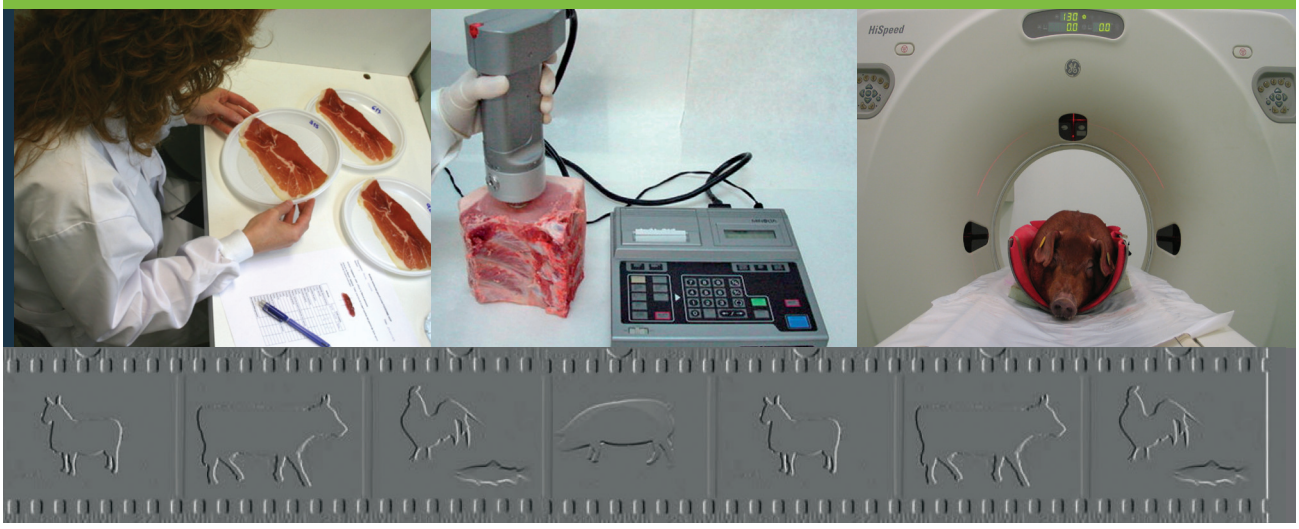
FAIM

Farm Animal IMaging

A handbook of reference methods for meat quality assessment



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A handbook of reference methods for meat quality assessment

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FOREWORD

Cost Action FA1102 "Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals (FAIM)" started in November 2011 and will end in November 2015. It has been a very ambitious program and 4 working groups have contributed to the delivery of the promised milestones and deliverables.

FAIM brings together > 300 experts from 23 (27) EU countries (and beyond). We aim to optimise non-destructive *in vivo* (iv) and *post mortem* (pm) imaging and spectroscopic methods for the measurement of body composition and meat quality (MQ) in major farm animal species and to devise standardised principles of carcass classification and grading (CCG) across countries. Such work is necessary for the development of value-based- payment and marketing systems (VBMS) and to meet the urgent need for market orientated breeding programmes.

FAIM encompasses a collaboration of hard- and software manufacturers with livestock and imaging academic experts to develop the required products for implementing the scientific work. FAIM helps to coordinate and strengthen EU scientific and technical research through improved cooperation and interactions. This is essential for achieving the required advances in CCG systems to measure carcass yield and MQ, to meet the industry need for VBMS, and to improve production efficiency throughout the meat supply chain.

FAIM also supports EU legislation on individual animal identification through showing additional benefits of feeding back abattoir data on individual animals for optimising management, breeding and providing phenotypic information, which helps to facilitate the implementation of genome-wide- selection.

The main aim of FAIM is to identify, optimise and standardise non-invasive iv and pm imaging and spectroscopic methods for the measurements of body composition and meat quality in major farm animal species, to integrate automated systems for their objective assessment, and to facilitate effective data capture and management at the individual animal level.

The tasks were very complex and to make the "full circle", the feedback of recent and future valuable information obtained in the abattoir to the producer and breeders, we organised our network in 4 working groups.

