CHAPTER 1

INTRODUCTION

THE NEW ANALYTICAL INSTRUMENTS

A new generation of analytical measurement equipment is advancing in industrial applications. These new measurement devices are yet another step in the industrial development, and the concept on these measurement techniques is a total integration of many sciences and technologies. The integration typically combines rapid measurement techniques with advanced data analysis and many years of practical experiences and laboratory testing.

Examples of new measurement equipment based on this concept are numerous and so are the objectives. The food industry is one of the fields where the new tools are becoming more common. Increased requirements from the consumers, growing markets and competition, and new food processing techniques are among the driving forces for the development of many new analytical tools in the food industry.

One common feature of the modern analytical instruments is the prevailing use of computers. The computers are e.g. used for data sampling, measurement optimisation, information decomposition, and regression studies. These issues are examples of the immediate advantage of using computer technology: the ability of rapid collection and handling large amounts of data. It is, however, striking that this approach only recently has been gaining the wider acceptance among instrumentation developers. In many applications traditional scientific approaches are still being used and the computer is often used to replace an obsolete traditional technique with no emphasis on the new advantages of the computer technology.

The inclusion of the vast quantity of data supplied by the computerised measurement in the measurement system is defined as multivariate data techniques. This definition covers both the data acquisition technique for the inspected object, and the multivariate statistical and mathematical analyses performed on this data. The latter subject is becoming known as chemometrics in the chemical sciences.

This study is dedicated to the possibilities of introducing new multivariate techniques in the area of meat quality evaluation. As discussed later in this chapter, despite a strong world-wide competition, and maybe actually due to this, the meat industry is currently paying extensive attention to the field of technological measurement equipment.

MULTIVARIATE DATA ACQUISITION TECHNIQUES

The modern multivariate data acquisition techniques are characterised by being fast and collecting numerous data points using adequate sensors. Two main groups within the multivariate data acquisition techniques are ultrasound techniques and spectroscopic techniques. Ultrasound measurements are based on audio pulses and spectroscopic techniques are based on light absorption, reflection and/or transmission, or vibrational spectroscopy as e.g. the measurement of nuclear magnetic resonance.

ULTRASOUND IMAGING

Ultrasound has been used for meat measurements for decades. The technique has been adapted from medical applications because of the ability to segment different biological tissue types. The separation between the tissue types can be located by measuring ultrasonic echoes (Whittaker *et al.* 1992) from the intersection between materials with difference in the acoustical impedance (Swatland 1995a). The ultrasound is advantageous by being a very fast acquisition technique, and this is utilised in the meat applications. Especially A-scanning transducers have been used with the Ultrafom (SFK Technology, Herlev, Denmark) or the A-Scan Plus (Sonic Industries, Hartboro, PA, USA). More advanced are the medical image scanners, which have been introduced for meat scanning in e.g. the AUS (Animal Ultrasound Services, Itchaca, NY, USA) and the Aloka (Simonsen & Weel, Taastrup, Denmark) systems. The images from the medical scanners are collected manually, and the output information from the images is supplied with a manual readout of the images or in combination with automatic image analysis software. Recently, SFK Technology introduced the Autofom as a fully automated ultrasound system for pork grading. The Autofom possesses several functional advantages compared to the manual operated ultrasound equipment, mainly the objectiveness and the total scanning of the carcass length.

SPECTROSCOPY

Spectroscopy has been a known and used analytical technique in many years. The basic principle of spectroscopy is to radiate the sample with a controlled wavelength and measure the response from the sample.

In optic spectroscopy the sample is excited by illumination from a light source. The light is transmitted, absorbed and reflected by the sample and this response can be measured with a detector (Kress-Rogers 1993). Among the mostly used optic spectroscopic techniques are UV reflectance (UV), visual reflectance (VIS), near infrared reflectance (NIR), and fluorescence. In UV, VIS, and NIR the surface reflection spectrally ranges below 400 nm, from 400-800 nm, and at 800-2500 nm respectively. In fluorescence measurements the illumination and the reflection are optimised to specific wavelength characteristics to account for fluorophors in the sample. A subdomain of the spectroscopic techniques is the vibrational spectroscopy, e.g. low field nuclear magnetic resonance (LF-NMR), where the excitation of the sample is not made with light frequencies but consists of a magnetic field and a radio frequency pulse. By controlling the spin of the hydrogen nuclei in the sample, different responses are obtained depending on the composition of the sample.

