CHAPTER 12

# DISCUSSION

In this chapter a discussion of the results and experiences obtained throughout the project is presented. Many subjects have been touched upon, and many leads have been followed in the project. Cutting edge technologies for both the analytical measurements and the data analyses have been used, and the complex link between these techniques and the meat quality applications are summarised in this discussion. In the end of this chapter a summarising table reviews the results obtained in this work is found.

The multivariate data acquisition techniques used in this project cover Autofom ultrasound imaging, visual reflectance (VIS), fibre optical probe (FOP), near infrared reflectance (NIR), fluorescence, Raman spectroscopy, and low field nuclear magnetic resonance (LF-NMR). Common to all these multivariate screening techniques is the generation of vast data amounts. The multivariate data from the biological material is generally very co-linear. Traditional statistics is not optimal for dealing with these kinds of problems (Munck *et al.* 1998) and therefore new multivariate data evaluation techniques have been developed. In this work principal component analysis (PCA), partial least squares regression (PLS), N-way PLS, Tucker and PARAFAC decomposition, and image analysis have been used.

## QUALITY COMPOSITION INFORMATION

### *LEAN*

Grading based on pork carcass composition has in the previous decades been one of the key-points in the Danish pork success. The Danish industry early formed into a co-operative, and a common payment system was introduced throughout the country. This situation was (and is to a large extent still) unique world-wide.

The use of the composition information in the payment system has brought a tradition of a high quality and standardised products to the Danish pork industry. The use of ultrasound used for meat composition measurements has gone from single transducer A-scanning systems, over medical image scanners to the most recent innovation, the Autofom. The Autofom posses all possibilities for being the future standard for assessment of the grading information. The system is characterised by being very rapid (Less than two seconds are needed to measure an entire carcass). In the results presented here the Autofom has been proven to be superior to the simpler technologies for measurement of lean in grading situations in pork. The efficiency of the Autofom compared to the other ultrasonic measurement principles mainly seem to reside in the functionality of the system implying no movable parts other than the pig carcass itself. The Autofom system has been designed specifically for the carcass grading purpose. Therefore, features such as carcass representation, longitudinal images of the entire carcass, measurement automation and rapidity of the measurement are in favour of the Autofom.

Other techniques for pork carcass grading have been used more in the past decade. The manual ruler for measuring the carcass backfat was the initial technique and it is still being used in American abattoirs. The NIR insertion probes (Fat-O-Meater and Henessy Grading Probe) measures the backfat and the meat thickness on the carcass in a more objective manner, although still being manually operated. Both these techniques are far inferior to the Autofom in grading performance. The automated Danish Classification Centre (KC) is based on robotic control of 9 NIR insertion probes in the loin, ham and shoulder. The system has shown to be slightly lower standard error of prediction of the lean meat percentage (SEP=1.70 and SEP=1.95 respectively), but with a zero bias between the two systems, which is important for the abattoirs payment system. The KC is limited by the requirement to a low line speed due to the robotic control of the probes. The system is not capable of giving a total three-dimensional information of the carcass, but is more depending on indirect relations between especially the fat layers and the compositional quality information. A maximum line speed of 360-400 c/hr. (Swatland 1995a) is a problematic constraint of the functionality, especially for abattoirs outside Denmark. Also the invasiveness of the probes of the KC can be problematic in the on-line use. Again, these constraints of the KC seem to favour the functionality of the Autofom. This work has demonstrated the possibility of extending the pork carcass grading information. Other research has touched the subject of advancing the composition information, but the Autofom work presented here is the first true on-line extended composition measure regarding the loin muscle area, intramuscular fat, and back bacon.

*LOIN MUSCLE AREA (LMA)*

The work with prediction of LMA from the Autofom images was approached with the active contour segmentation in the transverse images. Usually the image analysis in the Autofom system is performed on the longitudinal images (see Chapter 3). As presumed, the information detected in the transverse direction proved to contain additional information of the LMA. Using the active contour segmentation (see the preliminary work in Appendix C and Chapter 6), the Autofom detection of the LMA was improved from r=0.61 to r=0.76 for an independent test set. However, the results obtained required an interpolation of the transverse Autofom images. The interpolation virtually improves the transverse resolution by estimating the biological structures between the Autofom transducers. The interpolation showed problems in estimating the midline of the carcass, but generally provided a reasonable characterisation of the LMA structure (r=0.81; Table 1). Furthermore, the interpolation requires a relatively high processing time, and the solution to these problems is probably to improve the transverse resolution in the data acquisition by extending the number of transducers or to use a linear array in the Autofom frame in future Autofom generations. Hamlin *et al.* (1995b) reported a correlation coefficient of r=0.45 between live ultrasound estimated LMA and yield grade in beef. By adding the information from fat thickness and carcass weight, a regression model with a fit of $R^2$=0.62 was developed for prediction of final retail product. These results clearly demonstrate the value of the LMA information in beef. The situation is expected to be similar for porcine meat, where there is an even better facility for sorting the carcasses to obtain consistent products.