Working Group 1: Body/Carcass composition aimed at (i) knowledge exchange to develop harmonised procedures for *in vivo*, *post-mortem* and on-line imaging methods of predicting compositional traits; (ii) the development of a strategy for defining references for compositional traits and evaluating their robustness; (iii) the coordination of the creation of an imaging toolbox (e.g. phantoms, atlases) and to review the hardware and equipment available in Europe.

Working Group 2: Meat Quality had similar tasks but related to meat quality. A main task was to review existing procedures and equipment for *in vivo*, *post-mortem* and on-line imaging and spectroscopic methods of predicting MQ in livestock and suggest models to harmonise those.

Working Group 3: Algorithms and Working Group 4: traceability work towards (i) algorithms for data capture and automated or semi-automated image processing and to review available software; (ii) the coordination of building a "data warehouse"; (iii) a review and evaluation of existing systems and implementations of individual animal traceability systems with special focus on traceability in the abattoir.

One output of the work in working group 2 is now published in form of this handbook alongside with other FAIM outputs and we hope you will find these useful for your own work in this or related areas.

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and
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INTRODUCTION

Cost Action FA1102 "Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals (FAIM)" aims to optimise non-destructive *in vivo* and *post mortem* imaging and spectroscopic methods for the measurement of body composition and meat quality in the major farm animal species and to devise standardised principles of carcass classification and grading across countries.

These actions are necessary for the development of value-based payment and marketing systems and to meet the urgent need for market orientated breeding programmes. Work Group 2 (WG2) of FAIM, led by Maria Font i Furnols from Catalonia/Spain and Marjeta Čandek-Potokar from Slovenia focused on methodology of meat quality assessment with the main objective to review existing procedures and equipment for *in vivo*, *post mortem* and on-line imaging and spectroscopic methods for predicting meat quality in livestock. All these imaging and spectroscopic methods need reference methods for their calibration and validation, and, consequently, one of the FAIM milestones was to prepare a handbook of reference methods for the most important meat quality attributes.

To select the relevant meat quality parameters for pig, beef, sheep and poultry a questionnaire was sent to FAIM participants and distributed to the different stakeholders of the production chain, research centres and universities. A total of 106 questionnaires from 17 EU countries were collected by this work group: 34.9% for pig, 31.1% for beef, 17.9% for ovine and 9.4% for poultry (the rest were for rabbit, fish and game animals). According to the results of the survey and after discussion with meat scientists, a list of attributes by species was presented and discussed within WG2 at FAIM II conference and a unanimous agreement was reached. The agreed relevant meat quality parameters are those included in the different chapters of this handbook, and the most common reference methods used in various European laboratories to assess them are presented. Each chapter consists of an introductory section with a definition of meat quality attributes, a section with the sources of variation, and a section with the reference methods which includes some practical aspects and a comparison of different reference methods.

Furthermore, as the goal of FAIM is to replace these reference methods by other non invasive or non destructive imaging and spectroscopic technologies that can be used either *in vivo* or on/in line. For this reason a chapter giving an overview of the technologies that can be used for this purpose has been included. Since these technologies need to be calibrated and validated, an overview with some information regarding appropriate calibration and validation procedures as well as some of the most commonly parameters used to determine the prediction adequacy has been included in another chapter.

We are very grateful to the experts that participated in FAIM and contributed ideas and useful information for the handbook. In particular, we would like to thank the experts that participated in the writing of the different chapters of this handbook.

This handbook will not solve all the questions and difficulties related to reference methods, but we hope this document will be useful to scientists and technicians as it provides several reference methods for the most important meat quality attributes and gives guidelines for the accurate assessment of meat quality.

