eliminate the variation in sample size that otherwise would affect the model. The spectra in all three data sets showed slight fluctuating behaviour which was removed with the function “moving average smoothing” (window size 9). First a PLS2 (Partial Least Square analysis) model was calculated including all samples and reference data, in order to find correlations between *y* variables and to see whether some *y* variables were more important for the model than others (correlation coefficient). The usefulness of the models were evaluated by the correlation coefficients between the models and the *y* variable and which parts of the spectra or asymmetrical points that correlated best with each individual *y* variable. The PLS1 model was calculated in order to see if the model could be improved (higher correlations coefficients). For both PLS1 and PLS2 raw spectra, asymmetrical points or subtracted spectra were used as *x* variable, and the reference data was used as *y* variable. A full cross validation with an uncertainty test (test if a variable contributes significantly to the model) was used. Cross correlation coefficients and correlations under 0.40 were considered as poor, though this is not necessarily a criteria for ruling out this or these variables from the PLS1 models. Correlations between the *y* variables were also calculated and outliers were checked by performing a PCA (Principal Component Analysis) data compression.

3.5 Outline of all three data set

The table below lists all three data sets and provides a quick overview of the CT settings, reference data and the method used for each data set.

Table 4. All three data set.

		ProSafeBeef	Calibration study	Back fat
Species		Beef	Pork	Pork
Sample type		Whole muscles	Half carcasses	Back fat
Number of samples		100	27	149
CT settings	Energy level, kV	80 and 130	140	80
	Voxel size, mm	0.5x0.5x1	0.9x0.9x10	0.9x0.9x5
	Space between slices, mm	49	10	5
	Full body scan		x	x
Micro CT	Number of samples			5
	Energy level, kV			40 and 80
	Voxel size, µm			45x55 and 20x55
Reference data		Protein, collagen, moisture, fat, pH, WB, hardness, tenderness	Protein, collagen, moisture, fat	Fatty acids
Method	Image analysis	x		
	Spectral analysis	x	x	
	Spaciousness			x
	Multivariate data analysis	x	x	x

4 Results

4.1 ProSafeBeef

4.1.1 Computerized tomography

All 100 muscles were scanned twice at two different energy levels (80 and 130 kV) which resulted in 200 spectra. The two spectra from each muscle sample have been put together end to end and are plotted in Figure 19. The 80 kV spectra are located to the left and 130 kV spectra to the right. The spectra differ significantly ($p<0.001$) between the two energy levels. No significant difference between muscles was found. Spectra from the muscles scanned at 80 kV have a lower peak point and the spectra are wider than at 130 kV. Looking at each energy level separately, the spectra also deviate from each other both in width and height.

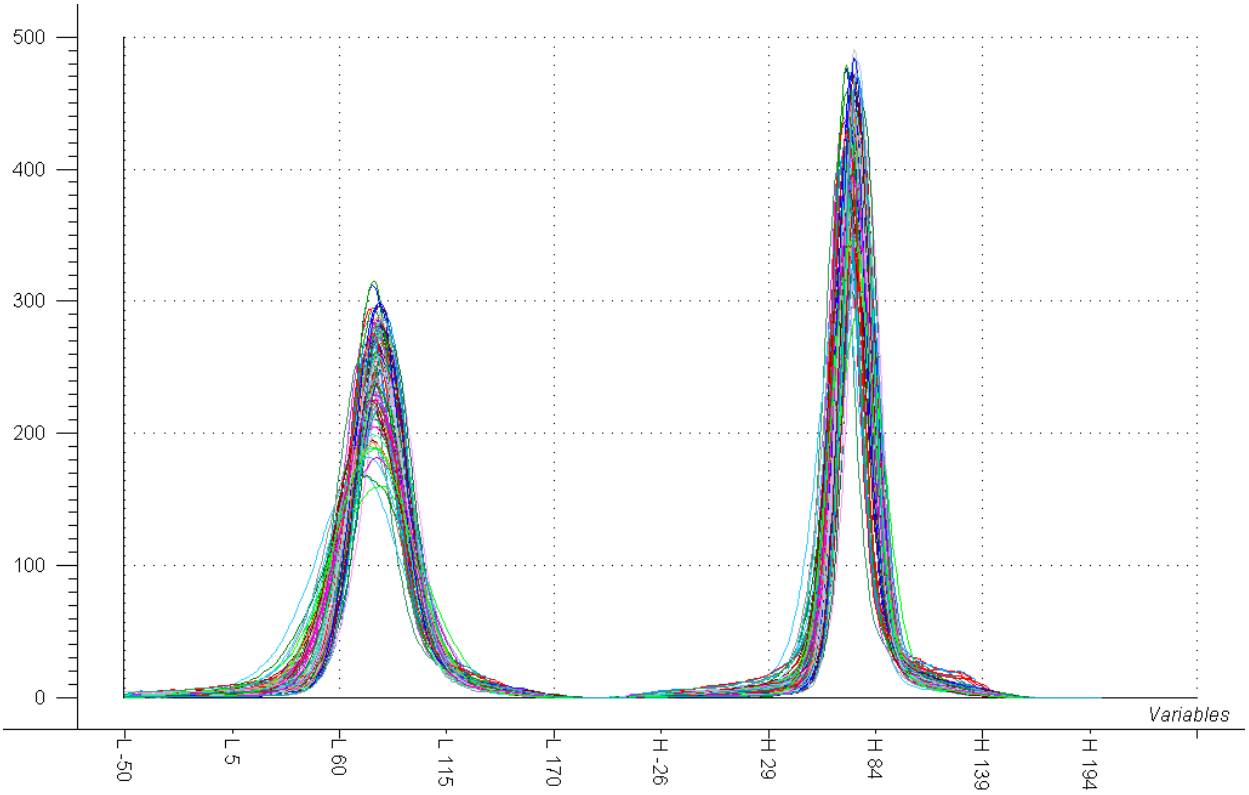


Figure 19. Spectra from all 100 samples at two different energy levels. The first spectra represent 80 kV (L = low energy) and the second represent 130 kV (H = high energy). X-axis represents Hounsfield Units, both spectra start at -50 HU and end at 200 HU. Spectra are smoothed and normalized by area. There is no significant difference between the spectra of the muscle groups. Spectra between the high and low energy level differ significantly ($p<0.001$).

4.1.2 Image analysis

This method was very time consuming therefore at first only 20 samples were picked out for image analysis. The idea was to see if the method showed useful results before all 100 samples were analysed. For each of the teen muscle groups, two samples were classified to be either least or most tender. The least tender sample was classified as having the highest WB force unit and hardness along with the lowest tenderness score. The tenderest sample was classified using the opposite criteria, low WB force unit and hardness along with high tender-

ness score. After classifying two samples from each muscle group the Dicom pictures were subtracted as described in the method. Dicom picture 80 kV, 0 mm (slice position) was subtracted/divided from 130 kV, 0 mm and so forth resulting in six pictures for each muscle (three from the sample most tender and three from the least tender).

In the figure below, two Dicom pictures (80 kV and 130 kV) from one meat sample of the muscle *infraspinatus* is shown. As can be seen from the Dicom pictures in Figure 12, scanning the meat samples with 130 kV results in a sharper picture along with a clearer separation between connective tissue (Red arrow), fat (Blue arrow) and meat compared with 80 kV. This difference might be the same as seen for the spectra that also varied between two energies. An example of the subtracted and divided pictures is illustrated in Figure 12.C and D. The pictures were noisy and not useful for further analysis.

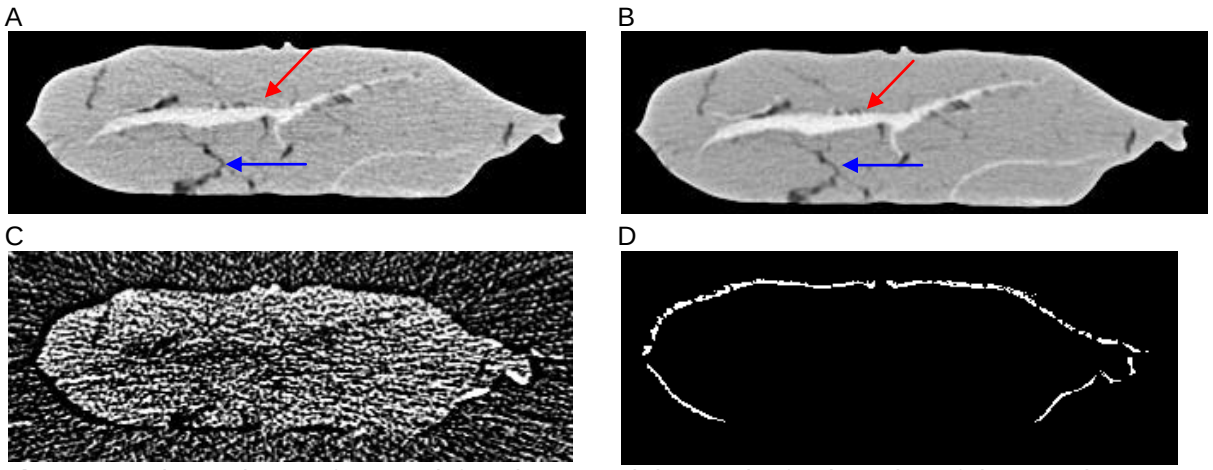


Figure 20. Dicom pictures from M. *infraspinatus* and the result of subtraction of the two pictures. A. M. *infraspinatus,* 80 kV. B. M. *infraspinatus* 130 kV. C. Result of subtracting picture A and B. D. Result of dividing picture A and B. Red arrow pointing at fat, blue arrow pointing at connective tissue. Same slice position and animal in both pictures.

4.1.3 Statistics

First correlations between *y* and *x* variable are studied to get an idea of how the spectra or asymmetrical points are affected by the different *y* variables. Usually these correlations are calculated. For the raw and subtracted spectra this would be very confusing to look at. This calculation would result in correlations between the eight *y* variables and 502 *x* variables (two spectra) a total of 502 x 8 correlations. Instead the x- and y-loading plot (xy plot) of both PLS1 and PLS2 models calculated on the raw and subtracted spectra are studied close. Figure 21 shows a xy plot for the raw spectra (PLS2).

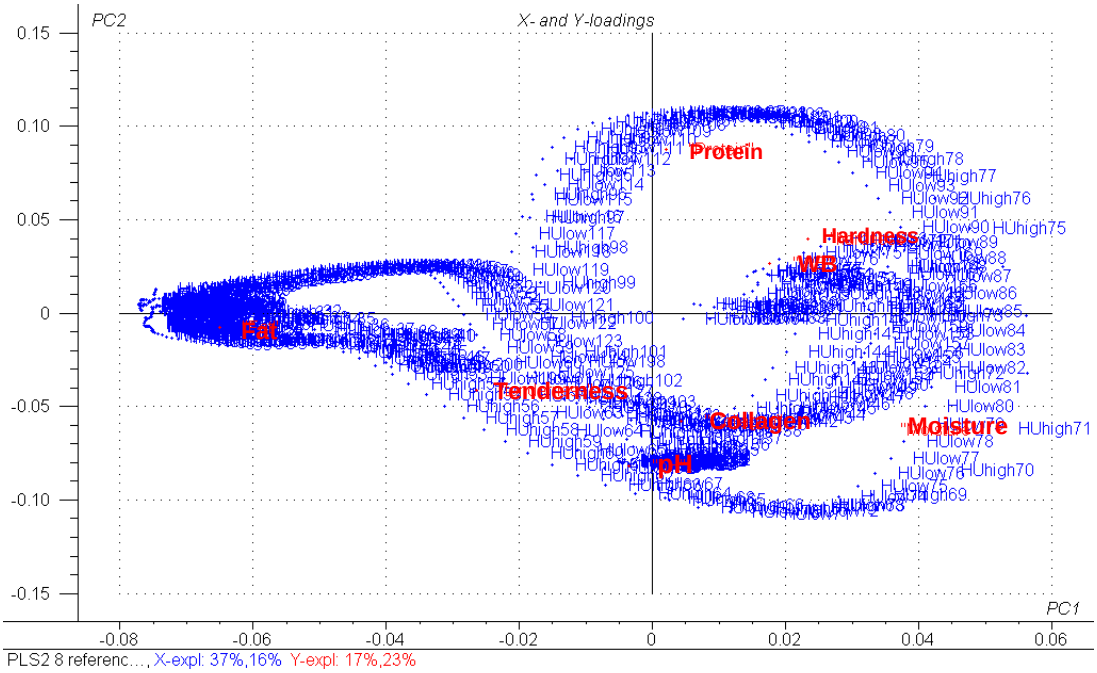


Figure 21. X- and y-loading of PLS2 model calculated on the raw spectra. As the *y* variables can be difficult to see they are highlighted (red).

Instead of showing all the xy plots, the different parts of the spectra that correlate with each *y* variable are listed in Table 5. Instead of listing a lot of HU values (pieces of spectra) that correlate with each *y* variable the spectra have been divided into five parts (Figure 22). The first part L represents the lower left side of the spectra (-50 – 30 HU) and R the higher right side of the spectra (126 – 200 HU). LS and RS represent the shoulder of each side of the spectra (31 – 74 and 86 – 125 HU). P represents the area around the peak (75 – 85 HU).

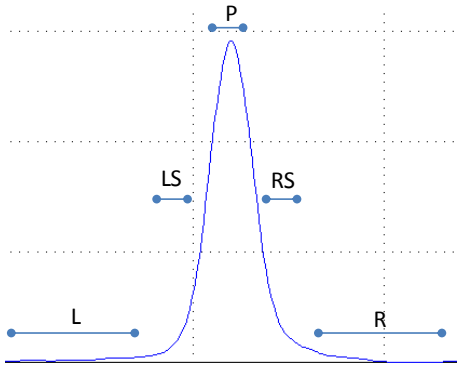


Figure 22. Interpretation of correlations between spectra and *y* variables. Each letters represents a spectra pieces as follows, L = -50 – 30 HU, LS = 31 – 74 HU, P = 75 – 85 HU, RS = 86 – 125 HU and R = 126 – 200 HU.

The variables protein, collagen, fat, pH and WB are correlated to both spectra (80 and 130 kV), using both PLS1 and PLS2 (Table 5). Moisture is correlated to both spectra in the PLS1 model and the spectrum of 130 kV in the PLS2 model. Hardness does not correlate to any of the spectra even though both PLS1 and PLS2 models are calculated. In general, fat and moisture correlates to the lower part of the spectra. Collagen and protein correlates to the right and high

end of the spectra. Tenderness correlates mainly to the lower end of the spectra and WB correlates to the high end of the spectra.

Table 5. *y* variable correlations to the different parts of the raw spectra. L = -50 – 30 HU, LS = -31 – 74 HU, P = 75 – 85 HU, RS = 86 – 125 HU and R = 125 – 200 HU.