*INTRAMUSCULAR FAT (IMF)*

IMF has long been one of the parameters of prime importance in beef research, but has been given surprisingly little attention in porcine research. In beef research, on-line prediction of IMF has been approached with vision and ultrasound.

The use of the Autofom related to IMF has been tested in this work. Relatively good results were obtained in predicting the IMF values with two-dimensional angular moment transformation on the Autofom images. Again, the prediction results were dependent on the Autofom resolution. The good results were achieved at reduced line speed and thereby with improved longitudinal resolution in the ultrasound images. The studies made at the normal resolution seem inadequate for sorting use. Other research results for on-line evaluation of the IMF in porcine meat have not been reported. Ragland (1997) showed that measurements made with a medical ultrasound scanner on live pigs could be used to classify the IMF values within one class out of five. In beef, Herring (1998) used four different ultrasound scanners to measure subjective marbling score and IMF. The best results were obtained with the CPEC (CPEC, Oakley, KS, USA; based on an Aloka 210 medical scanner) where correlation values of r=0.61 and 0.75 to IMF and marbling respectively were obtained. Also Whittaker *et al.* (1992) used an Aloka medical image scanner to predict marbling and obtained a correlation of $R^2$=0.45 on the slaughtered pigs. The results obtained on beef work and live pigs are similar to the results obtained for the Autofom, although the beef results seem to be with slightly lower correlation values. This indicates that the extensive representation of the meat with the Autofom (due to the scanning of the total carcass) is more important than the high local resolution of the medical scanners.

IMF was also evaluated with the spectroscopic techniques. Previous very good results in the prediction of fat with NIR have been achieved in many food products including fish, porcine and beef, despite that the fat absorption is considerable lower than absorption for moisture (Benson 1993). The NIR results obtained here on porcine meat are less optimistic than those reported previously. The main reason for this seems to be the strong water influence on the absorption pattern for water in the meat. Since approximately 75% of the meat is water, this seem to dominate the NIR spectra. Some of the previous work (e.g. Ellekjaer *et al.* 1994) was performed on meat emulsions, which had a greater variety in fat and water content. The VIS technique was surprisingly good at predicting the IMF values. The success for this technique seems to reside in the high reflectance from the bright fat in especially the low visual range around 400 nm.

The IMF predictions with the fluorescence technique were poorer than the VIS results. This is despite the fact that the fluorescence results are obtained in the UV range, where the fat is known to be highly reflective. The main reason for this is the meat representation, an essential problem in measurements of meat. Where the VIS and NIR measurements were made with an industrially optimised instrument measuring a surface with a diameter of 20 mm, the spectrofluorometer is a dedicated laboratory instrument measuring only 1×9 mm. This immense difference in the sample representation causes problems in the comparison between the instruments. The problem of the sample representation is one of the reasons for the success of the LF-NMR technique. The equipment is for laboratory use and is advantageous compared to the reflectance measurements mentioned above in the fact that the entire sample is measured and not just the surface. The results are not quite as good as the results obtained on the same LF-NMR equipment on fish meat by Jepsen *et al.* (1999), but the difference stems mostly from the very high variation in their data material. One of the future tasks of the LF-NMR system is likely to be IMF calibrations of the Autofom ultrasound data based on large data material.

A third approach for the evaluation of IMF is the imaging spectroscopy illustrated in Chapter 10. This method combines the spectral information from spectroscopic measurements with spatial imaging techniques where larger surfaces can be measured in a very short time. Based on the principles illustrated in Chapter 10, large sample sizes (up to 25×70 mm) can be measured using a UV macro objective. A homogeneity index was developed to calculate a measure of the variation of a spatial area by including the spectral variation in different subregions. The homogeneity index was applied to a set of porcine samples with a high variation in marbling content, and a significant correlation of 0.93 was found between the visual marbling score and the homogeneity index.