MULTIVARIATE DATA ANALYSIS

Multivariate data analysis techniques define the use of specific statistical and mathematical methods developed for dealing with the multivariate data. Basically there are two reasons for using these new data analysis methods: firstly the traditional statistical and mathematical methods have some drawbacks in dealing with the multivariate data, and secondly a decomposition of the multivariate data is usually necessary for human interpretation and validation. Two levels of multivariate data analysis are inspected here: the multivariate data processing (which in the chemical sciences is known as chemometrics) and image analysis.

CHEMOMETRICS

The term chemometrics describes the relation of measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods (Engelsen *et al.* 1998). The chemometric methods have been developed to account for the limitations of the traditional statistics, which suffer from two drawbacks when related to the multivariate data. First, the problem of dealing with the co-linearity of the multivariate data when, for instance, two NIR values at two adjacent wavelengths are very similar. Second, the problem of the usual statistical assumption of normal distribution rarely is fulfilled in chemical data series (Liang and Kvalheim 1996). Munck (1997) describes chemometrics as an explorative data analysis through a dialogue between the screening technique (the spectroscopic measurement) and nature (the reference information obtained with a traditional laboratory approach). With this explorative approach, a model between the data collection and reference information is established, and interpretation can be made using this model. Thus, the approach implies a backward hypothesis, where the information in the data limits what conclusions can be drawn.

The most used chemometric methods are principal component analysis (PCA) for decomposition and interpretation of large data set, and partial least squares regression (PLS) for linear regression of multivariate data (Martens and Næs 1993). Recently, methods for variable selection (Höskuldsson 1992; Leardi *et al.* 1992), non-linear regression (Berglund and Wold 1997; Bro 1995), multi-dimensional decomposition (Henrion 1994; Bro 1997), and multi-dimensional regression (Bro 1996) have been introduced to the field of chemometrics.

In food science chemometrics has especially been known within NIR research. However, in recent years, the techniques seem to be gaining wide use and respect in many industrial applications. An introduction to chemometric methods applied to meat quality applications is found in Næs *et al.* (1996). More specific examples of applications in meat quality is the use of neural networks used for pork carcass classification (Thodberg 1992) and for evaluating electronic magnetic scans (Berg 1998a), prediction of composition in LF-NMR (Jepsen *et al.* 1999) and NIR (Isakson *et al.* 1995).

IMAGE ANALYSIS

Image analysis defines a subgroup of modern digital signal analysis where two or more spatial dimensions are analysed. The techniques have proved efficient in fast and automated or semi-automated control of operations controlled by a computer. The main research in image analysis have been focused on medical applications, but in recent years also biological and multimedia applications are pushed forward in big scale.

Among the primary tasks in image analysis are feature extraction, object classification, colour vision and texture study. Russ (1994) and Gonzalez *et al.* (1992) are both general sources for image analysis. In the Autofom system, image analysis is used for feature extraction and combined with chemometrics. In meat research, image analysis has been used by for instance Buche *et al.* (1985), Chen *et al.* (1989), McDonald *et al.* (1989), Thane *et al.* (1990) Roudot *et al*. (1992), Ishii *et al*. (1992), McCauley *et al*. (1994), Ellekjaer *et al*. (1994), Ozutsumi *et al*. (1996), Sather *et al*. (1996), Borggaard *et al*. (1996), Horgan *et al*. (1995), Irie *et al*. (1996).

MUSCLE TO MEAT CONVERSION

The end quality of the meat product is a long integration of parameters and conditions. Some of these are connected to genetic material, handling and feed of the animals, transport to the abattoir, pre-slaughter stress, stunning method, electrical stimulation, cooling method and rate, maturing time, freezing and thawing, and cooking conditions. A review of the critical points in pork production is found in Berg

(1998b). For a comprehensive review of the muscle to meat conversion see Greaser (1986).