		Protein	Collagen	Fat	pH	WB	Moisture	Tenderness	Hardness
PLS2	80 kV 130 kV	RS	R	L	LS	R	- LS	-	-
PLS1	80 kV	LS	R	L	LS	R	L	L	-
		RS	RS	RS	P			P	
		R	R		R				
	130 kV	LS	LS	L	P	H	L	L	-
		P	P	LS	RS			LS	
		RS	RS		H			P	

The interpretation of the xy plot for the subtracted spectra is seen in Table 6. Here the correlations between the *y* and *x* variables behave differently than for the raw spectra. Hardness, tenderness, pH, collagen and protein correlations are spread out on the whole spectra low to high. For the raw spectra these variables were correlated more specific to only one end and for some also the middle. Fat and moisture still correlate to the lower part. WB has changes from correlating with the high end of the spectra to the lower end.

Table 6. *y* variables correlations to the different parts of the subtracted spectra. L = lower left side. LS = left shoulder. P = Peak area. RS = right shoulder. R = higher right side.

	Protein	Collagen	Fat	pH	WB	Moisture	Tenderness	Hardness
PLS2	P	LS	RS	LS	L	L	RS	P
PLS1	L	LS	L	L	L	L	L	L
	LS	P		LS	LS		LS	LS
	P	RS		P			P	P
	RS	R		RS			RS	RS
	R			R				

For the asymmetrical points, correlations are calculated instead of studying the xy plot. Here there are only 12 *x* variables, six for each energy level, which are more manageable to look at. In Figure 23 - Figure 26 it is possible to see which *y* variables correlate to which asymmetrical points. It is illustrated with the gray broken lines how the correlations would be expected to influence the spectra. The six asymmetrical points are tx, ty, 5a and b, 50a and b and is illustrated in Figure 18 in the method.

Collagen, hardness and pH are only correlated to the asymmetrical points of the spectrum at 130 kV (Figure 23). For collagen, the tx point moves towards the left on the HU scale and the 95 percent projection value (5b) to the right (the spectrum gets wider in this point) when the collagen content increases. The 5b correlation to collagen is below 0.40. Hardness correlates positively to the right side of the spectrum mostly 5b resulting in the spectrum getting wider the harder the sample. The 50 percent projection (50b) also correlates positively to hardness but is below 0.40. The spectrum of pH moves towards the left on the HU scale (negative correlation to tx) with increasing pH values and at the 5b point the spectra get wider (positive correlation).

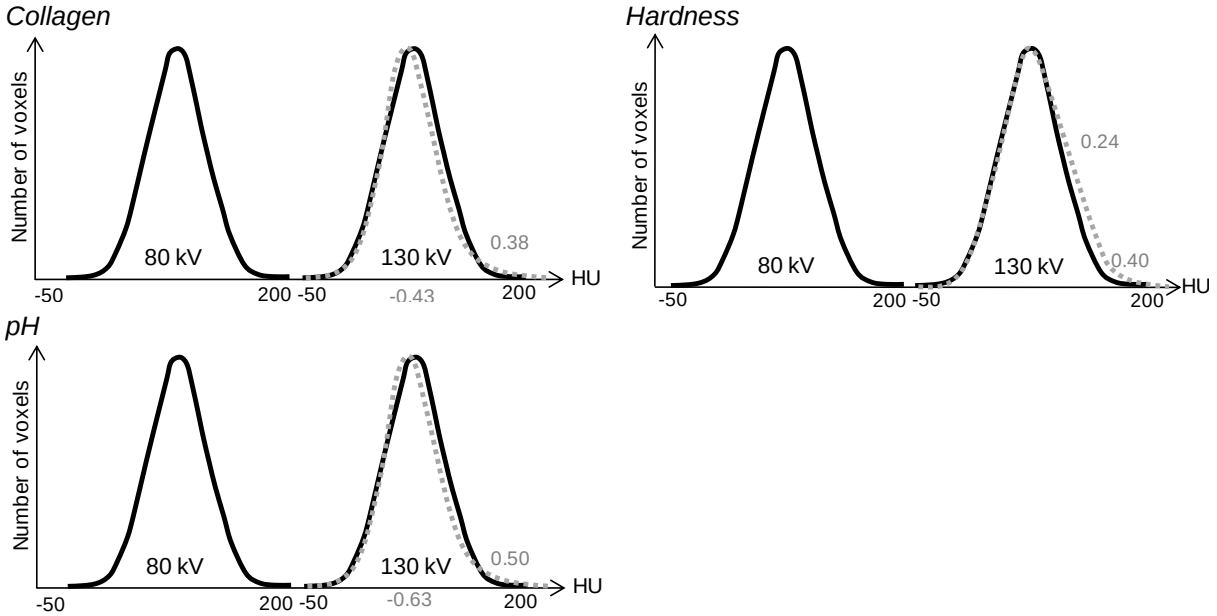


Figure 23. Illustration of correlations between asymmetrical points and collagen, hardness and pH. Gray numbers = correlations at the corresponding asymmetrical points tx, ty, 50a + b, 5a + b se Figure 18 in method. Gray broken line illustrates how correlations would be expected to influence the spectra at these points. Correlations lower than 0.40 are not illustrated though exceptions are made. As a minimum, two correlations are listed for each variable even though they might be lower than 0.40.

Tenderness and WB correlate to the 95 percent projection (5b) on both spectra thereby the spectra get more narrow in this area when WB force unit increases (negative correlation) and tenderness (positive correlated) decreases (Figure 24). Both correlations between the spectra and the 5b point are below 0.40. For tenderness the spectra of 130 kV is more important than the 80 kV spectra due to a higher correlation.

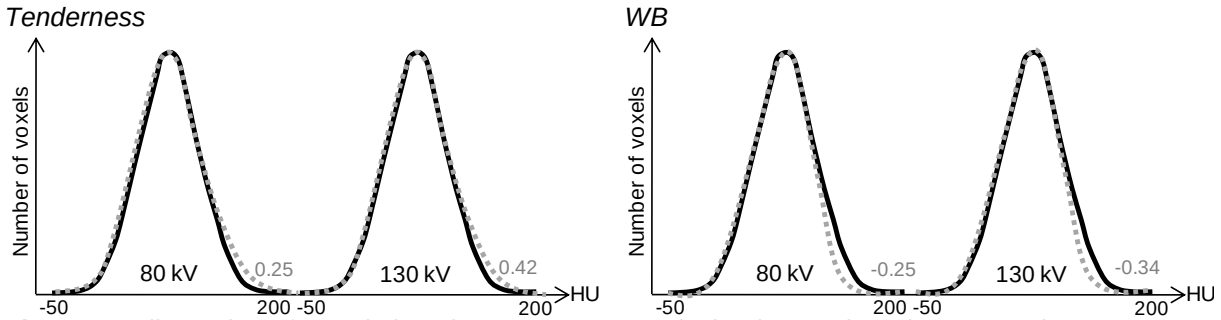


Figure 24. Illustration of correlations between asymmetrical points and tenderness and WB. Gray numbers = correlations at the corresponding asymmetrical points tx, ty, 50a + b, 5a + b se Figure 18 in method. Gray broken line illustrates how correlations would be expected to influence the spectra at these points. Correlations lower than 0.40 are not illustrated though exceptions are made. As a minimum, two correlations are listed for each variable even though they might be lower than 0.40.

When the fat content increases the lower left part of both spectra gets wider (positive correlation to 5a) (Figure 25). ty of the 130 kV spectrum decreases with increasing fat content due to a negative correlation. Moisture is the only variable affected by both ty and tx (Figure 25). When the moisture content increases, the spectrum at 130 kV moves towards the left at the HU scale and at the same time the ty value increases. Also the ty of spectrum at 80 kV increases. 5a of both spectra gets narrower with increasing moisture content.

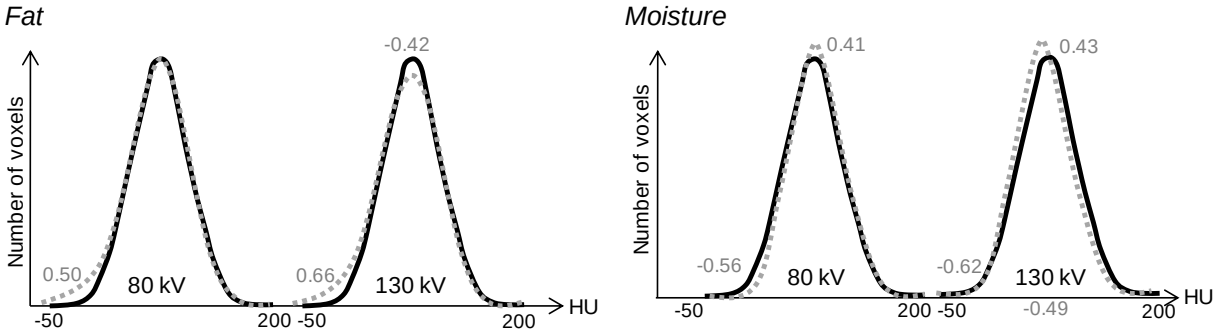


Figure 25. Illustration of correlations between asymmetrical points and fat and moisture. Gray numbers = correlations at the corresponding asymmetrical points tx, ty, 50a + b, 5a + b se Figure 18 in method. Gray broken line illustrates how correlations would be expected to influence the spectra at these points. Correlations lower than 0.40 are not illustrated though exceptions are made. As a minimum, two correlations are listed for each variable even though they might be lower than 0.40.

The last variable protein correlates positively to the tx point of both spectra (Figure 26). This means that the spectra move towards the right on the HU scale when the protein content increases. Furthermore, the 5b point of the 130 kV spectrum gets more narrow when the protein content increases due to a negative correlation.

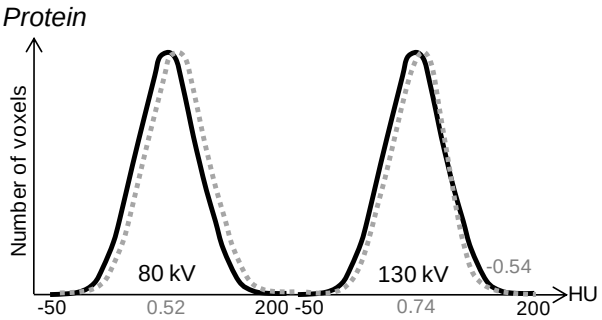


Figure 26. Illustration of correlations between asymmetrical points and protein. Gray numbers = correlations at the corresponding asymmetrical points tx, ty, 50a + b, 5a + b se Figure 18 in method. Gray broken line illustrates how correlations would be expected to influence the spectra at these points. Correlations lower than 0.40 are not illustrated though exceptions are made. As a minimum, two correlations are listed for each variable even though they might be lower than 0.40.

Correlations between the y variables have also been calculated and results are seen in Table 7. The correlation between tenderness and hardness is negative and is the largest of all correlations. This means that an increasing tenderness will result in a decrease in hardness. Protein is negatively correlated with pH, and moisture and protein also have a negative correlation below 0.40 with collagen. Therefore, when the protein content increases pH, moisture and collagen decreases. The correlation between fat and moisture is also negative resulting in the same pattern, if fat increases the moisture content decreases. WB correlates positively to hardness and negatively to tenderness as WB force unit increases so does hardness whereas tenderness decreases. Low correlations are seen for collagen which has a low positive correlation with WB and moisture, the same is seen between moisture and pH. Fat has a low positive correlation with tenderness and hardness and a low positive correlation to WB and moisture.

Table 7. Correlations between the *y* variables. Correlations lower than 0.40 are not listed, though exceptions are made. As a minimum two correlations are listed for each variable even though they might be lower than 0.40.

	Protein	**Collagen**	**Fat**	**pH**	**WB**	**Moisture**	**Tenderness**	**Hardness**
Protein	-	-0.39	-	-0.48	-	-0.48	-	-
Collagen		-	-	-	0.28	0.29	-	-
Fat			-	-	-0.29	-0.66	0.35	0.32
pH				-	-	0.38	-	-
WB					-	-	-0.59	0.62
Moisture						-	-	-
Tenderness							-	-0.97
Hardness								-

4.1.4 Correlation coefficient

The correlation coefficient (R) is a measure of how well the calculated models predict the eight *y* variables and are found in the predicted vs. measured plots. These plots are not shown, instead the correlation coefficient is listed for both PLS1 and PLS 2 models in Table 8. For all three PLS2 models hardness, tenderness, WB and collagen are below 0.50 except for collagen and tenderness in the model calculated on subtracted spectra. The remaining four variables

protein, moisture, fat and pH all lies above 0.50 ranging from 0.58 to 0.85. Moisture is the variable with the best correlation coefficient whereas WB has the lowest correlation coefficient. When PLS1 models are calculated most of the correlation coefficients improve (Table 8). Hardness is not improved by any of the PLS1 models. Tenderness is only improved when the raw spectra are used as *x* variable but the raw spectra does not improve the correlation coefficient of WB force unit.

Table 8. Correlation coefficient (R) of all eight *y* variables in PLS2 and PLS1 models calculated on the raw spectra, asymmetrical points and subtracted spectra. [a] If the muscle *infraspinatus* is ruled out from the PLS1 model, the correlation decreases to 0.05, se Figure 27. Bold script = highest correlation coefficient for each *y* variable.

	Hardness	Tenderness	WB	Collagen	Protein	Moisture	Fat	pH
Raw spectra								
PLS2	0.41	0.44	0.33	0.42	0.72	0.85	0.81	0.63
PLS1	0.46 (0.05)[a]	0.51	0.29	0.48	0.80	0.88	**0.83**	0.70
Asymmetrical points								
PLS2	0.44	0.47	0.34	0.41	0.75	0.85	0.74	0.58
PLS1	0.39	0.42	**0.38**	0.43	**0.81**	**0.90**	0.75	**0.72**
Subtracted spectra								
PLS2	**0.49**	**0.54**	0.27	0.42	0.73	0.84	0.77	0.66
PLS1	0.48	0.53	0.32	**0.54**	0.75	0.84	0.81	0.70

Taking a closer look at the score plot from the PLS1 model calculated on the raw spectra and hardness the muscle *infraspinatus* (C3) seems to group together which could indicate that the whole model is explained by this one muscle. If the muscle is removed from the model, the correlation drops to 0.05 (Table 8) confirming that *infraspinatus* (C3) did explain the whole model and that the model is useless without this muscle.

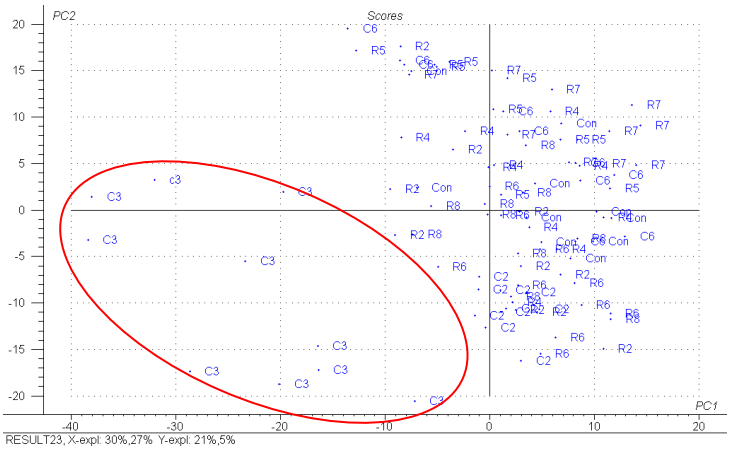


Figure 27. Score-plot of PLS1 model calculated on hardness with the spectra as x-variable. Red circle encircles the muscle *infraspinatus* (C3).