*WATER*

The spectroscopic techniques were used for predicting the water content as reported in Chapter 7. With the very low variation in the measured samples (mean of 72.9%

water and standard deviation of 1.4%), the prediction success is quite limited, and probably not interesting industrial applications. LF-NMR was the most efficient technique among the spectroscopic measurements, and the same equipment was also very efficient in evaluation of water content in fish meat, as reported by Jepsen *et al.* (1999). Similar to the work of Jepsen *et al.* no relation between water contents and water holding capacity was found.

#### *BACK BACON QUALITY*

Petersen *et al.* (1992) presented a back bacon study where the bacon slices were classified into five classes. Back bacon slices consist of three parts: the eye (the LD muscle), the tail (several supportive muscles, musculus serratus) and the subcutaneous fat surrounding the tail. Petersen *et al.* developed a semi-automatic classification procedure for back bacon with image analysis. By measuring the tail meat percentage, 87% of the bacon slices were classified according to a visual quality score. It was, however, concluded that the technique was destructive and could not be used for actual on-line classification, but was intended for support of further indirect measures.

Inspired by the work of Petersen *et al.* a back bacon study was performed with the Autofom (described in Appendix B). To the best of my knowledge, this is the first on-line work in which on-line sorting of the back bacon quality is investigated, and the potential is enormous for the pork industry. Being able to sort the pork sides based on the belly quality enables the pork industry to further optimise the profitable export of back bacon to especially UK. The results obtained are encouraging compared to the current non-existing facilities for sorting based on the belly standard. However, the best correlation of r=0.64 obtained by combining the lean information and two-dimensional AMT, is not sufficient for immediate use; future work is needed. The resolution of the Autofom on the sides of the carcass do seem to provide satisfactory images for the bacon information, although manual reading of the images had little relation to the back bacon quality. Inclusion of more transducers or even linear scanning ultrasound arrays in the bacon region should be considered in future Autofom reconstruction. The immediate continued work should focus on expanding the amount of data, including several subjective judges, and investigating other more structural based image analytical techniques like active contours.

### FUNCTIONAL MEAT QUALITY

#### *WATER HOLDING CAPACITY (WHC)*

For porcine meat the most important functional quality attribute is WHC. The problem with WHC in meat occurs due to genetic stress susceptibility, problematic animal handling, and environmental factors. Five different spectroscopic techniques

(VIS, NIR, FOP, Raman, and LF-NMR) are evaluated in relation to WHC in Chapter 7. The experiment included a population with the Halothane gene, ensuring a relatively high variation of WHC, which was also clearly pronounced in the statistical measures of the drip loss and the filter paper wetness. The multivariate predictions of drip loss made with the VIS spectra and the LF-NMR relaxation data were encouraging (r=0.72 and r=0.75 respectively; see Table).

The success of the VIS prediction is believed to lie in the indirect relation between myoglobin and WHC, and from the scatter effect from the difference in meat texture between the extreme meat quality and the normal meat. These results indicate that existing colour systems can be advanced to a more optimal measurement of meat.

Similar to the discussion regarding IMF, the representation of the meat is a critical feature of the various techniques. Both the FOP and the fluorescence techniques suffer from the lack of meat representation.

The success of the LF-NMR measurements is due to the influence of the spacing of the myofibrillar system in the muscles. Three water domains (free, intra- and extra-cellular) can be detected with LF-NMR as initially discussed by (Fjelkner-Modig and Tornberg, 1986). This study has further supported the characterisation of the water in the myofibrillar spacing by measurement of two muscles, the longissimus dorsi (LD) and the semitendisosous (LD). The exponential fit parameters, and especially $T_{21}$, was significantly different for the two muscles (38ms for LD and 43ms for ST). This can be directly related to the muscle fibre characterisation made by Petersen *et al.* (1996), where a similar proportion was found in the average cross-sectional areas for the muscle fibres in the two muscles (4763 $\mu m^2$ for LD and 5658 $\mu m^2$ for ST) determined by microscopic image analysis. This indicates that not only the distribution of muscle fibre types, but also the size of the myofibrillar space is influencing the WHC.