Maria Font-i-Furnols,
Marjeta Čandek-Potokar,
Maja Prevolnik Povše and
Charlotte Maltin - Editors

CONTENTS

Chapter 1

Protein, fat, moisture and ash

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1.1 Definition of the meat quality attributes

In general, meat is composed of moisture, protein, fat, minerals as well as a small proportion of carbohydrate, and the chemical composition of lean meat cuts is, on average, approximately 72% water, 21% protein, 5% fat and 1% ash. The most valuable component, from the nutritional and processing point of view, is protein. Moisture content is the most variable component of meat, and it is closely, and inversely, related to its fat content; the fat content is higher in entire carcasses than in lean carcass cuts. The fat content is also high in processed meat products, where high amounts of fatty tissue are used. The value of meat is essentially associated with its content of protein. In the animal body, approximately 65% of the proteins are skeletal muscle protein, about 30% connective tissue proteins (collagen, elastin) and the remaining 5%, blood proteins and keratin in hairs and nails.

1.1.1 Moisture

The largest part of meat consists of moisture, and it is important from both a sensory and technological point of view, as it influences eating quality factors, such as tenderness and juiciness, and the processing quality of the meat, as well as from an economical point of view as it contributes to the weight of the meat; moisture loss is weight loss. As moisture is the only component of meat that is substantially volatile at temperatures just above 100°C, the moisture content can be quantified by drying at such a temperature. Regarding the capacity for retaining the water in meat, in general beef has the greatest capacity, followed by pork, with poultry having the least.

1.1.2 Protein

Typically, meat contains about 19% protein of which 11.5% is structural proteins – actin and myosin (myofibrillar), 5.5% is the soluble sarcoplasmic proteins found in the muscle juice, and 2% is the connective tissues – collagen and elastin, encasing the structural protein. Collagen differs from most other proteins in containing the amino acids, hydroxylysine and hydroxyproline and no cysteine or tryptophan. Elastin, also present in connective tissue, has less hydroxylysine and hydroxyproline. Hence the protein value in cuts of meat that are richer in connective tissue is lower. The content of connective tissue in these cuts makes them tough and often lowering their economic and eating quality values.

Protein is the main component in meat that contains nitrogen, and the nitrogen content of meat is roughly constant. Therefore, the protein content of meat is determined on the basis of total nitrogen content, with the Kjeldahl method being almost universally applied to determine nitrogen content. Nitrogen content is then multiplied by a factor to give the protein content. This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen, and that nearly all of the nitrogen in the diet is present as amino acids in proteins. On the basis of early determinations, the average nitrogen (N) content of proteins has been found to be about 16%, which led to use of the calculation N × 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content. The factor 6.25 is also used to convert total nitrogen in meat to the total protein content of meat.

1.1.3 Fat

There are three main sites in the body where fat is found:

i) the largest amount by far is in the storage deposits under the skin (subcutaneous fat) and around the organs (visceral fat or flare fat). This constitutes the obvious, visible fat in a piece of meat, and can be as much as 40-50% of the total weight in fatty meat or fatty bacon. This adipose tissue is composed largely of triglycerides. Clearly this visible fat can be trimmed off the meat during processing, before cooking or at the table.

ii) in smaller cuts and streaks of fat can be visible between the muscle fibre bundles, i.e. in the lean part of the meat; this is known as intermuscular fat and can constitute approx. 4-8% of the weight of lean meat.

iii) there are often small amounts of fat (flecks) within the muscle structure, belonging to the intramuscular fat or marbling or part of the structural fat, which includes phospholipids and to some extent long chain fatty acids. The amount of this fat fraction varies with the tissue, and can constitute of 1-3% of the wet weight of muscle.

1.1.4 Ash

Meat contains a wide variety of minerals. The contents of iron, zinc and copper vary considerably in different species. High levels of minerals in the feed do not necessarily increase the level of mineral in the meat. Ash is the inorganic residue remaining after the water and organic matter (protein, fat, carbohydrates) have been removed by heating at high temperature (500-600°C) in the presence of oxidizing agents. This provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals can be distinguished from all the other components within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. The ash content of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, e.g. dried beef. Sodium chloride and phosphates are often the main component of the ash in many processed meat products.