The PLS model calculated on the *x* variable subtracted spectra is the best predictor of hardness and tenderness having the highest correlation coefficients. Calculating individual PLS1

models on WB, protein, moisture and pH with the asymmetrical points as the *x* variable results in the best correlation coefficient (prediction) compared to the other models. Prediction of the fat content is best using the raw spectra as the *x* variable in a PLS1 model. The last variable collagen is best predicted by the subtracted spectra also in a PLS1 model.

4.2 Calibration study

4.2.1 Computerized Tomography

The CT scanning resulted in average spectra from whole carcasses, though in this study we are only interested in *longissimus dorsi* and *biceps femoris*. The spectra from these two muscles have been extracted and can be seen in Figure 28. Both muscles have resulted in spectra with two peaks, one corresponding to fat (below 50) and the other to meat (above 50). No significant difference in the spectra between the two muscles was found though when comparing all the spectra they differed significantly ($p<0.001$) from each other. The spectra from *longissimus dorsi* have a higher fat peak than *biceps femoris*. The second peak (meat) is lower than for *biceps femoris*. For *longissimus dorsi* the second distribution (meat) looks a bit wider than for *biceps femoris*. Though as mentioned none of these observations are significant.

Longissimus dorsi *Biceps femoris*

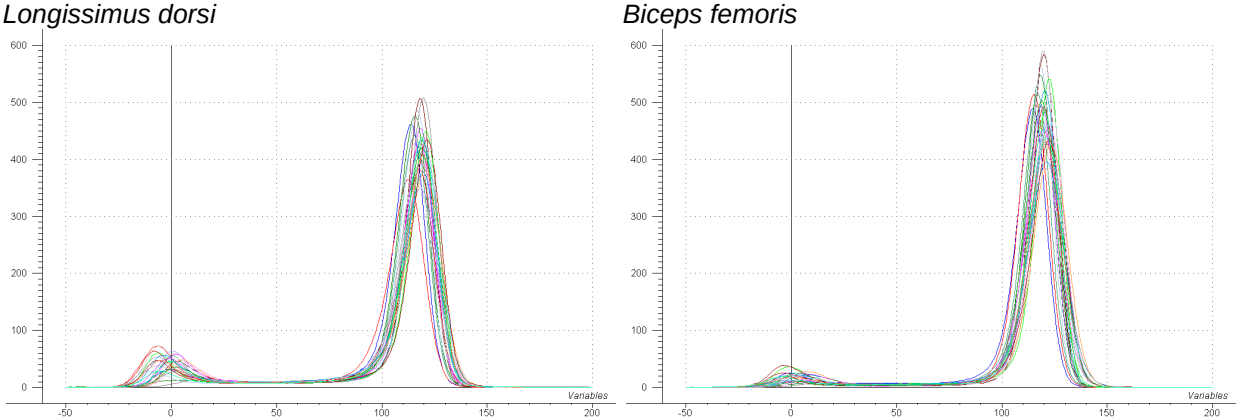


Figure 28. Spectra from *longissimus dorsi* and *biseps femoris* for all 27 samples. X-axis represent Hounsfield Units, both spectra start at -150 HU and end at 150. Spectra are smoothed and normalized by area. There is no significant difference in the spectra between the two muscles though all the spectra differed significantly ($p<0.001$) from each other.

4.2.2 Statistics

For this data set only one energy level is used therefore there are no subtracted spectra. Also here the spectra have been divided into five parts though they are not exactly the same as for the ProSafeData set (Figure 29). The first part L represents the lower left side of the spectra (-50 – 80 HU) and R the higher right side of the spectra (141 – 200 HU). LS and RS represent the shoulder of each side of the spectra (81 – 109 and 126 – 140 HU). P represents the area around the peak (110 – 125 HU).

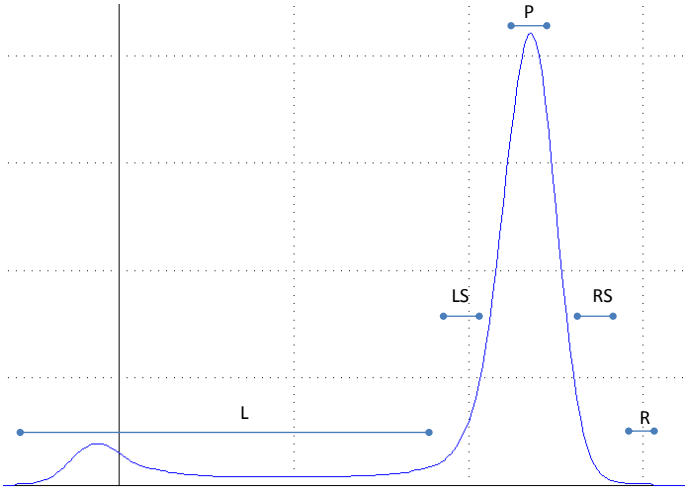


Figure 29. Interpretation of correlations between spectra and *y* variables. Each letters represents a spectra piece as follows, L = -50 – 80 HU, LS = 81 – 109 HU, P = 110 – 125 HU, RS = 126 – 140 HU and R = 141 – 200 HU.

How the y variables correlate to the different parts of the spectra are listed in Table 9. Protein correlates to L, LS and R meaning both the low and high end of the spectra. Correlations for fat are spread out on most of the spectrum except for the high end. Before collagen correlated to the higher end of the spectra now the correlation has change to the lower left side of the spectra. Water has also shifted from the low end of the spectra and correlates mostly to the shoulders. None of the *y* variables correlate to HU lower than 22.

Table 9. *y* variables correlations to the different parts of the raw spectra. None of the y variables correlated to HU lower than 22. L = lower left side. LS = left shoulder. P = Peak area. RS = right shoulder. R = higher right side.

	Protein	**Collagen**	**Fat**	**Water**
PLS2	L LS	LS	L LS RS	LS RS
PLS1	R	L LS	L LS P	RS

Correlations between the *y* variables and the asymmetrical points are illustrated in Figure 30. Water shows exactly the same as moisture did in the 80 kV spectra of the ProSafeBeef data set. ty increases with increasing water content and the 5a point of the spectrum gets more narrow. The other three variables show a slightly different pattern than in the ProSafeBeef data set. Protein now moves towards the left (negatively correlated to tx) on the HU scale when the protein content increases. The 5b point correlation to protein has also turned from negative to positive so it gets wider as the protein content increases. ty is affected negatively getting lower when the protein content decreases. Fat has correlations below 0.40 to the points tx and 50b showing that the 50b point gets wider and the spectrum moves towards the right on the HU

scale when the fat content increases. The correlation between collagen and the position tx has reversed their sign from negative to positive, the correlation to the 5b point is still positive and low.

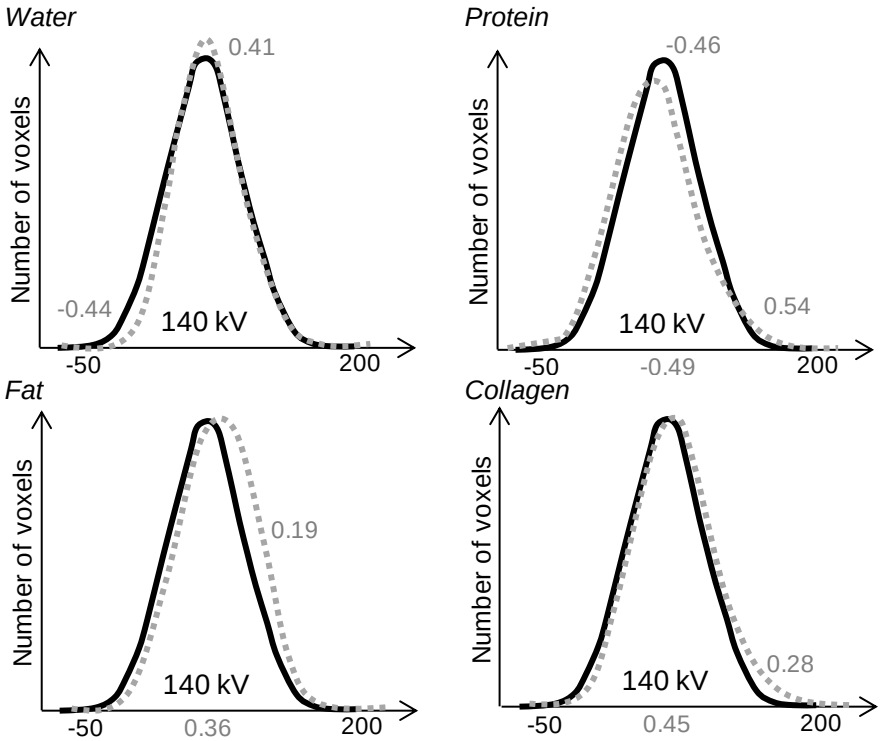


Figure 30. Illustration of correlations between *y* variables and asymmetrical points. Gray numbers = correlations which correspond to the asymmetrical points tx, ty, 50a + b, 5a + b se Figure 18 in method. Gray broken line shows how the spectra are expected to behave with the current correlations.

Correlations between the y variables are listed in Table 10. As for the ProSafeBeef data set protein is negatively correlated with moisture and collagen though for the correlation between protein and collagen is even lower and still below 0.40. The correlation between protein and moisture is at the same level. Also here fat and moisture are negatively correlated though the correlation is lower in this data set but above 0.40. In the previous data set, the correlation between fat and protein was too low to be listed and was therefore not of interest. In this data set this correlation is found to be much higher -0.47. When the fat content increases the protein content decreases.

Table 10. Correlations between the *y* variables. Collagen is shown even though it is below 0.40.

	Protein	**Collagen**	**Fat**	**Water**
Protein	-	-0.20	-0.47	-0.51
Collagen		-	-	-
Fat			-	-0.50
Water				-

4.2.3 Correlation coefficient

The correlation coefficient of both PLS1 and PLS2 models is listed in Table 11. For collagen and protein the raw spectra are a better predictor than the asymmetrical points when PLS2 models are compared. The opposite is seen for fat and water here the asymmetrical points are better in predicting (PLS2) than the raw spectra. However, for both variables the correlations are below 0.50 which means that both PLS2 models are poor predictors. The PLS1 models of the raw spectra clearly improve the correlation coefficient for protein and water. For the asymmetrical points the correlation coefficient for all four *y* variables is improved, all being above 0.50. For the protein content the raw spectra (PLS1) are the best predictor whereas for the other three variables the asymmetrical points are best (PLS1).

Table 11. Correlation coefficient (R) of all four variables in PLS2 and PLS1 models calculated on the raw spectra and asymmetrical points.

	Collagen	Protein	Water	Fat
Raw spectra				
PLS2	0.51	0.78	0.33	0.26
PLS1	0.50	0.85	0.51	0.22
Asymmetrical points				
PLS2	0.43	0.67	0.46	0.41
PLS1	0.70	0.70	0.57	0.53

4.3 Back fat

4.3.1 Computerized tomography

The CT scanning resulted in 147 spectra corresponding to 147 back fat samples (two samples were not able to be extracted from the raw data) (Figure 31). As can be seen the spectra varies in shape and size. It was also found that the spectra differed significantly ($p<0.001$) from each other but they did not differ between treatment groups.

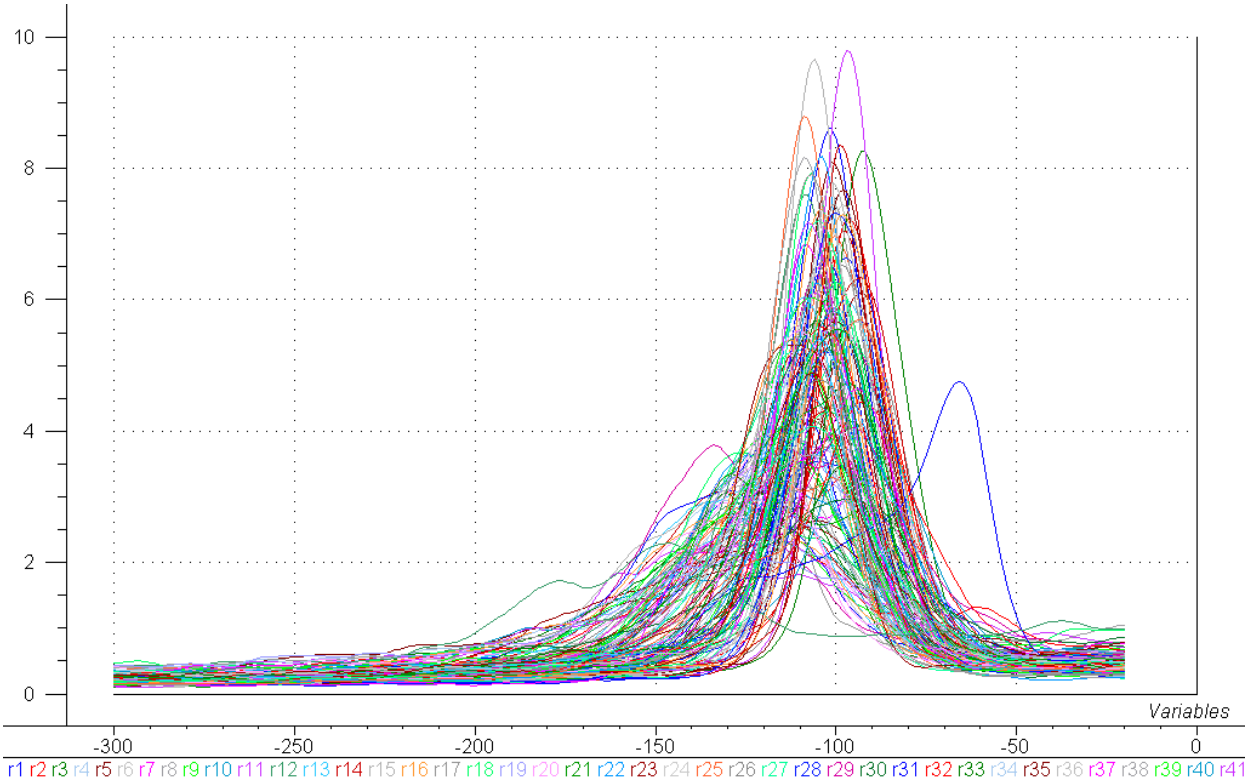


Figure 31. Spectra from all 147 samples. The skin peak was located above 0 and is not shown. X-axis represents Hounsfield Units -300 to -20. Spectra are smoothed and normalized by area. The spectra differs significantly ($p<0.001$) from each other bus does not differ significantly between treatment groups but.

The different spectra patterns were divided into five different categories (Figure 32). This gives a better survey of the different shapes and can be used to investigate if these different patterns are connected to the reference data. Picture A shows three different back fat samples and represent spectra containing one peak. Picture B, C and D could all fit into one category containing spectra with two peaks, but as can be seen they are very different from each other. Therefore they have been sorted into these three categories. B represents spectra with a left side shoulder, C a right side shoulder. Both categories do not show a clear picture of two peaks compared to picture D which contain spectra coming from back fat samples with two clear peaks. The last category, picture E shows spectra that did not fit into the other four categories A - D, they contain 3 or more peaks and are very bumpy in appearance.