In pork, the muscle metabolism is especially essential. Muscle metabolism describes the chemical and physical changes in the muscle between the time of slaughter and the rigor mortis. Upon slaughter, the oxygen and glucose supply of energy to the living muscle terminates. The dead muscle attempts to maintain the energy level, using energy reserves kept in the myoglobin protein and intramuscular storage of glycogen, creatine phosphate, and Adenosine Triphosphate (ATP). During the generation of energy in the aerobic metabolism lactid acid is generated, and a decrease in the pH value from 7.4 to 5.3-5.7 is observed in porcine meat (Forrest *et al.* 1997). The pH decrease can under certain conditions cause extreme decrease in meat quality particularly in porcine in meat. If e.g. the animal is very stress susceptible and the glycogen level is low before to the slaughter, the pH decrease will occur rapidly post mortem and the meat can become pale, soft and exudative (PSE). If, however, the pH decline happens slowly and to a less extent the meat can become dark, firm, and dry (DFD). Intermediate quality classes as red, soft and exudative (RSE) and red, firm and non-exudative (RFN) have also been used in the literature. E.g. Kauffman (1997) used the four terms to separate between desirable (RFN and DFD) and undesirable (PSE and RSE) meat.

In beef, the tenderness is the most relevant and most widely discussed quality characteristic. A historical review of the factors influencing the meat tenderness mechanisms can be found in Ouali (1990). Drainsfeld (1994) proposed to separate the tenderness relation into three components: the tenderisation, the ageing, and the tenderness. The tenderisation is the enzymatic proteolysis, which cannot be measured early post mortem because of the muscle contraction up to rigor mortis. The meat ageing is the maturing of the meat, which was the traditional method of enhancing the meat tenderness by storage in up to three weeks. Commercially, meat ageing is an unattractive way of ensuring the meat tenderness because of the large required cooling capacity. Also due to meat safety aspects long storage periods are undesirable. The last of the tenderness component is the tenderness (or the inverse toughness) of the end product, the cooked meat. This component is related to an integration of components such as connective tissue, muscle shortening, sarcomere length, fat and water.

MEASURING MEAT QUALITY

Many authors have addressed the subject of meat quality and often with different definitions as the framework. In this study I have chosen to classify meat quality into three groups: meat composition, functional quality and eating quality. As the names tell, the three groups differ in both the conceptual definitions, effects for the producer, and the method of measurement. Probably the most obvious group of quality characteristics is meat composition, which denotes histological and chemical aspects of the carcass. The term functional meat quality covers the combined product of genetic quality and muscle to meat conversion. Eating quality is well defined by the name itself. The term describes the quality impression for the end consumer. Some will argue that meat safety is also a meat quality issue, but with the increasing focus on this matter meat safety is becoming a separate science.

Recent reviews on instrumentation and methods for meat quality measurements have been given by Forrest et al. (1997), Monin (1998), Cross *et al.* (1994) and Chapter 2. The table at the end of this chapter presents a resume of the most important results obtained in meat quality measurements. The following sections present a summarising discussion of the main issues in meat composition, functional meat quality, and eating quality. The table presents a brief summary of different instrumental approaches of evaluating meat quality.

MEAT COMPOSITION

Traditionally, the mostly discussed and mostly used meat composition parameter for pigs has been the meat percentage, or the lean, of the carcass. The use of pork grading based on carcass composition in Denmark goes almost a century back, and objective grading has been utilised in Denmark since the 1950s (Kempster *et al.* 1982, pp. 138-39). This has been a key factor in the world leading position that the Danish pork production has had for many years. The grading instrumentation has developed from visual inspection with optical probes, through rulers, fat depth probes to ultrasound scanners.

Beef composition is less discussed in relation to grading than porcine meat composition. The main reason for this is probably the less flexible utilisation in Denmark of the beef carcass. This is due to both processing issues on the abattoir and the packing plants and to the bonds to the dairy industry. However, recently the EUROP standard was introduced on the European market (Borggaard *et al.* 1996). This is a proposed standard evaluation of the shape, fatness, and colour of the beef carcass. These features represent a value of the beef carcass, which has been evaluated visually for years, but recently the use of colour vision systems have been introduced.