1.2 Factors of variation

The limited effect of **feeding** on the nutrient composition of lean meat can be illustrated by a classical experiment by Harries et al. (1968), in which the composition of intensively-reared beef fed barley and protein supplements with grazing *ad libitum*, was compared with extensively-reared (grazing alone) as two extremes of husbandry practice. Analysis of the same muscles from animals from the two systems showed no significant differences in the protein and fat contents. There were greater differences between animals fed from the same system on different farms, than between different feeding systems. This shows that management practices had a larger effect.

As **animals grow**, the proportions of total nitrogen and fat increase as the animals approach maturity and more slowly thereafter. Collagen, which is a part of the connective tissue, becomes less soluble and less digestible, so poorly fed animals takes several years to reach an optimal size, provide meat of lower eating quality. Animals killed after a lifetime of work provide even tougher meat.

In pigs, when comparing the three **sexes** entire male, entire females and castrated males all with a live weight of 120 kg (IRTA, Zomeño et al. 2015), it was found regarding fat, that castrated males had a higher content of body fat than both entire males and females, and that females had more body fat than entire males. Regarding protein content of the carcasses, castrated males had a lower protein content compared to both entire males and

females. The moisture content showed an inverse relationship with the fat content, showing that castrated males had a lower moisture content compared to both entire males and females, and females had less moisture content then entire males. Finally, the ash content of the carcasses showed that entire males had higher ash content than both castrated males and females. This finding is supported by Latorre et al. (2003) who analysed chemical composition of the loin.

Latorre et al. (2003) found when comparing loins from different **genotypes**, Danish-Duroc (DD) with Pietrain × Large White cross (PLW) slaughtered at a live weight of 117 kg, that loins from DD had a higher fat content and lower water content, compared to the PLW cross. No difference was found regarding protein content in the same muscle.

Meat composition is also different depending on the **species**. Adeniyi et al. (2011) found higher lipid content in beef than broiler meat, while ash and nitrogen free were higher in broiler than beef. No differences were found in crude protein. Moisture and fat were found higher and protein lower in lamb than in broiler and beef by Karakök et al. (2008), and no differences were found in ash content.

Cooking does not affect the protein content in ground beef. It has been shown by the University of Wisconsin Extension that pan-frying or -broiling meat patties left the protein in the meat intact. In addition it did provide a healthy benefit for high-fat meats. Cooking reduced the amount of fat in the meat by almost half. Lean meat lost a very small amount of fat during cooking, but both high-fat and lean meats kept all of their protein and iron.

Cooking method can also affect at the proximate composition. In this sense, for instance, in camel meat, Nikmaram et al. (2011) studied the composition of raw meat, and cooked meat in microwave, roasted or braised and found as expected, that moisture was much higher in raw meat than in the three different cooking methods, ash content were higher in microwave cooked meat than raw meat and intermediate in the others, fat content was higher in microwave cooked meat than the others and protein was higher in microwave and braising cook meat compared with roasted meat and this higher than raw meat.

Brugiapaglia et al. (2012) carried out a study to evaluate the effect of two cooking methods on the nutritional value of *semitendinosus* muscle of Piemontese breed. The results showed little variation in values between roasting and grilling, but as expected the two cooking methods modified the chemical composition and nutritive value of the meat, but no differences between cooking methods were found. Cooked meat showed lower water contents and consequently higher energy values as well as protein and fat content than raw meat.

It was showed that moisture, fat and protein is not affected by **pH** of the meat, at least in *longissimus thoracis* of beef muscle (Holdstock et al., 2014).

1.3 Reference methods of measure

1.3.1 Protein

All references used are based on Kjeldahl total nitrogen determination (ISO 5983-1:2005) based in the transformation of the organic nitrogen in ammonium ions by acidification followed by a distillation in basic environment and a final valuation (Figure 1). It is important to use a precise scale (0.1 mg) for the weightings. The percentage of nitrogen total is obtained using a factor of 0.14. From this percentage a factor of 6.25 is applied to convert the nitrogen to meat protein.