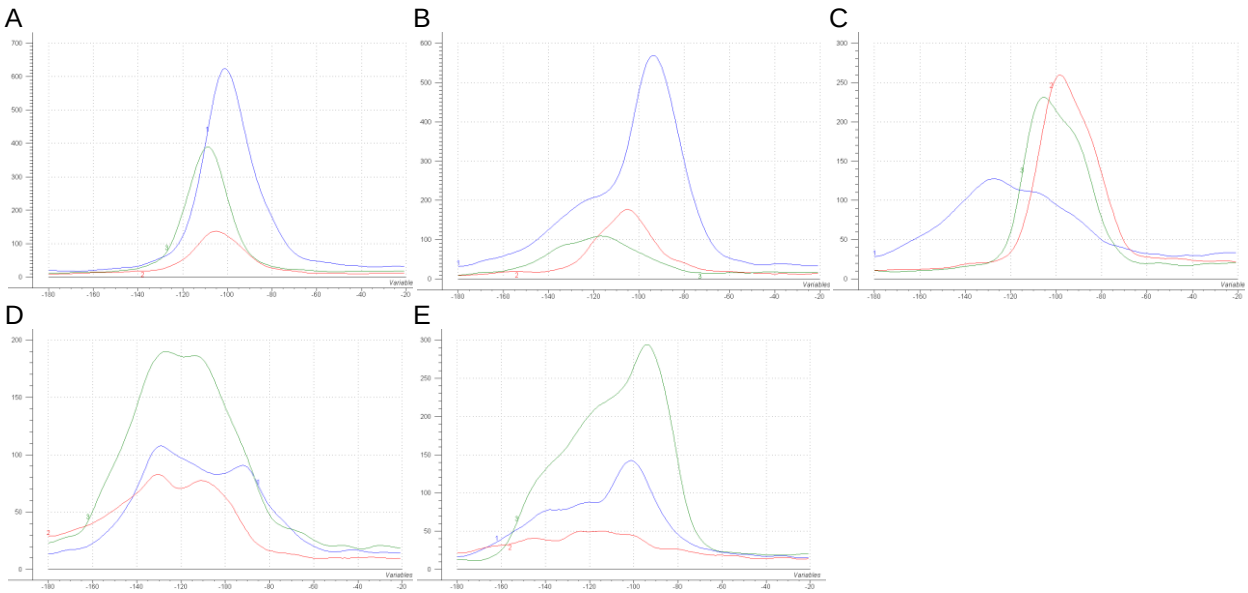


Figure 32. Spectra from back fat samples sorted into five spectra shape categories A - E. Only three spectra are shown per category and represent the shape variation within each category. A. Spectra with one peak. B. Spectra with two peaks, left shoulder. C. Spectra with two peaks, right shoulder. D. Spectra with two peaks. E. Spectra with three or more peaks.

After sorting spectra from all 147 back fat samples into the five categories, the number of spectra in each category for each diet treatment were counted and listed in Table 12. Category A has the highest number of spectra, both in total and for each diet treatment, followed by B and E. The last categories C and D have the lowest number of spectra with only one or two spectra per treatment. As can be seen, there is no connection between the five spectra shape categories and the diet treatment. Spectra with one peak are found in all five diet treatments. The same is seen for the other four categories B - E. Whether these different categories could be connected to the number of layers is investigated in the paragraph of spaciousness.

Table 12. Distribution between diet treatment and the five spectra shape categories A - E for all 147 spectra.

	Diet treatment					
	1	**2**	**3**	**4**	**5**	**SUM**
A (1 peak)	12	12	16	15	15	70
B (Left shoulder)	6	11	9	7	8	41
C (Right shoulder)	1	2	1	2	1	7
D (2 peaks)	1	2	1	1	2	7
E (≥ 3 peaks)	9	2	3	4	4	22

The scale used for the micro CT scanner is not in HU therefore it is not possible to compare these two data sets directly. Instead it is possible to compare spectra shape. The micro CT scanning resulted in 10 spectra corresponding to five samples scanned twice at two different energy levels (Figure 33). The first spectrum represents 80 kV and the second 45 kV. The red, green and light blue spectra differ significantly between the two energy levels. For each spectrum the first peak corresponds to fat and the second peak to skin. Here the skin has not been

eliminated as done in Figure 31. The spectra do not look like the spectra from the medical CT scanned back fat samples. Only one peak in the fat area is seen. The transition in the spectra from fat to skin is different for the spectra at the two energies. Pretending that two distributions can be drawn, one for each tissue, they cross each other around 0.5 on the Y-axis in the 45 kV spectra. For the 80 kV spectra the two distributions cross each other further up the Y-axis around 1. The low energy spectra have a higher fat and the spectra at 45 kV is located further to the left on the X-axis than the 80 kV spectra.

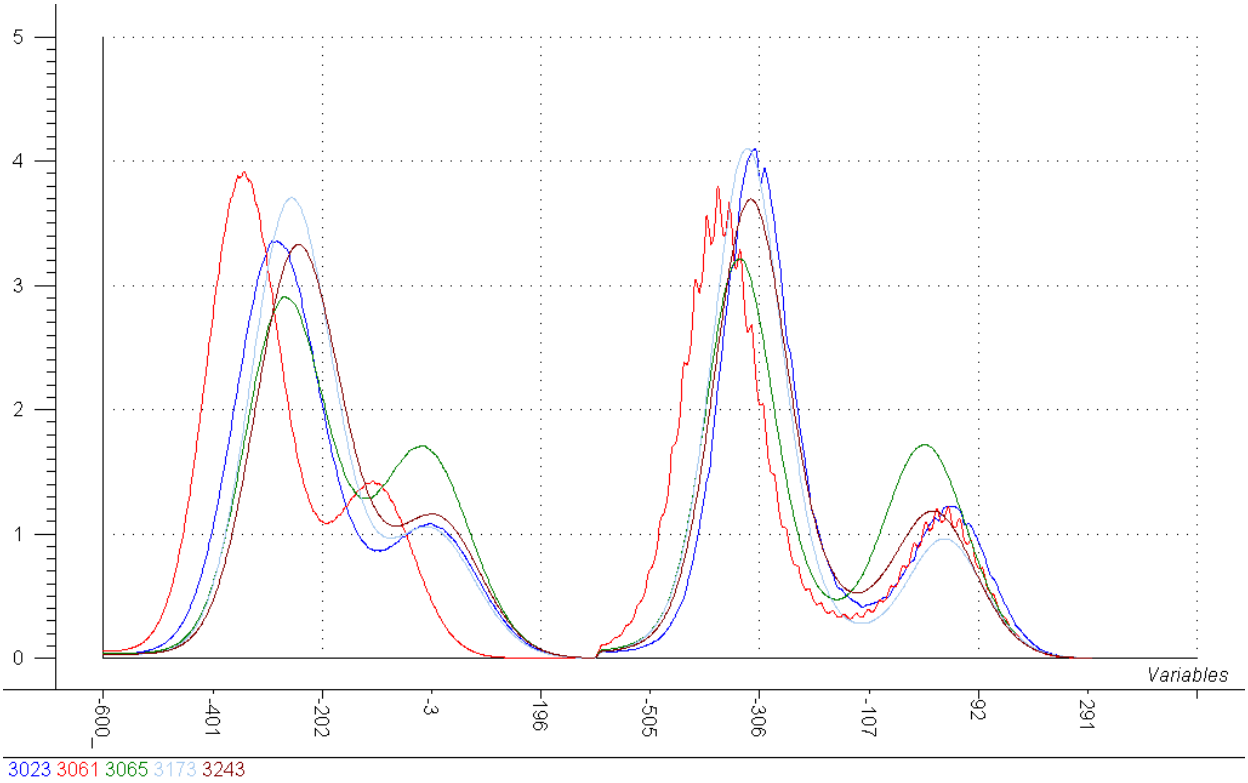


Figure 33. Spectra from the five samples scanned with micro CT at two different energy levels. The first spectra represent 80 kV (H = high energy) and the second represent 45 kV (L = low energy). X-axis does not represent the HU scale. The first peak of both the high and low energy spectra represents fat and the next peak represents the skin. Spectra are smoothed and normalized by area. The red, green and light blue spectra differ significantly between the two energy levels. Three of the samples differ significantly ($p<0.001$) in spectra between energy levels.

4.3.2 Statistics

After the back fat was CT scanned a variation was observed in the spectra shape. The method of asymmetrical statistics used for the meat samples did not fit these spectra therefore only the raw spectra were used for the multivariate data analysis. The correlation coefficient for the PLS2 model can be seen in Table 13. All the correlation coefficients are close to or below zero therefore the model cannot predict either of the *y* variables. It was also tried to see if the different slaughter dates had an influence on the data though here the correlation coefficients were also close to zero or negative. Due to these poor correlation coefficients no PLS1 models are calculated and therefore correlations to the spectra are not studied.

Table 13. Correlation coefficient (R) of all eight *y* variables in PLS2 and PLS1 models calculated on the raw spectra from the medical scanner. All 147 raw spectra are included in the calculation.

	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	SAT	MONO	POLY	UNSAT	OM 6/3	Iodin
PLS2	-0.44	0.05	-0.75	0.11	-0.12	0.06	-0.03	0.10	-0.15	0.06	-0.06	-0.17	0.07

A PLS2 model has also been calculated on the data from the micro CT scanning. Only five samples were scanned which means that this model is not solid and can therefore not be used for any conclusions. All correlations coefficients except for one were found to be even worse than for the PLS2 model calculated on the medical scanning results. Palmitic acid differs having a correlation coefficient of 0.81 which is probably a coincidence. If a PLS1 model is calculated for palmitic acid, the correlation coefficient decreases to 0.62. Calculating cross correlations on palmitic acid and the spectra makes it possible to plot correlations between variables (Figure 34). Some of the correlations are very high especially for the first part of the spectra (red circles). The highest correlation is found at a spectra value of -339 (not HU). Plotting a 2D scatter plot of the -339 spectra value against palmitic acid, the correlation is found to be -0.94. As the spectra in Figure 34 do not show widespread fluctuating behaviour, these correlations can be considered to be reliable and indeed interesting. However as said before, concluding on the basis of five samples should be done with caution. PLS2 models were also calculated on subtracted spectra which resulted in correlations all below zero also for palmitic acid.

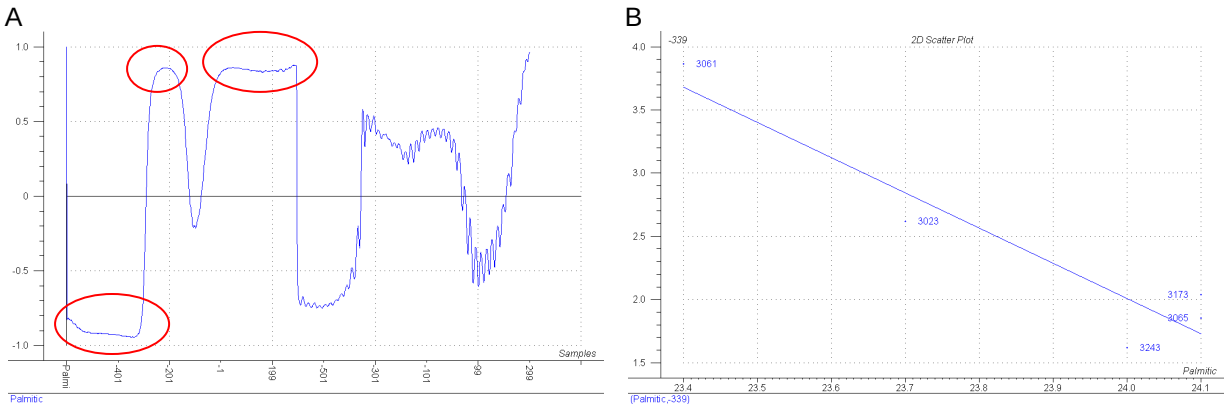


Figure 34. A. Line plot of correlations between spectra and palmitic acid. B. 2D scatter plot between the spectra value -339 and palmitic acid showing a correlation of -0.94.

4.3.3 Spaciousness

After the 149 spectra were divided into the five categories A – E, they were coloured according to their number of distributions as described in the method. The left side of the distribution was given the colour green and the right side red (see Figure 17 method). If the spectra had more than two distributions an extra colour was added (blue). Examples of how the colour is distributed in the back fat samples can be seen in Figure 35. The coloured back fat samples showed two different patterns. Either the coloured pixels were mixed as seen in sample 1 and 3 or they were divided into two different layers, one being green the other red (2, 4 and 5). For back fat samples where pixels were divided into two layers, the inner layer was always found to be green and the outer layer towards the skin, red. As mentioned the red colour of the spectra

corresponds to the high end of the spectra whereas the green corresponds to the low end of the spectra. Therefore, when the pixels in a back fat sample are divided into two layers, the density will be lower for the inner layer (red pixels) than for the outer layer (green pixels). None of the back fat samples showed pixels that were divided into three or more layers. This could be expected for the spectra containing three peaks or more. When the spectra containing more than two distributions was coloured the blue pixels either mixed as did the red and green pixels. Or if the sample divided in to two pixel layers the blue pixels was located in the middle of the sample in between the green and red pixels (not shown).

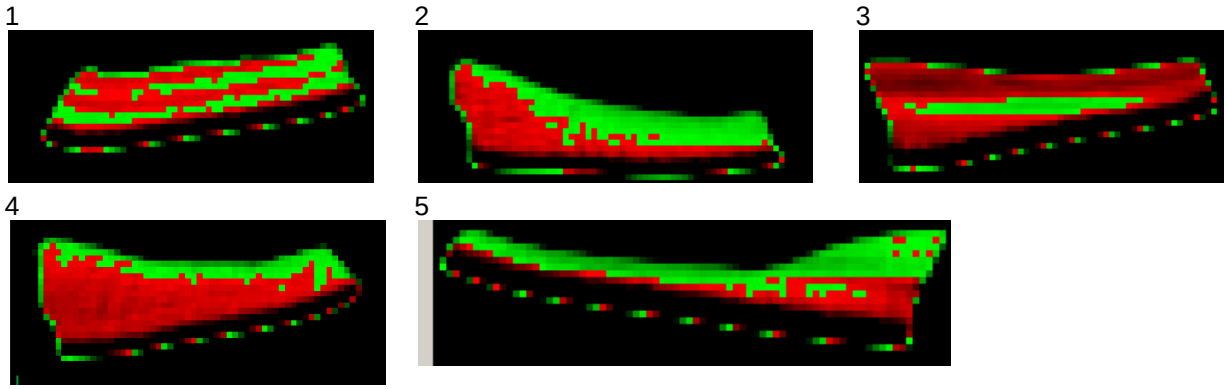


Figure 35. Results from the colouring of the back fat samples according to the number of distributions. Samples scanned with the medical CT scanner. 1 = 1, 2 = 2 and so forth. Colouring of 1 and 3 are mixed. For 2, 4 and 5 the colours are divided into layers.

Whether the colouring can be connected to the categories defined above can be seen in Table 14. In general, the pixels stay mixed when the spectra from back fat samples contain only one peak, if the spectra contained a left shoulder or more than three peaks the pixels mainly divided into layers. None of the five spectra shapes has only mixed samples or samples only dividing into layers. Therefore the pixel colouring is independent of the spectra shape or number of distributions.