*MYOFIBRILLAR CONTRACTION*

In beef, the tenderisation mechanism has by far been the most studied functional quality characteristic. In the work headed by Tornberg described in Chapter 9 spectroscopic techniques (NIR and NMR) were combined with physical measurements (isometric tension and shortening, W.-B. shear force and cooking loss). The tenderness of the meat (as measured with W.-B.) was affected by three glycolytic rates and two cooling regimes. The glycolytic group with the fastest post-mortem pH decrease showed the best tenderness. During the post-mortem metabolism, the first score of the multivariate LF-NMR followed the pH development with time, as interpreted from the loading plots. NIR provided only little information of the tenderisation process, but LF-NMR provided useful information in the interpretation of the biochemical and biophysical post-mortem changes.

$T_{21}$, which according to Tornberg *et al.* (1993) characterises the water protons within the muscle fibres, was inversely correlated to pH early post mortem. For the fast glycolytic group the T21 decreased post-mortem, which was explained by a leakage of the myofibrillar proteins. For the slower glycolytic groups, first an increase of the $T_{21}$ was observed (explained by a swelling inside the muscle fibres) followed by a decrease, once the destruction of the cell walls begins. It was concluded that $T_{21}$ was influenced by the intracellular protein concentration, and, as such, changes dramatically during the breakdown of the myfibrils and the following water leakage. The use of LF-NMR alone provided a good segmentation of the three glycolytic groups using Parafac decomposition (Appendix D).

The $T_{21}$ parameter has provided very qualitative information regarding the myofibrillar space in both beef and pork (see the WHC experiment described above). An early post-mortem experiment using LF-NMR should be also conducted on porcine meat, similar to the described beef experiment. The intervals of the measurements should be smaller than for the beef experiment due the earlier occurrence of rigor mortis in for porcine meat. This experiment will further help to understand the muscle swelling and contraction in relation to both water holding and tenderness.

## EATING QUALITY

### *TENDERNESS*

In beef, the tenderness of the meat is the predominant quality attributes. In relation to spectroscopy, NIR was investigated by Park *et al.* (1998), who found a relatively good relationship to tenderness (see Table in Chapter 1). Also the work by Hildrum *et al.* (1994) was promising for NIR in relation to tenderness. The NIR information is hypothesised to reside in scatter effects from the muscle contraction. Protein denaturation information can be expected around 1500 nm and 2000 nm, but the measurements reported in this work are troubled by the dominating water absorption information in this spectral area.

The pre-rigor work by Tornberg *et al.* (1999, Chapter 9) included spectroscopic NIR and LF-NMR measurements as well as physical measurements (shortening and myofibrillar length), and chemical measurements (pH) has been continued with a post-rigor inspection (to be published). The preliminary indications observed in this data are less successful for tenderness prediction than the previous reported work, but includes the potential of interpreting the post-mortem conditions because the date are measured at 8 time intervals post-mortem. The post-mortem changes are, due to the shortening of the muscle, one of the parameters influencing the toughness (the inverse tenderness) of the meat (Drainfeld 1994).

*WARMED-OVER FLAVOUR (WOF)*

The WOF characteristics in ready-to-eat and other convenience foods are of increasing interest in the food industry. The relation between the pre-slaughter stress and WOF has recently been addressed in porcine meat by Byrne *et al.* (1999a). Chapter 8 presents an extension to this leading work where spectroscopic analyses have been included in the experiment. The results showed that especially VIS was capable of describing the stress groups involved in the measurements and the storage period (0 to 5 days) after cooking. LF-NMR was successful in describing the stress groups and fluorescence provided a good separation of the samples according to the storage period. A sensory and chemical validation of the WOF samples was also included in the experiment, and this showed that especially VIS, fluorescence, and LF-NMR were successful in predicting the sensory terms developed especially for WOF characterisation (see Table 1). VIS and fluorescence was further capable of predicting thiobarbituric acid reactive substances (TBARS), phosphatidylethanolamin (PE), phosphatidylcholin (PC), and conjugated dienes, which are accepted as descriptors of the WOF characteristics. The spectroscopic results are interesting due to the on-line prospect of the methods when the sampling problem is overcomed. It is furthermore interesting that LF-NMR and VIS both works so well. Due to the nature of the two techniques, they measure different phenomena in the samples and complements each other well.

## MULTIVARIATE DATA ACQUISITION TECHNIQUES

The Autofom has been manifested as the leading technique for on-line evaluation of the total lean and the lean in the meat primals. The advantage of the Autofom is clearly outlined in both the grading performance and the functional features of the system with regard to the objectivity of the measurement, the non-invasiveness, and the speed of the measurement. Introduction of advanced data analysis in off-line tests of the Autofom has indicated that by overcoming the problems of the coarse transverse and longitudinal resolution of the system, it can be used for advanced composition measures in pork grading or sorting. Thus, future Autofom generations should aim for an optimal local resolution as well as the current successful global resolution.