Meat composition, however, also covers qualitative issues. Primal cut meat percentage, where the quantity of the meat in the major carcass parts is determined, is a natural extension of the total meat percentage. Furthermore, intramuscular fat (IMF), marbling, water and protein are examples of qualitative composition information.

The table at the end of the chapter reviews some of the approaches that have been taken to evaluate compositional meat quality. For additional literature concerning on-line measurement of compositional meat quality the reader is referred to sources as Swatland (1995a) who among others review fat depth probes. For more information on the use of ultrasound, see sources like Liu *et al*. (1958, 1995), Thane *et al*. (1990), Perkins *et al*. (1992), Whittaker *et al*. (1992), Perkins *et al*. (1992), McCauley *et al*. (1994), Ophir *et al*. (1994), Park *et al*. (1994), Hamlin *et al*. (1995a,b), Stanford *et al*. (1995), Ozutsumi *et al*. (1996), Sather *et al*. (1996), Herring *et al*. (1998). Video imaging is discussed in Scholz *et al*. (1995), Borggaard *et al*. (1996) and Gerrard *et al*. (1996).

FUNCTIONAL MEAT QUALITY

The biochemical changes occuring in post mortem meat are highly relevant for the meat quality. The muscle energy metabolism and the protein degradation describe the biological processes during the post mortem muscle contraction when the muscle is being converted to meat. Also these processes, which continue until the meat is being consumed, are highly relevant for the meat quality. These biochemical changes occurring during the muscle to meat conversion and the post rigor tenderising processes are integrated into the term functional meat quality.

As discussed in one of the previous sections, the functional quality problem in porcine meat are mostly related to the water binding conditions. Therefore, most research in functional quality has been addressed to measuring the water holding directly (with drip loss, filter paper wetness, cooking loss or protein solubility) or indirectly (with e.g. pH, colour or conductivity). The tenderisation process is especially relevant for the discussion of whether beef quality can be monitored with several approaches including muscle fibre shortening, isometric tension, water and mechanical toughness (e.g. the Warner-Bratzler shear force; W.-B.).

Reichert (1996) and Monin (1998) gave a review on some of the techniques for measuring functional meat quality. Some of the principal results are reviewed in Table 1, where Kauffman (1997) compared and integrated the combinations of pH, FOP and conductivity. A review of pH relationships with pork quality is given by Bendall *et al*. (1988). Optical and electrical probes are discussed by Irie *et al*. (1992), Garrido *et al*. (1995), Swatland (1995), and fluorescence assessment of beef palatability is discussed by Swatland *et al*. (1996).

EATING QUALITY

The eating qualities describe the properties of the meat as interpreted by sensory panels, i.e. any of the human senses as discussed by Brockhoff (1994). In meat research the sensory attributes normally measured in user panel evaluations are colour, tenderness, flavour and odour as described by Bechtel (1986). Recently more scientific approaches are used, like in Byrne (1998), where 45 descriptive terms are reduced to 16 by an explorative approach, where the sensory panel is used to select the 16 most descriptive terms as evaluated by principal component analysis. The eating quality can be regarded as the ground truth of the meat quality, but due to the meat preparation and cooking, the fresh meat quality is often difficult to relate to the sensory quality. Measurements of eating quality with rapid techniques are attempted with e.g. fluorescence (Swatland 1995a) and with NIR (Park *et al.* 1998) (see the Table).