Table 14. The distribution of the coloured back fat samples for each category A - E. Layers = the coloured pixels of the back fat samples distributed into two layers. Mixed = the coloured pixels of the back fat samples are mixed.

	Colour	
	Layers	**Mixed**
A (1 peak)	5	65
B (Left shoulder)	27	14
C (Right shoulder)	4	3
D (2 peaks)	5	2
E (≥ 3 peaks)	13	9

Scanning the back fat samples in the micro CT scanner gives a whole new view on the samples (Figure 36). First of all, the resolution is higher and therefore it is possible to see more details in the pictures. For example, it is now possible to see the different fat layers and also the connective tissue layer in between the layers. The upper blue area is the skin. Below the skin,

the first fat layer (outer) is seen (red and green). Next to the outer layer, a layer of connective tissue is seen, this layer is also blue. Now the inner layer follows, and if a subinner layer is present it comes after the inner layer. Both layers are divided by a layer of connective tissue. Samples 1 and 3 have three layers which is most distinctive in sample 1. Another interesting discovery is the colouring of the samples. Before the samples showed either a clear splitting of the pixels into two different colours or the pixels stayed mixed. Now the pixels are mixed completely. There is a chance that the green and red pixels are not distribute equally between the two layers resulting in more red/green pixels being seen in one layer compared with the other. This is very difficult to see therefore the spectra of each layer are studied.

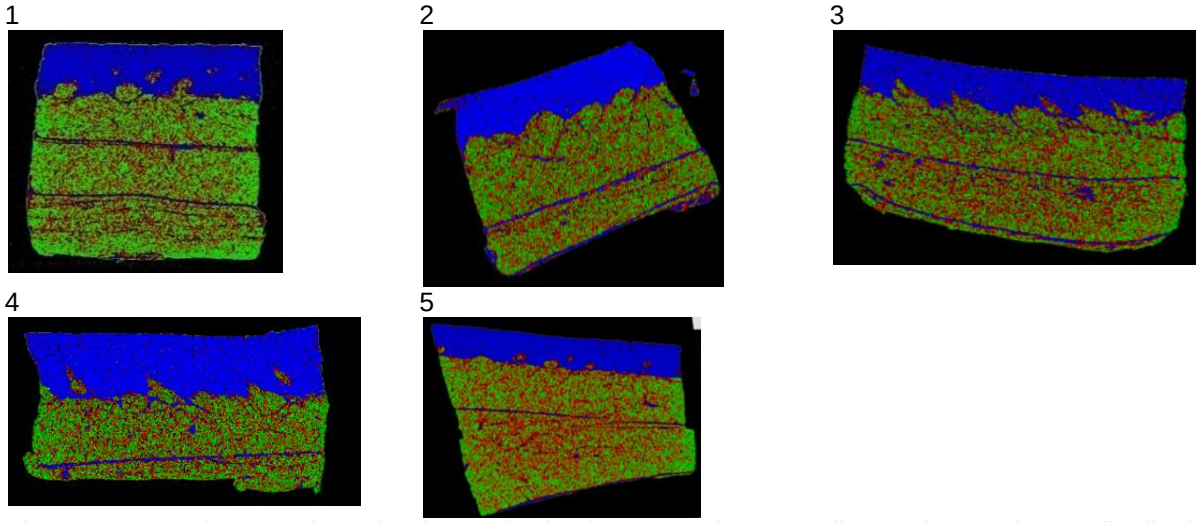


Figure 36. Results from the colouring of the back fat samples according to the number of distributions. Samples scanned with the medical CT scanner. The five samples are the same as in Figure 35. 1 = 1, 2 = 2 and so forth. Colouring of 1 and 3 are mixed. For 2, 4 and 5 the colours are divided into layers. Only images from the high energy (80 kV) scan is shown.

From each layer at each energy level the spectrum has been extracted and is plotted in Figure 37. It is clear for all five samples that spectra from the two different energies differ. However the spectra do not differ significantly between the different layers (within same energy) but has shown to differ significantly between energy levels ($p<0.001$). Even though they did not differ between layers the following observations should be noticed. The low energy spectra (45 kV) are located further down the X-axis than the high energy spectra (80 kV). It is also interesting that the outer layer spectra at both energies are lurched further to the left than do the inner layer spectra. This is best seen for sample number 2 whereas looking close one at the other samples they show the same.

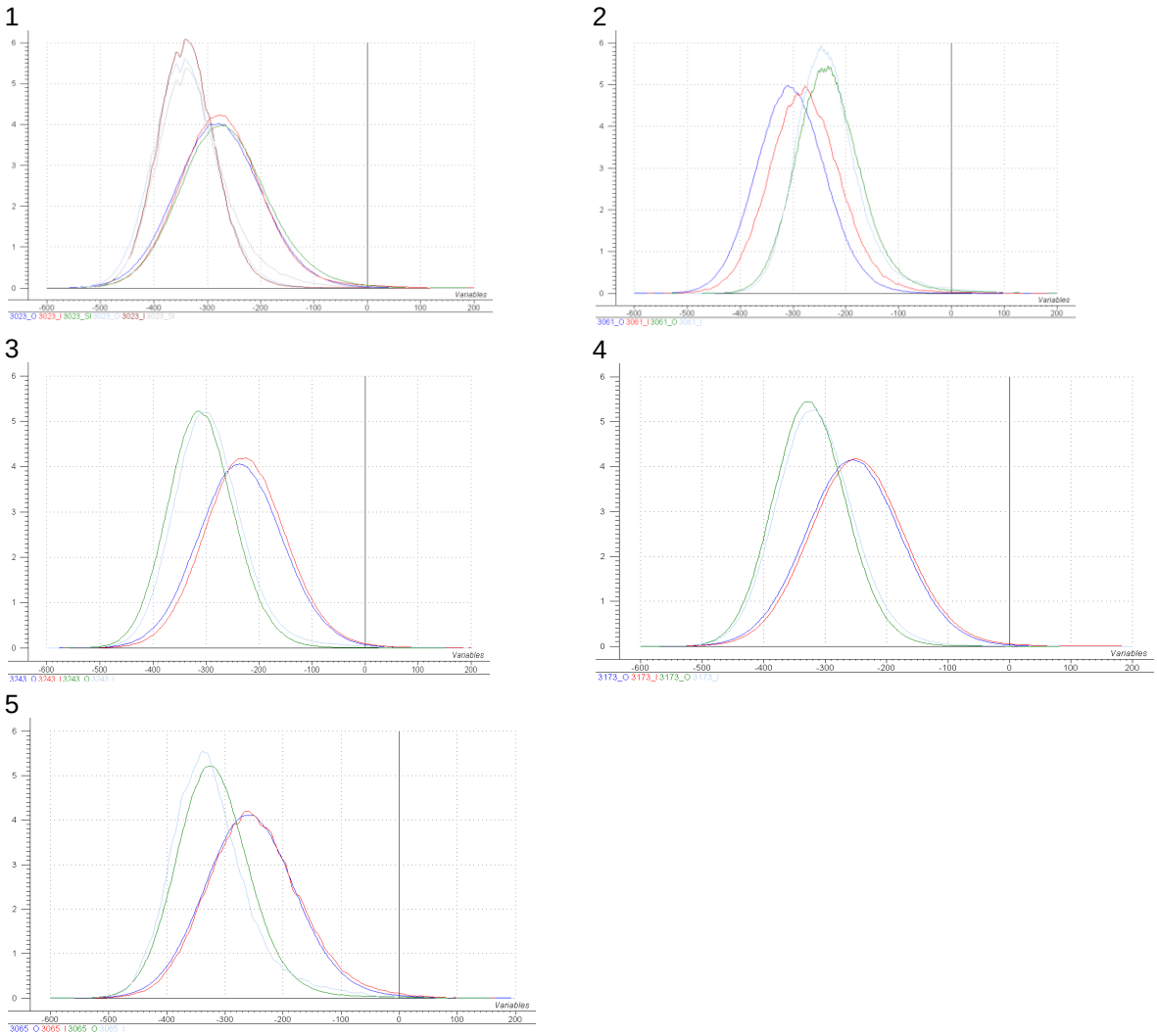


Figure 37. Extracted spectra from each layer of the back fat samples scanned with the micro CT. Spectra represent each fat layer (two or three) at each energy level. All spectra from one sample are plotted together. Dark and light green correspond to the spectra of 45 kV. Red and dark blue correspond to the spectra of 80 kV. For sample 1 dark green spectrum corresponds also to 80 kV and dark red to 45 kV. Samples are scanned with the micro CT scanner. The five samples are the same as in Figure 35 and Figure 36. 1 = 1 and so forth. X-axis does not represent the HU scale. None of the spectra differ significantly between the different layers (within same energy) but the spectra between the energy levels have been found to differ significantly ($p<0.001$).

5 Discussion

5.1 Meat quality parameters

5.1.1 ProSafeBeef

The standard variation for different meat quality parameters in the ProSafeBeef data set showed to vary within each muscle group especially for WB, collagen and fat where the standard deviations was high compared with the mean values. The animals for this study were picked out randomly which resulted in both a huge variation in age (19 – 30 months) and weight (250 – 439 kg) of the animals. There is no questioning that the variation within the muscle groups was affected by these two parameters. The meat composition changes with age. Another important factor that also affects the meat quality parameters is how the cattle are reared, what feed have they eaten and so forth. As they have been selected randomly, these factors vary between the ten animals also resulting in a variation within the muscle groups. The variation could in principle be an advantage since a variation might make it easier for the CT scanner to predict the meat quality parameters. However, when experiments are performed it is important that the variation is systematic. It would be easier to compare animals fed the same diet, reared under the same conditions and so forth. This would eliminate a lot of errors making it easier to compare different muscle groups from different animals at different ages.

It was questioned whether connective tissue and other fragments were cut off before analyses for total protein and collagen were performed. It was tried to subtract the collagen content from the total protein content for each animal to see if this might give an answer. For three different individual muscles a negative result was obtained meaning that there was more collagen than protein. Analyses of the collagen and protein content were not necessarily performed on the same meat slice and the composition within one muscle can also vary. There can be a large amount of connective tissue in one end of the muscles whereas in the other end no connective tissue is found. If the collagen content is measured on the sample containing the large amount of connective tissue the collagen content could be higher than the total protein content of the other slice or vice versa. Also if connective tissue and other fragments have been removed prior to analysis this might also give some variation between the two parameters. Removing fragments before analysis and comparing results with the CT scanning can also be a problem. The whole muscle was placed in the scanner though only three slices were recorded for each muscle. If these fragments were removed before the chemical analysis, results might be affected negatively as the two results obtained from the chemical analysis and the CT scanning not being completely identical.

5.1.2 Calibration

The Calibration data set is in some ways identical to the ProSafeBeef data set. Also here the animals have been randomly chosen though the variation within each of the two muscles was very small compared to the mean value. This small variation is probably caused by the fact that

growing-finishing pigs sent to the Danish slaughterhouses are very uniform in age, weight and also carcass composition. So even though they were collected to cover the whole population fat and lean, they would still be expected to vary less than the cattle in the ProSafeBeef. There is also the possibility that the pigs are not reared under the same conditions. Even though all of them came from conventional herds, none of them were reared under free range conditions or in other alternative systems. This also limits the variation.

5.1.3 Back fat

In the back fat study, all of the animals were reared under the same conditions, slaughtered at approximately the same weight and they were fed the same basic diet. The variation between diets was mainly seen in the fatty acid composition. As expected, this has given a significant variation in the back fat samples between the different diet treatments especially in linoleic acid and the total amount of polyunsaturated fatty acids. Different studies have shown that linoleic acid in the feed can be directly found in the fat of the animal as it was found in this study. Linoleic acid increased in the back fat with an increasing linoleic acid level in the feed.

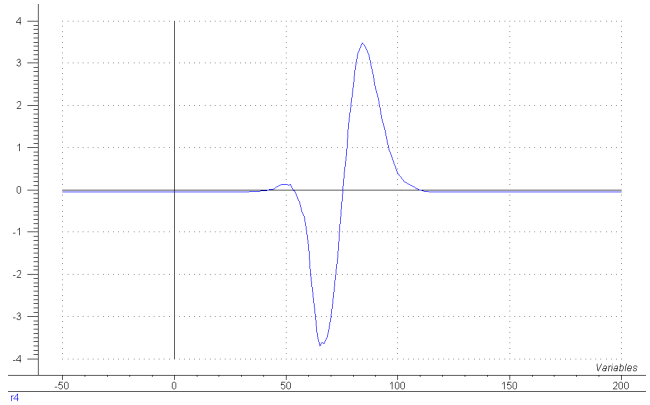
Neither the micro nor the medical CT scanner gave any hope of predicting the fatty acid composition. An explanation could be the method used for measuring the fatty acid composition. The fatty acids of adipose tissue are in the form of triglycerides. The reference data are single fatty acids and also show the level of saturation. The density of triglycerides will differ from single fatty acids so maybe the reference data are wrong compared with the information seen in the spectra of the back fat samples. The difference in density between the saturated and unsaturated fatty acids proved to vary in size (Pietrobelli *et al.,* 1996). C20:0 and C20:4 only differed by 3 HU. Between C18:0 and C18:2 the difference were approximately 12 HU. The small difference between C20:0 and C20:4 could be caused by *trans* formations instead of a *cis* formation thereby giving the unsaturated fatty acid (C20:4) a density closer to the saturated fatty acids. The polyunsaturated C18:2 contain two *cis* double bonds resulting in a lower density than the saturated C18:0.

5.2 Image analysis

The hypothesis of the image analysis method was that the variation seen in the images between the two energies might be connected to meat quality. Images seemed to be very noisy and were also at first defined as this. Later it was discovered that it might not be as simple as that. When spectra from two different energies are subtracted the result looks like Figure 38.A. Here both positive and negative numbers are seen. The negative numbers correspond to the left side of the spectrum and the positive to the right. The spectrum represents the image or vise versa, therefore when two images are subtracted also here negative and positive numbers/values would be expected. When the program used for this analysis subtracts two images it does not differentiate between negative and positive numbers. Instead it classifies each side of the spectrum as having the same gray tone values which results in a histogram as shown in

Figure 38.B. This might remove important information therefore the program ImageJ is not suitable for image analysis. When the spectra are used for analysis, the spacious resolution are hidden. The spacious resolution is the information that image analysis is expected to interpret. Image analysis would show were the pixels and information might be located in the muscle scanned instead of the spectra which show a total count of all pixels found for each HU/gray tone value. Since useful results were not obtained with this method the hypothesis could not be neither confirmed nor rejected. However, if another program that differentiates between the difference in the gray tone values was used, the image analysis might provide useful results.

A



B

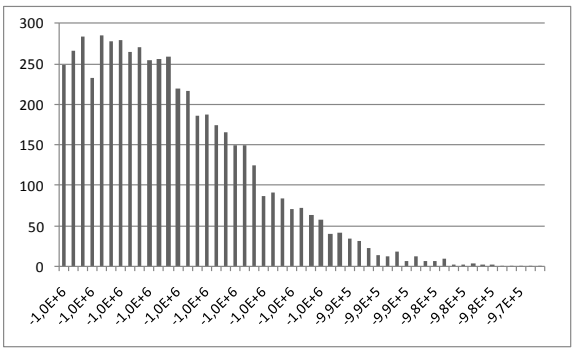


Figure 38. A. Difference spectra when two spectra with two different energies from the same sample are subtracted (normalized by area and smoothed). B. Result from ImageJ when two images are subtracted from each other. A and B represent the same sample.