The VIS technique has proved to be a very successful analytical technique with good results for fresh as well as cooked porcine meat. This indicates the generality of the multivariate technique. The work of e.g. Swatland (1989) presents the biochemical validation of the results obtained in an exploratory manner in this work. The efficiency of the VIS technique calls for the development of an advanced colour system optimised for meat measurements. In usual colour metres the spectral range is optimised for the human eye (through the CIELAB standard; Hunt 1991) and not for the characteristic wavelengths of meat. The Fat Quality Meter (FQM; Chapter 2) developed by the Danish Meat Research Institute (Roskilde, Denmark) presents an excellent frame for such an advanced colour metre. The FQM is by nature a hand-held probe, but it is possible to implement as an automated control of the system. Another option could be to implement an imaging spectrograph similar to the technique outlined in Chapter 10. If the visual range is the spectral range of interest, the proposed system can be simplified with regard to the UV objective and the cooling facility, and a more robust and on-line realistic system can be developed. An ultimate modification of either the FQM or the imaging spectrograph into an advanced colour system should consider the traditional wavelengths for the CIELAB measurements (with integrating curves peaking at 450, 550, and 600 nm) and the wavelengths for the myoglobin characterisation (in the different oxidated and oxygenated states) (430, 418 nm; Swatland 1995a). Other specific wavelengths should include 460 nm (because of NADH, although fluorescence cannot be measured with the FQM) to facilitate a more generally optimised meat quality inspection. As a complement to pH measurements such a multi-wavelength tool is likely to provide valuable meat quality inspection.

The LF-NMR system proved to be an efficient analytical tool for evaluating the functional quality (WHC on porcine meat and muscle contraction or beef) and eating quality in the cooked and reheated porcine meat. The success of the measurements is mainly du to structural characterisation of the myofibrillar space, but it is interesting to notice this characterisation is observed even in the cooked meat. The technique is not fully investigated, and especially the early post-mortem investigation should be continued. The indications seen in this study as well as the previous studies by particularly Tornberg (1993, 1999) indicates that the LF-NMR technique can be a very powerful tool for interpretative investigation and related to physical measurements (e.g. shortening, and microscopic analyses). The LF-NMR is, however, not immediately realistic as an on-line tool. Instead the future perspectives is more in the line of using the technique as an advanced tool for calibration of e.g. the Autofom and other fast techniques, or as a laboratory system for occasional sampling at abattoirs.

The use of FOP systems has been investigated in meat applications in especially pork abattoirs since the initial work by Swatland (1982). In this study a set of different probe designs has been investigated (Appendix A), all with the purpose of increasing the representation of the meat. The majority of the probe designs revealed durability problems when used in realistic measurement situations, and this problem needs more attention before a realistic FOP system can be prototyped. The proposed probes are connected with optical fibres to a two-channel portable spectrophotometer, which combined cover the spectral range from 200 to 980 nm. The FOP system showed a reasonable performance tested off-line on an abattoir and with three insertions made into the meat (Chapter 7). Off-line tests made in a German plant also revealed success in 24 hours post-mortem separation of PSE extremes from normal carcasses (Appendix A). However, when used on-line the performance of the FOP was discouraging due to sampling problems and probe durability (Appendix A). Future work with the FOP should include the facility of internal reference, fibre stability (guide), higher representation, probe representation and durability.

The imaging spectrograph outlines in Chapter 10 posses some features extremely relevant for measuring meat, especially the high the sample representation. Using macroscopic vision, spectroscopic information from relatively large sample regions (up to 25×70mm) can be measured. In the experiments carried out in this study, the imaging spectrograph was compared and found similar in accuracy to a laboratory spectrofluorometer and a motorised scanning fluorescence microscope. The imaging spectrograph is advantageous compared to the spectrofluorometer in the sample representation, and advantageous compared to the microscope in the measurement time. Experiments carried out on fish meat showed that he instrument was capable of following the post-mortem changes on both the fish skin and the fish muscle. Using a binning technique of the CCD sensor in the imaging spectrograph, the spectroscopic information could be collected from up to 330 subregions on the sample. This is utilised in a homogeneity study of IMF, where a dedicated homogeneity index (HI) deducted from the spectroscopic information, was superior to a traditional image texture measure (co-occurrence matrix features; COM) when compared to visual marbling evaluation (r=0.94 for HI and r=0.87 for COM). Despite the positive results of these demonstrations, the imaging spectrograph is a dedicated laboratory system and not for industrial use. The potential of the system does, though, outline an efficient frame for laboratory optimisation of applications before the development of a prototype can take place.