ON-LINE MEASUREMENTS IN MEAT ABATTOIRS

The meat industry has traditionally been very conservative. Compared to e.g. the medical industry, the research and development in meat science is far less advanced. However, the industry is currently undergoing dramatic changes, and there are no indications that this development will cease. The changes are noticed in the following observations:

- there is an increasing demand from the consumers and the media for optimal quality, consistency, safety, animal welfare, and environmental issues
- control of genetics and growth conditions has been used more widely
- countries from e.g. Asia, Eastern Europe and South America are becoming strong competitors among the producers
- the technological inventions are rapidly gaining accept and respect
- many new applications and meat products are being pushed forward

These observations suggest one thing: there is a strong need for a flexible meat industry. One of the most important tools in ensuring this flexibility is the control of meat quality and consistency. The control of meat leanness in Danish pigs has for instance been one of the main reasons for the success of the Danish pork export. Currently, however, the total lean is the only grading information used for feed back to the producers in the payment system. For sorting and processing optimisation on the abattoir processing line the lean is typically also the only information used. This has, especially for both pork and beef production, lead to the breeding for very lean animals, which also have also decreased the intramuscular fat and thereby the eating quality (Wood 1990).

On-line measurement is by Kress-Rogers (1993) defined as provision of data on a continuous production process that can be used to adjust process variables using feedback or feed-forward control. This requires that data are available in real-time or with a short delay.

The on-line measuring conditions naturally differ extensively for the different types of meat. This study focuses on the pork and beef, which are the types of meat with the most relevant potential of control of the end products. The control of the processing line depends on on-line available quality information, which is currently very limited and this has resulted in very inconsistent meat quality. In the past decade, the poultry industry has grown widely but has also been able to homogenise the products to a very consistent level. Therefore, the poultry issues are kept out of the discussions in this thesis, although many of the techniques treated will have a potential use in the poultry industry.

Pork abattoirs typically have a throughput of 300-1,000 carcasses per hour (c/hr). In Europe the typical line speed is 400-700 c/hr, whereas the line speed in US typically is 700-1,000 c/hr, although the line speeds occasionally go up to 1,250 c/hr. In a typical slaughtering process, the pigs are stunned, killed, and dressed in 30-45 minutes depending on the line speed and then brought into the cooler. Usually the grading measurement of the carcasses takes place at approximately 40 min. post-mortem, but with the recent Autofom ultrasound application the measurement is made at approximately 20 min. p.-m. In Danish pork abattoirs there are often possibilities of sorting the carcasses directly in the cooler, which highly improves the possibility of utilising the on-line information. This option is rarely seen in other countries.

In beef abattoirs, the throughput is considerably smaller than in the pork situation, normally 50-300 c/hr. The carcasses are slaughtered within 1 hour after stunning. As a natural consequence of the lower line speed, the slaughtering process is less automated than for pigs. There is often time for manual grading of the beef carcasses, and the newly introduced EUROP standard is most often followed in Europe. This standard evaluates the shape, colour and fatness of the carcasses. In USA, the intramuscular fat is also included in the grading situation, and this information is evaluated either manually by an inspector or with technological systems based on vision or ultrasound. Recently in Denmark automatic grading of the beef carcasses was introduced with the Beef Classification System (BCC-2, SFK Technology), which is based upon computer vision. Similar systems has been introduced in Germany (VBS2000, BGT), Canada (CVS, Lacombe), France (MORMACLASS, INRA) and Australia (VIASCAN, MLA).

SCOPE OF THE STUDY

The objective of this study is to investigate the possibility of introducing new multivariate techniques for evaluating meat quality to compliment the current grading equipments, where the industry is well equipped with e.g. the Autofom for pork grading and the BCC-2 for beef grading.

The Autofom ultrasound system and spectroscopic techniques set up the framework for the multivariate data acquisition. All methods investigated have potential for on- or at-line applicability. Not necessarily all animals on an abattoir line are measured in this introductory work, but the methods posses significant improvements compared to the existing quality methods. The spectroscopic techniques involved are the VIS, NIR, fluorescence, Raman, and LF-NMR. The system for measuring VIS and NIR is an industrial system, whereas the remaining measurements are made with laboratory equipment. Special attention is paid to a fibre optical system for measurement of VIS with an insertion probe. No such system is available for multivariate use in meat, and a dedicated system is developed based on an existing multi purpose equipment.