Other problems or errors are combined with CT scanning and the method of image analysis. When two images are subtracted, divided or in other ways converted with image analysis it is very important that the object (muscle or back fat sample) are located exactly at the same place in both images. The purpose of subtracting the images was to see the difference between two pixels from two different energies but located at the same spot in a muscle or back fat sample. If the sample is moved between the two scans, the object position of the images will no longer be identical. Subtracting two images where one of them has a small displacement compared to the other, the pixels subtracted would no longer be the same and can give erroneous results. A displacement was seen as a thick white line around the meat sample when the Dicom picture showed in Figure 20 were divided.

Another way for the object to shift from one image to another is through the voxel size. For the five back fat samples scanned with the micro CT scanner the voxel sizes differed between the two energies used. This gave a lurch in the images from the two energies therefore image analysis was not possible. It was suspected that the lurch was mainly caused by the way the images were treated after scanning.

5.3 Spectral analysis

For the spectral analysis three different interpretations were chosen. The raw spectra, asymmetrical points and subtracted spectra. For the back fat samples the spectral analysis with the asymmetrical points was not used. This analysis was not applicable for all of the spectra as

some of them had more than one peak. When the raw spectra is used, the calculated PLS1 and PLS2 models can freely choose between all variables and find those points of the spectra that are most important. When the model chooses variables of importance there is a possibility that the rejected variables also have some importance for the model. When the model is calculated and it finds two variables (points on the spectra or in the x-variables in general) with the same correlation (negative/positive) the model randomly chooses between these two variables. When asymmetrical points were chosen, the spectra were narrowed down, in this case to six points on the spectra. In other words, the information thought to be of interest was chosen. This gives the model fewer variables (points) to calculate on which in turn give a smaller chance of two variables having the same correlation and thereby one of them getting rejected. The six points were chosen because it was found in the theory that the spectra were affected by the reference data in some of these areas of the spectra. The spectra prove to move towards the left on the HU scale when the fat content increased. Choosing the 95 percent projection (5) it was tried to get as much as possible of the lower part of both sides of the spectrum within the points (5a and b) without getting so low as the spectrum had straightened out. The x and y values of the peak point was chosen to get an idea if the spectra moved towards the left or the right. The 50 percent projection was chosen to see how and if the spectra were affect in this area. The information might as well be located at the 10 and 80 percent projection.

Subtracted spectra were used in the ProSafeBeef data set as two energy levels were used. This method was expected to be the same as for the image analysis. The information might be located in the difference between the two scannings. Manipulating with the spectra as with the asymmetrical points and subtracted spectra might remove some important information.

5.4 Computerized Tomography

5.4.1 Medical contra micro CT

When using Computerized Tomography, there is a chance that the pixels are mixed (containing more than one tissue). This affects the average μ-values and thereby the numerical value or CT number. This problem increases with an increasing voxel size. Therefore scanning of the fat and meat in the medical scanner results in more mixed pixels than in the micro CT scanner. This is especially seen in the images where more details are seen in the micro CT images than in the images from the medical CT scanner. The two main differences in the scanning methods micro and medical CT scanning are the resolution and the scale. The scale from the micro CT scanner was not in HU even though results were compared to air (Styrofoam). The samples used in the micro CT scanner were only 25 mm in diameter, the thickness varied between the five samples. The size of the micro CT samples therefore corresponded to approximately 2 - 3 voxels in the medical scanner. The higher resolution along with the different scales means that the two scanning methods cannot be compared directly.

In theory, a typical spectrum from half a pig carcass was shown (Figure 5). Two distributions were seen corresponding to fat and meat. None of these two tissues had more than one peak in the spectra. The same was seen for the meat scanned in this study also here none of the spectra showed tissues with more than one peak. However, when the back fat samples were CT scanned (medical) the spectra turned out to deviate some having more than one peak. When half a pig carcass is CT scanned, the adipose tissue is much higher in quantity than one back fat sample. In the theory it was found that the fatty acid composition varied between the fat depots. Therefore, the differences found in the spectra when only small back fat samples was CT scanned equalizes when fat from half a pig is CT scanned.

The main reason for using the micro CT scanner was to see if it was possible to recreate or confirm what was seen in the medical CT scanner. The five samples for the micro CT scanning were chosen to cover the different spectra shapes (not spectra containing $>$ 2 peaks) and the two different colour patterns, mixed and splitted. It was found that spectra containing two peaks, when they were scanned with the medical CT scanner, only had one peak when the micro CT scanner was used. The biggest difference between the two scanning methods is the resolution which is higher for the micro CT scanner. It would be expected for the micro CT scanner to show more details, which was also seen in the images but apparently not in the spectra as only one fat peak was found for all of the five samples. The energy level was the same 80 kV for both methods therefore this could also not be an explanation.

The back fat samples were also scanned at 40 kV with the micro CT scanner. This was done to see if the same pattern as Pietrobelli *et al.* (1996) showed was seen. If this was the case, the results were expected to be seen when multivariate data analysis was used. If the models had given better and useful correlations there might have been different correlations between the spectra and the reference data between the two energy levels due to the difference in absorption of the fatty acids between the two energy levels. It could for example be that linoleic acid correlated to the low end of the spectra at an energy level of 40 kV and the high end at 80 kV. But none of this was seen or studied further, since correlations coefficient (R) was poor.

The function and information of the micro CT scanner is very limited. Usually, the current scanner is used for measuring very small objects such as plastics, spare parts and other objects in this category. The operators of this scanner had only used it for half a year and did not calibrate it before it was used. Also they guesstimated by adjusting the power where the spectra approximately should be placed before they started the scanning in order to get good images. This is also the reason why the voxel size is not the same for the two scans (45 and 80 kV).

5.4.2 Energy level

It was discussed in the theory whether one energy level was better than another when different information was of interest. The medical scanner has a natural limitation as it is not capable of scanning at energy levels below 70 kV. Also it was found that the difference between tissues

decreases with increasing energy levels and that the density of the unsaturated and saturated fatty acids at 40 kV had changed compared to 70 kV. For the ProSafeBeef and the Calibration data set the energy level and scannings were decided and performed prior to this study and therefore could not be altered. Also no one has ever tried to predict meat or fat quality using CT scanning. Therefore it was not possible to use former experience in this area. As mentioned in the introduction CT has already showed to be able to predict carcass composition in pig and lamp carcasses (Kongsro *et al.,* 2008; Vester-Christensen *et al.,* 2009). Energy levels used in these studies would therefore not be a bad place to start. Vester-Christensen *et al.* (2009) used 140 kV whereas this is not stated in Kongsro *et al.* (2008). In this thesis, the attenuation coefficient and energy levels have been examined a bit closer. It turned out that at energy levels above 100 kV there is almost no difference in the attenuation coefficient between tissues therefore energy levels above 100 kV might not be the most optimal levels to choose when meat and fat quality are of interest. Information about the quality of these two tissues might not be showed very clearly at these high energy levels. When meat tissue is CT scanned one energy level could be enough though two energy levels might give more information. Scanning the back fat samples 80 kV instead of 70 kV was used. At 70 kV Pietrobelli *et al.* (996) showed exactly the information of interest, that the saturated fatty acids have a higher density than the unsaturated fatty acids. The difference between these two energy levels will be in the contrast and the attenuation. The attenuation at 70 kV will be higher though the contrast is worse than at 80 kV. Though it is difficult to say that if 70 kV had been chosen the study had provided better results as the density pattern of the fatty acids at 80 kV is not known.

5.4.3 Temperature

The temperature of the back fat samples during scanning is a factor that might affect the results. Increasing temperatures can result in some fatty acids going from solid to liquid which changes their density. The most important aspect for the temperature during scanning is to stay as constant as possible. The micro CT scanning stretched for a long time period. Measurements taken in the first period might be different than those from the last time period due to changes in their physical state and the samples drying and shrinking. The temperature of the micro CT scans was at room temperature. If some of the fatty acids changed their physical state it would be the unsaturated fatty acids which have the lowest melting points. Preparation for the medical CT scanning and the scanning took approximately 30 minutes. The temperature change for the back fat samples during this time period was approximately 3.8 ± 1.2 °C for both the core and the surface. The actual scanning took approximately five minutes, and the temperature change would therefore not be higher than approximately 1 °C (1 °C / 7.5 min.) during the scanning. This was considered to be an acceptable temperature change and assessed not to be a problem for the results. Due to the higher temperature of the micro CT scanned samples, the density might be higher than for the samples scanned with the medical scanner.

Therefore the different melting points of the fatty acids should be studied in order to see to what extend this might be a problem.

5.5 Spaciousness

Colouring of the back fat samples from the medical scanner resulted in two different colour patterns. Pixels in some samples were mixed, whereas some pixels in other samples were divided into two colour layers. For the samples where the pixels were divided into two layers the red colour, which corresponded to the high end of the spectra and thereby having a higher density, was located towards the skin in what would correspond to the outer fat layer. The opposite was seen for the green layer which was located in what would correspond to the inner fat layer and the lower part of the spectra (low density). The fatty acid composition is also varying between the fat layers. The outer fat layer contains a higher amount of unsaturated fatty acids than the inner fat layer(s). It was therefore expected that when the back fat samples were coloured, the outer layer would be coloured green due to a lower density than the inner layer, and the inner layer would be coloured red due to the higher density. However, this was not the case, as the opposite was seen. In the study of Whittington *et al.* (1986), the collagen content of the outer layer was found to be higher than of the inner layer. Collagen has a higher density than lipids. The higher collagen content in the outer fat layer might affect the density and therefore the pixels in this layer are coloured red. The water content will also affect the density though it was found that the water content did not vary between the two layers (Whittington *et al.,* 1986). Therefore the water content would influence both layers equally. The collagen or water content were not measured in the back fat samples, therefore this cannot be confirmed. Another explanation why the outer layer shows a higher density than the inner layer could also be the deposition of the different fatty acids between these layers. In this data set it is only the fatty acid composition for the whole back fat samples that is known. It was found that the total amount of for example linoleic acid increased with an increasing linoleic acid content in the feed. This increase does not say anything about how the fatty acids are distributed in the layers. When linoleic acid is changed in the feed to higher levels the inner layer might deposit more than the outer layer. This could mean that even though the outer layer is still more unsaturated, the ratio between these two layers might change. This would mean that the S/U (saturated/unsaturated) ratio between the layers becomes smaller with increasing linoleic acid content of the feed. The studies found in this area do not show unequivocal results. The deposition of linoleic acid was higher in the outer layer than in the inner (Koch *et al.,* 1968b; Warnants *et al.,* 1998). Whittington *et al.* (1986) observed the opposite. For the rest of the fatty acids, the same random variation was seen (Koch *et al.,* 1968b; Whittington *et al.,* 1986; Warnants *et al.,* 1998).

The spectra of three out of five back fat samples scanned with the micro CT scanner differed significantly between the energy levels. The low energy spectra were located lower towards the right compared with the high energy level spectra. When spectra were extracted from each layer for all five samples they also differed significantly between energy levels. It was hoped

that the same pattern as when the back fat samples was scanned with the medical CT scanner was seen. However, due to a much higher resolution and the fact that the two scanning methods could not be compared directly this was not the case where the pixels were only mixed. Extracting the spectra from each layer was a way to see if the layers (inner and outer) differed between each other, and maybe in this way finding the same patterns as in the medical scanner. The spectra of the different layers within the same energy level did not show any significant difference. Though it is striking that when the spectra are studied closely the lower left side of the spectra of the outer layer is located just a small tad the left compared with the inner layer for all five samples corresponding to nine obeservations. Only one sample deviates at an energy level of 80 kV what are the odds of that? This corresponds to throwing a coin 10 times and nine of these times the coin lands on heads and only once at tail.

5.6 Statistics

5.6.1 ProSafeBeef and Calibration

Correlation coefficients between the y variables and PLS1 and 2 models were calculated. The correlation coefficient is an estimate of how well the model or *x* variable predicts the *y* variables. The three different spectral analyses the raw spectra, asymmetrical points and subtracted spectra were used as the *x* variables. It was stated in the method that correlations below 0.40 were considered as poor, at the same time, correlations above this level can also be questionable. Aiming at correlations close to one would be optimal and most useful trying to predict meat quality. The aim of this thesis is to see if it is possible to predict different meat and fat quality parameters. No studies have previously been published in this field therefore there is no experience within this subject. This means that even a poor result give useful information about the CT as an analytical tool. None of the Correlation coefficients (R) for the two data sets have correlations close to one; therefore CT has not shown to be able to predict any of the *y* variables. At the same time, they are not so poor that it should just be concluded that the results are useless. Looking at protein, moisture, fat and pH they have high R values in the ProSafeBeef data set, and protein and collagen in the Calibration data set (above 0.70). The highest correlation was found between moisture and the asymmetrical points (0.90). Suppose that this model is accepted to predict the moisture content in meat, 81 percent (R^2) of the variation is described by the CT scanning. What can they be used for, when they are not good enough for predicting? The R value was found to differ for each variable depending on which of the three spectral analyses that was used. This shows that it matters which method of analysis is chosen for predicting each *y* variable. It might be possible to improve some of the *y* variables even more, maybe by choosing other projections for the asymmetrical points or by finding a fourth spectral analysis method. Also the asymmetrical points chosen for one *y* variable might not be optimal for other *y* variables.

Looking at the cross correlations between the *y* variables for both the ProSafeBeef and Calibration data set they show the same pattern. Fat, protein and water are the three major components of meat and interact proportional with each other. If one component increases/decreases it would be expected that the other two would act opposite, one might be affected more than the other. Negative correlations between these three parameters would therefore be expected. In both studies, the moisture/water content was found to correlate negatively to protein and fat. The correlation between collagen and protein is for both data sets negative and below 0.40. The negative correlation means that an increasing protein content results in a decreasing collagen content. For the rest of the variables in the ProSafeBeef data set the largest correlation was seen between tenderness and hardness (-0.97). This makes perfect sense, when the tenderness increases it would be expected that the hardness decrease. It would also be expected for the WB shear force, both peak force and initial yield, to increase with increasing hardness, whereas the tenderness would decrease due to tougher meat. This is seen in the correlations between WB, tenderness and hardness, where WB correlates positively to hardness and negatively to tenderness. WB also showed a positive correlation to collagen (0.28) while increasing collagen content would result in increasing WB shear force as would be expected.

In the Calibration study, two peaks in the spectra were seen where the first peak (low HU) was defined as a fat peak. However, the fat content did not correlate to this peak or this part of the spectra therefore defining this peak as fat might be false. Also none of the other three *y* variables correlated to HU lower than 22, and the “fat” peak was located around 0 HU (normally it is seen in the area of -60 HU). The Calibration data originates from half a pig carcass. When spectra from the two muscles are extracted, this peak is most likely caused by shadows from bones. Therefore, the definition that the first peak of the Calibration spectra is fat is wrong. Comparing the Calibration data set with the ProSafeBeef data set the spectra are not located at the same place at the HU scale. The peak of the Calibration spectra was located around 115 HU, and the peak of ProSafeBeef spectra was located around 80 HU. This difference might be caused by a difference in the meat tissue from pork and beef. There is a natural variation between the protein, fat and water content. Also, as mentioned above, the spectra from the Calibration study were extracted from half a pig carcass which can result in shadows.