The inhomogeneity and anisotropy of the meat is generally a critical aspect in measurements of the meat. Extensive scanning of the carcass is one of the advantages of KC and Autofom relative to the manual single-point measurements. The same problem is relevant, when discussing the spectroscopic techniques. Especially the FOP, which is basically a single-point measurement, and the fluorescence measurement, which is made with a laboratory equipment measuring only 1×9 mm of the meat surface, are sensitive to the homogeneity of the meat. Jeremiah (1982) evaluated the variation in muscle characteristics within the same animal. Thus, it must be stressed that measurement equipment intended for meat should pay attention to the sample representation.

Considering the measured sampling area, the speed of the acquisition technique and the sample preparation, the spectroscopic techniques analysed in this work can be characterised into three groups: the on-line applicable (FOP/VIS/NIR), the off-line applicable (fluorescence), and the laboratory techniques (Raman, LF-NMR).

Promising results have been obtained with the spectroscopic techniques in this study. However, with regard to the functional and eating quality perspectives, no general technique for use in a grading situation can be justified from these results. A temporary compromise could be to include the spectroscopic techniques in an ongoing quality measurement programme or as a guiding tool for meat quality inspectors as also proposed by Belk *et al.* (1998) with regard to beef grading.

## MULTIVARIATE DATA ANALYSIS

The multivariate data analysis, or chemometric, methods have been very powerful in the study of multivariate data. The extensive amounts of data generated by the multivariate data acquisition techniques are approached in an exploratory manner. In this way, the data can relatively easily be interpreted, despite the complex data organisation. Two main groups of chemometric techniques have been used in the study of meat, namely the decomposition and the regression techniques.

In this study, decomposition of the multivariate data is made by three methods: principal component analysis on the one-dimensional spectra and PARAFAC and Tucker decomposition on the higher dimensional spectra (the two-dimensional AMT and the NIR and NMR data from the beef evaluation). The decomposition reduces the dimensionality of the data and enables a simple interpretation of the interactions between the samples and the initial variables. This has been successfully used in the evaluation of the tenderisation processes described in Chapter 9. The N-way decomposition methods (PARAFAC and Tucker) have a potential ability to resolve unknown components, such as chemical constituents of the measured sample (Bro 1998). This was not within the scope of this study, but could be included in areas as fluorescence detection of different kinds of connective tissue (Swatland 1995a) or early post-mortem metabolic changes measured over time with LF-NMR, which have been outlined in Appendix D. Due to the fact that much basic work still needs to be done in spectroscopic analysis of meat, the potential of combining chemical knowledge with the exploratory resolvements with N-way methods could provide a powerful tool. In this study the Tucker method proved superior to the PCA in decomposing the AMT data of the Brodatz natural textures to fewer dimensions (Chapter 5), but by including more principal components, the difference disappeared.

Regression analysis has been performed using two approaches: partial least squares regression on one-dimensional data and N-PLS on decomposition on higher dimensional data. All these regression techniques are linear, which appears to be sufficient in this study. However, in the unpublished extension to the work headed by

Tornberg (Chapter 9) in which post-rigor tenderness is predicted with rapid methods, there seems to be a non-linear relationship between the sensory evaluation and the shear force measurements. This problem can be dealt with by applying a non-linear pre-processing transformation to the reference information (e.g. the square root) before performing the linear regression analysis. Another simple non-linear technique is e.g. the addition of the squared variables transformation in PLS (INPLS; Berglund and Wold 1997). Neural networks have also been used in meat applications (Berg 1998b, Thodberg 1993), but neural networks are limited by requiring large calibration data sets, which is often a problem in meat applications. A compromise is the combination of the scores from a PCA and neural networks (Bro 1995).

Image analysis is an efficient method for analysing signals from spatial domains, which differs from the spectral domain by a lack of variable specificity. Therefore, special signal analysis is usually required to compare several measurements. In the Autofom the image analysis is made with feature extraction of anatomical structural in the carcass. Successive to the image analysis, the extracted features can be compared for several measured carcasses. In the Autofom this comparison is facilitated by chemometrics.