Porcine meat is studied for composition quality (meat percentage, meat primal percentage, intramuscular fat, marbling, loin muscle area, and back bacon) and functional quality (WHC) and eating quality (sensory scores of warmed-over flavour properties in cooked meat). Beef is investigated for functional meat quality related to tenderness (WB, isometric tension, muscle fibre shortening, pH, and cooking loss).

The purpose of the work is to outline the possibilities for providing sorting (feed forward) information and/or grading (feed back) information based on fast multivariate techniques for measuring meat quality and consistency.

Dell Allen (1998) has said: *You can’t manage what you can’t measure!*. Let these wise words conclude the introduction to this dissertation describing the possibilities of introducing new techniques for measuring quality of meat on-line.

Table 1. On- and off-line approaches for measuring meat quality. MQM: Meat quality meter. FOP: fibre optical probe. W-B: Warner Braztler.

INSTRUMENT	TECHNIQUE	REFERENCE	MEAT	RESULT	LITERATURE
Fat-O-Meater	NIR	Lean	Porcine	R^2=0.68-0.90	Walstra (1989)
Henessy	NIR	Lean	Porcine	R^2=0.66-0.86	Walstra (1989)
Classification	NIR	Lean	Porcine	R^2=0.77-0.81	Walstra (1989)
MQM	NIR	Pigment	Beef	R^2=0.91	Andersen *et al.* (1995)
	NIR	pH	Porcine	r=-0.73	Swatland (1983)
Henessy & Meat-Check	NIR	PSE, RSE, RFN or DFD	Porcine	55-74% correct classified	Kauffman (1997)
NIR Systems	NIR	Collagen solubility	Porcine	r<0.88	Young *et al.* (1996)
	NIR	W-B	Beef	r=0.79	Hildrum *et al.* (1994)
	NIR	W-B	Beef	R^2=0.67	Park *et al.* (1998)
Technicon	NIR	Moisture, protein, fat	Beef	r=0.98-0.99	O'Keeffe (1987)
Zeiss MCS 210	VIS	Pigment	Porcine	r=0.96	Andersen *et al.* (1989)
Colormet	VIS	Dark cutting Grade	Beef	3 classes observed	Gariépy *et al.* (1994)
DSR	VIS	Pigments		Positive relations	Osawa (1995)
	VIS	Quality index	Porcine	r=0.83	Swatland *et al.* (1992)
Optical probe & Quality Meter	VIS El. cond.	Prot. solubility	Porcine	R^2=0.49 R^2=0.58	Olivier *et al.* (1991)
TOBEC	El. cond.	Lean Ham lean	Porcine	R^2=0.85 R^2=0.89	Kuei *et al.* (1990) Meseck *et al.* (1997)
	El. cond.	Visual score	Pork	r=0.71	Schmitten *et al.* (1985)
TOBEC	El. cond.	Lean	Pork	R^2=0.90	Berg *et al.* (1994)
Aloka	Ultrasound	IMF Marbling	Beef	r=0.61 r=0.75	Herring *et al.* (1995)
Aloka	Ultrasound	Marbling	Beef	R^2=0.45	Whittaker *et al.* (1992)
	Ultrasound	IMF	Live pigs	Within 1 class	Ragland *et al.* (1998)
Aloka	Ultrasound	Yield grade	Pork	R^2=0.75	Hamlin *et al.* (1995b)
Aloka	Ultrasound	Loin area	Pork	R^2=0.62	Hamlin *et al.* (1995a)
	Colour vision	Marbling	Beef	R^2=0.86	Gerrard *et al.* (1996)
BCC-2	Colour vision	Conformat., fatness, fat colour	Beef	R^2=0.57 R^2=0.97 R^2=0.59	Borggaard *et al.* (1996)
	Fluorescence	Toughness, taste,juicin., hardness	Beef	r=0.66-0.83	Swatland *et al.* (1995a)
Brueker	NMR	IMF	Pork		Geers *et al.* (1995)
Lunar DPX-L	X-ray	Protein Water	Pork sides	R^2=0.97 R^2=0.99	Mitchell *et al.* (1998)
	Filter paper and vision	Area	Beef, pork	r=0.92	Irie *et al.* (1996b)