As mentioned before studying the possibility of using CT as a meat quality predictor has not been done before. This does not mean that no literature is available and can be used. In the studies performed on fish and humans it was shown that the spectra and HU were affected by the fat content. In these studies, the muscle tissue was studied and it was found that the dispersion of the muscle tissue spectra increased in the lower left side (Gjerde, 1987; Rye, 1991; Goodpaster *et al.*, 1999; Goodpaster *et al.,* 2000). Also the density of the muscles decreased with an increasing lipid content (Nordal *et al.*, 1988; Soldevilla *et al.,* 2005). In the ProSafeBeef data set the lower left side of the spectra (low HU) correlated to the fat content. This was seen when asymmetrical points and the raw spectra were used as *x* variables. For the Calibration

data set the same pattern was not seen. Here fat correlated to several different parts of the spectra, though when the raw spectra were used as *x* variable it correlated mostly to the lower left side (low HU). Nordal *et al.* (1988) expected that an increasing density in the muscle tissue would be caused by fibrosis (connective tissue development in organs and tissues) though this was not observed. Along with this statement and the fact that collagen has a higher density than both adipose and muscle tissue it would be expected for the right side of the spectra (high HU) to be affected by the collagen content. The collagen content of the ProSafeBeef data set correlated with the high end of the spectra, as expected. The collagen content of the Calibration data set correlated to the lower part of the spectra. On the HU scale, moisture is located between fat and muscle tissue therefore it would be expected that the spectra are affected in the same way as for the fat, in the lower left part of the spectra. This is seen when the asymmetrical points and subtracted spectra are used as *x* variable for both data sets and also for the raw spectra in the ProsafeBeef data set. In the Calibration data set, the water content correlates to the right shoulder of the spectra. Water and pH are indirectly connected due to the shrinking of myofibrils post mortem. This is also seen in the ProSafeBeef data set where the cross correlation between pH and the moisture content was positive (0.38). Therefore it would be expected that the spectra is affected in the same area of the spectrum as water and fat. However this was not seen as pH correlated was spread out on most of the spectrum.

It is difficult to say what is expected for the correlations between the sensorial and WB measurements and the spectral analysis. Collagen content is found to correlate to toughness (WB shear force) (Torrescano *et al.*, 2003) also in the results correlations were found between WB force unit, tenderness and hardness. Also the PLS1 and 2 models in the ProSafeBeef data set did not show very good correlations coefficient (R) for tenderness, hardness and WB not being higher than 0.54. This might be caused by the fact that the muscles scanned were raw or uncooked and the three quality parameters were all performed on cooked meat samples. The meat texture and structure change during heating. Therefore, when the CT results of raw meat are compared to these three variables performed on cooked meat they do not correlate as well as some of the other variables. The variables protein, collagen, water, fat and pH are all variables measured directly in the raw meat and are therefore closer to what the CT scanner detect (higher correlations). Some of the variation between the results from the two data sets could be caused by a natural variation between two meat types, pork and beef. There is also a variation in the number of samples, ProSafeBeef has three times as many as the Calibration data set. In the ProSafeBeef there are ten different muscles compared with only two in the Calibration data set. At last two energy levels are used for the ProSafeBeef data set, giving the model twice as much information to calculate on.

5.6.2 Back fat

When the method multivariate data analysis was used on the back fat sample scanned in the medical CT scanner, no correlations were found to the reference data neither for individual fatty

acids nor the saturation level etc. Not even small correlations to give some hope of predicting these data. One reason could be the resolution of the scanning which was small. Maybe the medical scanner did not provide enough details. The difference in the density between the fatty acids is small as was seen by Pietrobelli *et al.* (1996) and when the voxel size is big these small details might be equalized due to mixed pixels. Therefore when we try to predict the fatty acid composition with the medical scanner we actually try to predict something very detailed with something having almost no detail. It was hoped that when the back fat samples were measured with the micro CT scanner having more details it might obtain better results in the method of multivariate data analysis. However, this was not the case as correlations were found to be even worse than in the medical CT scanner. A reason why the micro CT scanner was not able to predict the fatty acid composition could be the skin peak being closer to the fat peak. In the medical CT scanned back fat samples the skin was separated from the fat tissue. The skin is out of interest when the fatty acid composition should be predicted therefore eliminating this distribution from the spectra would give the multivariate data analysis less *x* variables to work with. As the fat and skin distributions in the back fat samples are located very close together it is not possible to separate them.

One fatty acid actually differed from the rest, palmitic acid which suddenly showed a correlation coefficient (R) of 0.81. It did not look like noise as no fluctuating behaviour was seen in the line plot between the spectra and palmitic acid. The five samples were not chosen with any concern for the fatty acid composition which could also be a reason for the poor correlations between the fatty acids and the spectra. However, concluding that the spectrum is able to predict palmitic acid and not any of the other reference data is not realistic with only five samples. Therefore it could be interesting to scan more samples and see if the same result was obtained.

5.7 Theory

It was difficult to find literature on CT scanning of meat regarding the density of both fatty acids and collagen. Two studies gives an idea of how fatty acids or adipose tissue might be connected to the CT scanning results (ICRU, 1992; Pietrobelli *et al.,* 1996). These data can be connected to the theory of the structure of fatty acids, and how the structure might affect the density of these. Pietrobelli *et al.,* 1996 does show that the pattern of the fatty acids at 40 kV is different than expected. This can be caused by the attenuation changes depending on the energy. Also this pattern is not necessary wrong though it just has to be considered when the energy level is chosen so the information of interest matches the information that comes from the current energy level. Therefore, at 70 kV the pattern corresponds to the theory found, therefore an energy level in this area (80 kV) was chosen.

In the theory, the density of the fatty acids and proteins was illuminated. It was expected that better results had made these paragraphs even more relevant and useful. It is clear that the density of especially the fatty acids varies depending on for example the level of saturation and

thereby double bonds, *cis* and *trans* bonding etc. Finding literature on the density of proteins showed to be more difficult than for the fatty acids though also here there was a limited amount of literature. Looking at the meat proteins, especially collagen consists of cross-linkages. It is expected that these cross-links contribute to the higher density of collagen compared with the other proteins. Results did show how CT results might be connected to the reference data though if even better results had been obtained it might have been possible to include more of the theory and maybe connect the limited theory on the density to these.

Studies have been found, performed on both fish and humans. There is a natural variation between these two species and also in comparison with pig and cattle. The density of the fat or fatty acids is the same, regardless of the samples being derived from humans, fish or pigs, though the fatty acid composition will vary. The same is valid for the protein composition. The adipose tissue can be found in the same area of the HU scale but will be expected to vary between the species. Both species showed the same pattern for the muscle spectra when the fat content varied therefore it is expected that the spectra from pork and beef will also behave in the same way.

6 Conclusion

The main statement of this thesis was: It was not possible to predict meat or fat quality with Computerized Tomography. However, if the methods are improved or modified in future work it might be possible. The ProSafeBeef data set, which was scanned at two different energies, had better correlations coefficients (R) than the Calibration data set. This might be caused by two energy levels providing more information.

Image analysis did not provide any useful results as the program was not suitable for this method. The principle of image analysis and subtracting spectra are the same though image analysis is expected to interpret the spacious resolution. When working with the method of image analysis different problems influencing the results were found. Lurching between two pictures along with different voxel sizes give erroneous results.

Animals in the ProSafeBeef data set were chosen randomly therefore also a random variation was obtained. When performing new experiments, a systematic variation will be easier to handle whereas a random variation can affect results both negatively and positively thereby making it difficult to conclude. Systematic variation was seen in the two other data sets where the random variation was limited. The composition within a muscle is not homogeneous, therefore the samples CT scanned and analysed chemically or sensorial should be the same. When chemical analyses are performed they should be made without taking away fragments as for example connective tissue so they match what is CT scanned. Instead of analysing the fatty acid composition the composition of triglyceride should be measured and compared with the CT scanning results.

Results varied depending on the spectral analysis method, raw spectra, asymmetrical points or subtracted spectra and the meat quality parameter. This shows that if CT in the future should be able to predict meat quality and maybe fat quality, different methods of analysis should be chosen depending on the meat quality parameter. Limiting the number of *x* variables for the PLS1 and 2 models to calculate, as with the asymmetrical points and the subtracted spectra, makes the data interpretation more reliable as the chance of important variables being rejected is lower. However, there is a chance that important information is removed. Choosing other projections for the asymmetrical analysis might improve correlations for some of the variables.

The correlations between the spectra or asymmetrical points to each individual *y* variable did not show the same results for the two meat data sets. In general, the ProSafeBeef data set fitted better to earlier studies performed on humans and fish. When predicting the sensory variables, tenderness and hardness along with WB they are most likely to be detected through the collagen content as they are measured on cooked meat and the samples scanned are raw. The cross correlations found to be above 0.40 between the *y* variables fitted well with the expectations. Even though correlations are not good they give an idea of how the CT scanning results can be connected to the reference data and how the spectra are affected by the different y variables.

Colouring of the medical CT scanned back fat samples pixels either divided the samples into two layers or they were mixed. In samples with pixels that divided into two layers, the outer layer and the inner layer would be assigned with red and green colours respectively. This shows that the outer layer has a higher density than the inner layer which does not correspond to the theory on the fatty acids. The higher density of the outer layer might be caused by the collagen and water content though confirming this hypothesis, the collagen and water content should be analysed in each layer. Extracting spectra from each layer of the back fat samples scanned with the micro CT scanner the spectra from the outer layer were located further towards the left than the inner layer though it was not significant. However, even though no significant difference was found it would be interesting to see if results could be repeated or if the same pattern was obtained when more samples are used.

Energy levels above 100 kV were used, though the variation between tissues at this high energy levels show limited difference. Larger difference between tissues is seen at lower energy levels. Since correlation coefficients close to one were not obtained, it cannot be concluded which energy level is suitable for which *y* variable and neither if more than one energy level should be used depending on the *y* variable predicted. However, for both the ProSafeBeef and the back fat study scanned with two different energies, the spectra were found to deviate between the two energy levels. This means that different information is obtained when using the two energy levels.

Eliminating random variation in the data set having age, weight etc. under control does not mean that other errors cannot arise. There are also parameters (energy level, resolution, intensity, temperature) from the CT scanner that can give variation or in other ways influence results.

7 Perspective

The three data sets provide many results. However, it was discussed if some of these results should have been left out from the thesis and maybe only the data sets on pork (meat and fat) should have been included. The work performed before and during this thesis has never been done before. For that reason, it was decided to show as much as possible for future studies to learn from these mistakes and maybe improve the method of CT scanning. The thesis could be compared with a shotgun that aims and shoots at the meat and back fat samples and subsequently tries to sort out the different pieces that were hit, hopefully finding something useful.

It was concluded that CT as applied in this work is not able to predict meat and fat quality due to a number of reasons. The usefulness of a method will actually depend on the purpose and perception of the supplier and purchaser. For the slaughterhouses (supplier) measurements giving low correlations could be acceptable for classification even though we do not receive a precise estimate of the quality. If for example beef should be classified according to tenderness it might be acceptable for a certain percentage to be classified wrongly. CT might still be better than the equipment or methods available for classification at the moment (actually no online methods are available for assessing tenderness). Whereas for purchasers such as processing companies correlations not being close to one are more important because they rely on this information to produce products with consistent quality.

All three experiments should be repeated though with different adjustments. This should be done in order to either confirm or falsify the results obtained in this thesis. Repeating the ProSafeBeef data set, the random variation should be limited by choosing animals with the same age, weight and from the same supplier so the variation is more systematic. In order to confirm or reject results obtained in this thesis additional as well as the same energy levels should be used. Maybe the detail level in the medical scanner is not high enough for some of the *y* variables. By increasing the resolution, the models might be improved therefore meat samples should be scanned in the micro CT scanner. When the back fat samples were scanned with the micro CT scanner results did not improve though they were also poor in the medical scanner. Thus it could be concluded that the micro CT scanner did not provide more information than the medical scanner. However, both the ProSafeBeef and Calibration data set gave better results for the back fat samples than in the medical scanner. It is therefore believed that it is just a question of finding a method to improve these results, and micro CT scanning could be one of them. When using the micro CT scanner, settings and output should be given great consideration and the scanner should also be calibrated to at least air and water so the results end up on the HU scale.

One of the problems set up for this thesis has proven not to be as simple as expected. How many energy levels are needed to obtain proper information? First of all, the method of CT scanning has not been used for predicting meat quality before, therefore limited experience was available. As the results showed, CT was not able to predict particularly fatty acid composi-

tion of back fat from pigs. Therefore it was difficult to conclude which and how many energy levels should be used and if the right energy levels had been chosen. The theory showed that the difference in the attenuation between tissues was higher at energy levels below 100 kV, and above this level the difference was low or almost lacking. Therefore, results might be improved if the energy level was lowered. In the back fat samples 40 kV was tried with the micro CT scanner but this did not confirm the hypothesis. Though, as mentioned before, several different sources of errors were found for this scanning method. It would therefore be interesting to scan meat and also fat (again) - next time under more controlled conditions.

8 Reference

Allen, P. & N. Finnerty (2001). *Objective beef carcass classification – A report of a trial of three VIA classification systems*. [www.teagasc.net]. Agriculture and Food development Authority, Ireland. [Cited December 4th 2008]. Available on the internet: http://www.teagasc.net/publications/beefgrading/beefgrading.pdf

Aaslyng, M.D. (2002). Quality indicators for raw meat. I: J. Kerry & D. Ledward (ed.). *Meat processing*. First edition. Woodhead Publishing Limited, Cambridge England. Page 157-174.

Bailey, A.J. (1985). *The role of collagen in the development of muscle and its relationship to eating quality*. Journal of Animal Science 60; 1580-1587

Bajerholm, C., H.P. Mortensen & A. Madsen (1992). *Fodring af han- og sogrise med stigende mængder majs – indflydelse på kød- og spisekvalitet*. Slagteriernes Forskningsinstitut, Rapport nr. 01.695/28

Baker, G.L. (1969). *Human adipose tissue composition and age*. The American Journal of Clinical Nutrition 22; 829-835

Beringues, J.C. (1999). *Sorption isotherms and waterdiffusitivity in muscles of pork ham at different NaCl contents*. Thesis from University of Politècnica de Catalunya. Available on the internet: www.tdr.cesca.es

Bragagnolo, N. & D.B. Rodriguez-Amaya (2002). *Simultaneous determination of total lipid, cholesterol and fatty acids in meat and backfat of suckling and adult pigs.* Food Chemistry 79; 255-260

Brienne, J.P., C. Denoyelle, H. Baussart & J.D. Daudin (2001). *Assessment of meat content using dual energy x-ray absorption*. Meat Science 57; 235-244

Brindley, D.N. (1984). Digestion, absorption and transportation of fats: general principles. I: J. Wiseman (ed.). *Fats in animal nutrition*. First edition. Butterworths, London, UK. Page 85-103.