In this study, extensive structural image analysis has been proposed for the detection of the loin muscle area (LMA) in the transverse Autofom images. Using bipolar interpolation the 16×635 point transverse ultrasound images are transformed into a 318×318 image where the intermediate biological structures between the Autofom transducers are estimated. The interpolated images are still of low transversal resolution and especially the structures in the mid-line of the carcass seemed poorly estimated. Specific segmentation approach was therefore developed to meet the problem of the low resolution (Chapter 6). The developed segmentation principle combines principles from deformable templates which for contours was introduced as snakes by (Kass *et al.* 1988), and score images, which were used in e.g. (Geladi *et al.* 1989). The score image is included as an a priori information with which the error function determining the direction and step length of the motion the active contour is iteratively updated. A segmentation contour is thereby deliberately assured to match a certain common shape of the LMA structure, but without introducing an assumed fit with a geometrical model, which is the common approach in deformable templates. This results in a robust segmentation and ensures convergence despite missing data (e.g. the midline in Autofom images). The proposed method is especially relevant for the low resolution ultrasound images observed in the transverse dimension for the Autofom, but could well be appropriate in other applications where the structure of the segmentation object is complexly defined geometrically and/or the contrast in the images is poor. In future perspectives, a potential use of the active contour principle could be to segment the muscle structure into several transverse image slices of the Autofom image sequence. The muscle contour detected in one image is used as the initial curve in the following image, and the active contour principle is used to update the segmentation. This approach is similar to the time development used in 3D segmentation of real-time medical ultrasound images (Caselles *et al.*. 1997). The use of the active contour in three dimensions also enables the use of a three-dimensional score image, which can be calculated with e.g. Parafac/Tucker3 (Bro 1998).

The detection of IMF with the Autofom has also been approached with an image analysis technique specifically developed for the purpose. This technique, two-dimensional angle measure technique (2D-AMT), is eventually an image texture method, extended from the initial use to characterise geometric formations (Andrle 1994) to one-dimensional image texture analysis by Esbensen *et al.* (1995). AMT works by transforming intensity differences in the spatial image domain to a scale domain. Thus, the scale representation, which is a common problem in image texture studies, is implicitly included in AMT. Compared to the initial image texture approach made by Esbensen *et al.* (1995) and the subsequent application by Kvaal *et al.* (1998), the 2D-AMT differs by the two-dimensional sampling in the spatial domain and by the optional two-dimensional scale domain utilised. The 2D-AMT has proposed three different modes involving 1) random spatial sampling, 2) directional dependency, and 3) rotational independency. The AMT data are multivariate and require multivariate post processing such as PCA or Tucker. For the two-dimensional scale domain data provided by 2D-AMT, the N-way techniques Tucker and N-PLS are used for the post-processing of the AMT data. Results obtained on tentative classification of the Brodatz natural textures and prediction of IMF from the Autofom images suggest that the 2D-AMT introduces additional information due to the sampling methodology. The 2D-AMT even displayed some structural capacity from the back bacon study (Appendix B) where the performance of the 2D-AMT was superior to manual reading of the ultrasound images. The main advantages of the AMT are: 1) the generalisation and non-essential parameter optimisation makes the approach an easy to use tool for empirical texture screening; 2) when combined with multivariate decomposition the scale domain makes the AMT able to overcome changes in scale and magnification; 3) the possibility of mapping texture information from images (or signals) from different sizes and even different dimensions onto the same domain for comparison. One of the disadvantages of the 2D-AMT texture information seems to be the generality due to the ease of use, and very specific texture profiles may be better characterised with more traditional texture modelling.

In an attempt to combine the advantages of the specificity of spectral data with the advantages of spatial information, the imaging spectrograph was outlined in Chapter 10. One of the tasks with the spectrographic system was to study homogeneity of food products. For this purpose, a homogeneity index was developed inspired from statistic textural measures in traditional imaging. By combining the spectral information with the spatial one-dimensional image with the use of variance and entropy, the homogeneity index expresses both the spectral and the spatial variation. In the example, the homogeneity index proved superior to traditional co-occurrence matrix texture features, where only the grey-scale information is included.