Brooks, C.C (1971). *Fatty acid compostion of pork lipids as affected by basal diet source and fat level.* Journal of Animal Science 33; 1224-1232

Burrow, H.M., S.S. Moore, D.J. Johnston, W. Barendse & B.M. Bindon (2001). *Quantitative and molecular genetic influences on properties of beef: a review*. Australian journal of Experimental Agriculture 41; 893-919

Cameron, N.D. & M.B. Enser (1991). *Fatty acid composition of lipid in Longissimus dorsi muscle of Duroc and British Landrace pigs and its relationship with eating quality*. Meat Science 29; 295-307

CDD, (2008). *Fatty acids*. [www.cdd-tum.de]. Center for diet and disease at the Weihenstephan Campus of the Technische Universität München (TUM). [Cited October 18th 2009] Available on the Internet: http://www.cdd-tum.de/index.php?id=58

CFS (2004). *Continuous fat analysing with the CFS MasterTrack*, Equipment brochure from January 4th 2004, [www.cfs.com]. CFS, Netherlands. [Cited January 9th 2009]. Available on the internet: http://www.cfs.com/media/CFS_MasterTrack_EN.pdf

Commission Regulation (EC) No 1249/2008 of the European Parliament and of the Council 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcases and the reporting of prices thereof.

Council Regulation (EEC) No 3220/84 of the European Parliament and of the Council of 13 November 1984 determining the Community scale for grading pig carcases.

Council Regulation (EEC) No 1208/81 of the European Parliament and of the Council of 28 April 1981 determining the community scale for the classification of carcasses of adult bovine animals.

Daza, A., J. Ruiz-Carrascal, A. Olivares, D. Menoyo & C.J. Lopez-Bote (2007). *Fatty acids profile of subcutaneous backfat layers from ibirian pigs raised under free-range conditions.* Food Science and Technology International 13(2); 135-140

Enser, M. (1984).The chemistry, biochemistry and nutritional importance of animals fats. I: J. Wiseman (ed.). *Fats in animal nutrition*. First edition. Butterworths, London, UK. Page 23-51.

Enser, M., E. Dransfield, P.D. Jolley, R.C.D. Jones & M. Leedham (1984). *The composition and consistency of pig backfat as it affects the quality of vacuum-packed rindless bacon rashers.* Journal of the Science of Food and Agriculture 35; 1230-1240

Fortin, A. (1986). *Development of backfat in individual fat layers in the pig and its relationship with carcass lean.* Meat Science 18; 255-270

Foss (2009). *MeatMaster – Know what's in to get the best out*, [www.foss.us]. Foss North America. [Cited January 9th 2009]. Available on the internet: http://www.foss.us/Solutions/ProductsDirect/MeatMaster.aspx

Gjerde, B. (1987). *Predicting carcass composition of rainbow trout by computerized tomography*. The Journal of Animal Breeding and Genetics 104; 121-136

Goodpaster, B.H., D.E. Kelley, R.R. Wing, A. Meier & F.L. Thaete (1999). *Effect of weight loss on regional fat distribution and insulin sensitivity in obesity*. Diabetes 48; 839-847

Goodpaster, B.H., D.E. Kelley, F.L. Thaete, J. He & R. Ross (2000). *Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content*. Journal of Applied Physiology 89; 104-110

Goodsitt, M.M., R.F. Kilcoyne, R.A. Gutcheck, M.L. Richardson & D.I. Rosenthal (1988). *Effect of collagen on bone mineral analysis with CT*. Radiology 167; 787-791

Gurr, M.I. & J.L. Harwood (1991). *Lipid Biochemestry – an introduction*. Fourth edition. Chapman & Hall, London, UK.

Hansson, I. (2003). *Pork production and classification of pig carcasses in European countries*. [www.eupigclass.net]. Ingemar Hansson, Department of Food Science, SLU, Uppsala, Sweden. Eupigclass growth project GRD-1999-10914. [Cited December 4th 2008]. Available on the internet: http://www.eupigclass.net/Monitoring%20systems%20in%20Europe.htm

Harris, P.V. & W.R. Shorthose (1988). Meat texture. I: R. Lawrie (ed.). *Developments in meat science – 4*. First edition. Elsevier Applied Science, London. Page 245-296

Helmholdt, R.B., A.F.J. Ruysink, H. Reynaers & G. Kemper (1972): *Electron density distributions on single, double and triple C-C bonds*. Acta Crystallographica B28; 318-319

Hubbell, J.H. & S.M. Seltzer (1996). *Tables of X-ray mass attenuation coefficient and mass energy-absorption coefficients from 1 keV to 20 MeV for elements Z=1 to 92 and 48 additional substances of dosimetric interest*. [www.nist.gov]. U.S. Department of Commerce, NIST standard Reference Database 126. [Cited November 15th 2009]. Last updated April 1996. Available at the Internet: http://physics.nist.gov/PhysRefData/XrayMassCoef/cover.html

Hugo, A. & E. Roodt (2007). *Significance of porcine fat quality in meat technology: a review.* Food Reviews International 23; 175-198

ICRU (1992): *Photon, electron, proton and neutron interaction data for body tissues - ICRU report 46.* International Commission on Radiation units and measurements, Maryland USA.

Insull, Jr.W., G.E. Bartsch, M. Erickson & G. Curtis (1967). *Fatty acid composition of human adipose tissue related to age, sex and race*. The American Journal of Clinical Nutrition 20; 13-23

Jurie, C., J. Robelin, B. Picard, G. Renand & Y. Geay (1995). *Inter-animal variation in the biological characteristics of muscle tissue in male Limousin cattle*. Meat Science 39; 415-425

Kalender, W.A. (2005). *Computed Tomography – Fundamentals, system technology, image quality, applications*. Second edition. Publicis Corporate Publishing, Erlangen, Germany.

Kauffman, R.G., R.L. Russell & M.L. Greaser (1999). *Using pork to teach students quality variations and how they are measured. 1998 UW-AS305 Class*. Journal of Animal Science 77; 2574-2577

Koch, D.E., A.F. Parr & R.A. Merkel (1968a). *Fatty acid composition of the inner and outer layers of porcine backfat as affected by energy level, sex and sire.* Journal of Food Science 33; 176-180

Koch, D.E., A.M. Pearson, W.T. Magee, J.A. Hoefer & B.S. Schweigert (1968b). *Effect of diet on the fatty acid composition of pork fat*. Journal of Animal Science 27; 360-365

Kongsro, J., M. Røe, A.H. Aastveit, K. Kvaal & B. Egelandsdal (2008). *Virtual dissection of lamb carcasses using computer tomography (CT) and its correlation to manual dissection*. Journal of Food Engineering 88; 86-93

Kröger, C., C.M. Bartle, J.G. West, R.W. Purchas & C.E. Devine (2006). *Meat tenderness evaluation using dual energy x-ray absorptiometry (DEXA).* Computers and Electronics in Agriculture 54; 93-100

Larick, D.K., B.E Turner, W.D. Schoenherr, M.T. Coffey & D.H. Pilkington (1992). *Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet.* Journal of Animal Science 70; 1397-1403

Larson-Meyer, D.E., S.R. Smith, L.K. Heilbronn, D.E. Kelley, E. Ravussin, B.R. Newcomer & the Look AHEAD Adipose Rasearch Group (2006). *Muscle-associated triglyceride measured by computed tomography and magnetic resonance spectroscopy*. Obesity 14; 73-87

Lawrie, R.A. & D.A. Ledward (2006). *Lawrie's meat science*. Seventh edition. Woodhead Publishing Limited, Cambridge England

Liu, Y., B.G. Lyon, W.R. Windham, C.E. Realini, D.D. Pringle & S. Duckett (2003). *Prediction of color, texture, and sensory characteristics of beef steaks by visible and near infrared reflectance spectroscopy. A feasibility study*. Meat Science 65; 1107-1115

Maltin, C., D. Balcerzak, R. Tilley & M. Delday (2003). *Determinants of meat quality: tenderness.* Proceedings of the Nutrition Society 62; 337-347

Mitchell, A.D., J.M. Conway & W.J.E. Potts (1996). *Body composition analysis of pigs by dual-energy x-ray absorptiometry.* Journal of Animal Science 74; 2663-2671

Mitsumoto, M., S. Maeda, T. Mitsuhashi & S. Ozawa (1991). *Nearinfrared spectroscopy determination of physical and Chemicals characteristics in beef cuts.* Journal of Food Science 56; 1493-1496

Moody, W.G. & S.E. Zobrisky (1966). *Study of backfat layers of swine*. Journal of Animal Science 25; 809-813

Navajas, E.A., C.A. Glasbey, K.A. McLean, A.V. Fisher, A.J.L. Charteris, N.R. Lambe, L. Bünger & G. Simm (2006). *In vivo measurements of muscle volume by automatic image analysis of spiral computed tomography scans*. Animal Science 82; 545-553

Nordal, H.J., P. Dietrichson, P. Eldevik & K. Grønseth (1988). *Fat infiltration, atrophy and hypertrophy of skeletal muscles demonstrated by X-ray computed tomography in neurological patients*. Acta Neurologica Scandinavia 77; 115-122

Ockerman, H.W. (1996). *Chemistry of meat tissue*. Eleventh edition. [http://kb.osu.edu]. Department of Animal Science, Ohio State University. Available at the Knowledge Bank at OSU (Ohio State University Libraries and office of the chief information officer. http://hdl.handle.net/1811/5957

Olsen, E.V., M. Candek-Potokar, M. Oksama, S. Kien, D. Lisiak & H. Busk (2007). *On-line measurements in pig carcass classification: Repeatbility and variation caused by operator and the copy of instrument*. Meat Science 75; 29-38.

Park, B., Y.R. Chen, W.R. Hruschka, S.D. Shackelford & M. Koohmaraie (1998). *Near-infrared reflectance analysis for predicting beef longissimus tenderness*. Journal of Animal Science 76; 2115-2120

Pietrobelli, A., C. Formica, Z. Wang & S.B. Heymsfield (1996): *Dual-energy X-ray absorptiometry body composition model: review of physical concepts*. American Journal of Physiology – Endocrinology and Metabolism 271(6); E941-E951

Purchas, R.W., X. Yan & D.G. Hartley (1999). *The influence of period of ageing on the relationship between ultimate pH and shear values of beef m. Longissimus thoracis*. Meat Science 51; 134-141

Purves, W.K., D. Sadava, G.H. Orians & H.C. heller (2003). *Life: The science of biology*. Seventh edition. Sinauer Associates and W. H. Freeman, New York, US.

Rye, M. (1991). *Prediction of carcass composition in Atlantic salmon by computerized tomography*. Aquaculture 99; 35-48

Scheeder, M.R.L, K.R.Gläser, B. Eichenberger & C. Wenk (2000). *Influence of different fats in pig feed on fatty acid composition of phosolipids and physical meat quality characteristics*. European Journal of lipid Science and technology 102; 391-401

Searcy, D.J., D.L. Harrison & L.L. Anderson (1969). *Palatability and selected related characteristics of three types of roasted porcine muscle*. Journal of Food Science 34; 486-489

Sebranek, J.G. (2009). *Functional properties of muscle proteins: implications for processed meat product characteristics*. Reciprocal Meat Conference (pp. 1-7). Proceedings of the 62nd American Meat Science Association June 21-24, Rogers, Arkansas.

Soldevilla, M.S., M.F. McKenna, S.M. Wiggins, R.E. Shadwick, T.W. Cranford & J.A. Hildebrand (2005). *Curvier's beaked whale (ziphius carvirostris) head tissue: physical properties and CT imaging*. The Journal of Experimental Biology 208; 2319-2332

Stolowski, G.D., B.E. Baird, R.K. Miller, J.W. Savell, A.R. Sams, J.F. Taylor, J.O. Sanders & S.B. Smith (2006). *Factors influencing the variation in tenderness of seven major beef muscles from three angus and Brahman breed crosses*. Meat Science 73; 475-483

Stryer, L., Berg, J.M. & J.L. Tymoczko (2002). *Biochemistry*. Fifth edition. W. H. Freeman and Company, New York

Swatland, H.J. (1995). *On-line evaluation of meat*. First edition. Technomic Publication Company, Lancaster, Pennsylvania USA.

Toldrá, F. (2003). *Muscle foods: water, structure and functionality*. Food Science and Technology International 9(3); 173-177.

Torrescano, G., A. Sánchez-Escalante, B. Giménez, P. Roncalés & J.A. Beltrán (2003). *Shear values of raw samples of bovine muscles and their relation to muscle collagen characteristic*. Meat Science 64; 85-91

Van Laack, R.L., S.G. Stevens & K.J. Stalder (2001). *The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization*. Journal of Animal Science 79; 392-397

Vester-Christensen, M., S.G.H. Erbou, M.F. Hansen, E.V. Olsen, L.B. Christensen, M. Hviid, B.K. Ersbøll & R. Larsen (2009). *Virtual dissection of pig carcasses*. Meat Science 81; 699-704

Vestergaard, C., J. Risum & J. Adler-Nissen (2004). *Quantification of salt concentrations in cured pork by computed tomography*. Meat Science 68; 107-113

Vestergaard, C., S.G. Erbou, T. Thauland, J. Adler-Nissen & P. Berg (2005). *Salt distribution in dry-cured ham measured by computed tomography and image analysis*. Meat Science 69; 9-15

Warnants, N., M.J. Van Oeckel & Ch.V. Boucqué (1996). *Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products*. Meat Science 44; 125-144

Warnants, N., M.J. Van Oeckel & Ch.V. Boucqué (1998). *Effect of incorporation of dietary polyunsaturated fatty acids in pork backfat on the quality of salami.* Meat Science 49(4); 435-445

Warnants, N., M.J. Van Oeckel & C.V. Boucque (1999). *Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues*. Journal of Animal Science 77; 2478-2490

Warriss, P.D. (2000). *Meat science – An introductory text*. First edition. CABI Publishing, Oxon, UK.

Watanabe, A., C.C. Daly & C.E. Devine (1996). *The effect of the ultimate pH of meat on tenderness changes during ageing*. Meat Science 42; 67-78

Whittington, F.M., N.J. Prescott, J.D. Wood & M. Enser (1986). *The effect of dietary linoleic acid on the firmness of backfat in pigs of 85 kg live weight*. Journal of the Science of Food and Agriculture 37; 753-761

Wood, J.D. (1984). Fat deposition and the quality of fat tissue in meat animals. I: J. Wiseman (ed.). *Fats in animal nutrition*. First edition. Butterworths, London, UK. Page 407-435.

Wood, J.D., P.J. Buxton, F.M. Whittington & M. Enser (1986). *The chemical composition of fat tissues in the pig: effects of castration and feeding treatment*. Livestock Production Science 15; 73-82

Wood, J.D., M. Enser, F.M. Whittington, C.B. Moncrieff & A.J. Kempster (1989). *Backfat composition in pigs: differences between fat thickness groups and sexes.* Livestock Production Science 22; 351-362

Wulf, D.M., R.S. Emnett, J.M. Leheska & S.J. Moeller (2002). *Relationships among glycolyticn potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability*. Journal of Animal Science 80; 1895-1903