The combination of the multivariate data techniques and the reference information measured on the carcasses and the meat samples have successfully been tied together with chemometrics. Using these techniques in an explorative approach, interesting conclusions regarding both expected hypotheses (like the characterisation of the myofibrillar space with the LF-NMR $T_{21}$ parameter) and new theories induced by the data itself (like the relation between the visual data and the sensory properties of the warmed-over flavour) can be drawn. Much work, however, still remains to be completed before some of these techniques eventually can be introduced for on- or at-line quality measurement systems. This work generally needed are: 1) massive collection of data to facilitate the development of artificial intelligence system to cope with global regression models. 2) Solving the sampling problems for most of the techniques, where both non-invasiveness and sample representation ultimately must be dealt with. 3) The continued development of trust-worthy reference measures, which are closely related to a better basic knowledge of the quality influencing processes occurring in the meat.

*Table. Overview of the meat quality and composition results obtained throughout this study.*

| INSTRUMENT | TECHNIQUE | DATA ANALYSIS | REFERENCE | MEAT | RESULTS |
|---|---|---|---|---|---|
| Autofom | Ultrasound | Structure detection,PLS | Lean | P | $R^2$=0.74-0.84<br>RSD=1.70-1.84% |
| | | Str. det.,PLS | Ham Lean | | RSD=0.31kg |
| | | Str. det.,PLS | Loin Lean | | RSD=0.15kg |
| | | Str. det.,PLS | Shoulder Lean | | RSD=0.15kg |
| | | 2D-AMT,PLS | IMF | | r=0.81 |
| | | Act. contour | LMA | | r=0.75 |
| | | Meat percentage + 2DAMT | Back Bacon visual score | | r=0.64 |
| Fibre Optical Probe | UV-VIS | PLS | Drip loss<br>Filter paper<br>IMF<br>Water | P | r=0.61,SEP=2.53%<br>r=0.26,SEP=12.9%<br>r=0.15,SEP=6.3%<br>r=0.35,SEP=1.2% |
| NIR Systems | VIS | PLS | Drip loss<br>Filter paper<br>IMF<br>Water | P | r=0.72,SEP=2.1%<br>r=0.54,SEP=18.0%<br>r=0.52,SEP=1.6%<br>r=0.46,SEP=1.0% |
| NIR Systems | VIS | PLS | Sensory terms<br>Conj. Dienes<br>TBA | CP | r<0.94,SEP>0.20cl.<br>r=0.98,SEP=7.5E-4<br>r=0.96,SEP=0.28 |
| NIR Systems | NIR | PLS | Drip loss<br>Filter paper<br>IMF<br>Water | P | r=0.64,SEP=2.4%<br>r=0.62,SEP=16.0%<br>r=0.70,SEP=1.3%<br>r=0.46,SEP=1.1% |
| NIR Systems | NIR | PLS | Sensory terms | CP | r<0.94,SEP>0.20cl. |
| PE LS-50 | Fluo. | PLS | Drip loss<br>Filter paper<br>IMF<br>Water | P | r=0.68,SEP=2.3%<br>r=0.55,SEP=19.0%<br>r=0.62,SEP=1.4%<br>r=0.16,SEP=0.9% |
| PE LS-50 | Fluo. | PLS | Sensory terms<br>Conj. Dienes<br>TBA | CP | r<0.80,SEP>0.24cl.<br>r=0.87,SEP=2.6E-3<br>r=0.64,SEP=0.75 |
| Maran | LF-NMR | Exp. fit | Drip loss<br>Filter paper<br>IMF<br>Water<br>Myofibrillar space | P | r=0.72,SEP=2.1%<br>r=0.61,SEP=17.1%<br>r=0.57,SEP=1.4%<br>r=0.55,SEP=1.0%<br>Cross-sectional area proportional |
| Maran | LF-NMR | PLS | Drip loss<br>Filter paper<br>IMF<br>Water | P | r=0.75,SEP=2.0%<br>r=0.53,SEP=18.6%<br>r=0.68,SEP=1.3%<br>r=0.67,SEP=0.8% |
| Maran | LF-NMR | PLS | Sensory terms | CP | r<0.94,SEP>0.21cl. |
| Maran | LF-NMR | Exp. fit | Myofibrillar space | B | Post mort. swelling characterised |
| Imaging spectrograph | Imaging | PLS | Ageing | F | r=0.86,RMSEP=76m<br>r=0.97,RMSEP=28m<br>r=0.95,RMSEP=35m |
| Imaging spectrograph | Imaging | HI | Marbling | P | r=0.94 |

Meats: P: porcine, CP: cooked porcine, F: fish, B: beef.