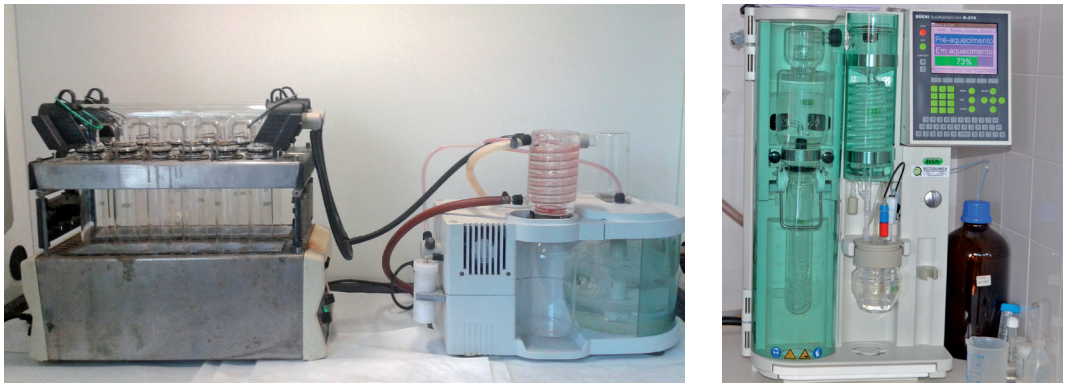

Figure 1: Digestion (left) and distillation (right) process to determine protein content.

1.3.2 Moisture

In general, moisture is evaluated by drying in an oven at 100-105°C until the sample reaches a constant weight (Figure 2). Alternatively it is also possible to use either freeze drying at room temperature for 96 h, or to use microwave (600 W) for 10 min. In any case it is important that the weighing is done precisely (0.1 mg) since weight is used for the calculation of the moisture content. The oven methodology the ISO (ISO 6496:1999) establishes that the difference between two repeated measures should be less than 0.1% (0.10 g for 100 g of samples).

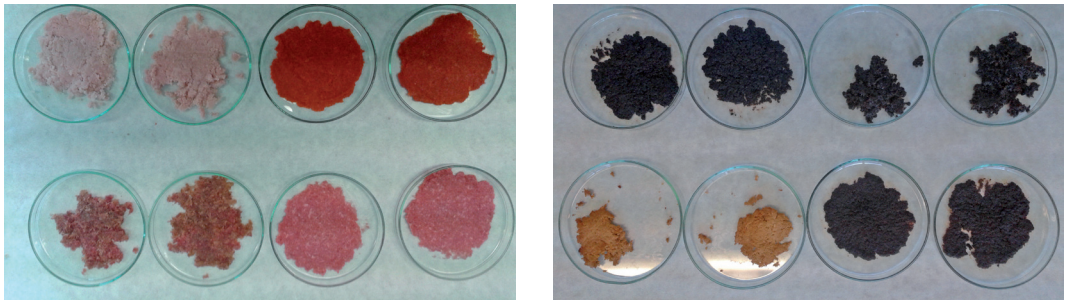

Figure 2: Meat before and after drying to obtain the moisture content.

1.3.3 Fat

There are basically two main methods to evaluate the fat content, a method based on Soxhlet extraction (ISO 6492:1999) with or without previous acid hydrolysis and petroleum ether (Figure 3) and a method based on Folch method (Folch et al., 1957; see more details in Chapter 6), extracting the fat with a mixture of chloroform and methanol. In the Chapter 2 about intramuscular fat determination changes in these methodologies are detailed as well as some photos of the procedure.

Figure 3: Equipment used to determine fat content with Soxhlet method.

1.3.4 Ash

Usually ash is evaluated by means of muffle oven 'ashing' at 500-550°C (Figure 4). However, it is also possible to use microwave 'ashing'. Since the measure is based in weighing, samples have to be weighed precisely (0.1 mg). The ISO (ISO 5984:2002) establishes that the difference between two repeated measures should be less than 0.10 g for 100 g of sample.

Figure 4: Ash evaluation process, from left to right, fresh sample, weighing, muffle oven and ash.

1.4 Parameters that can affect determination of chemical composition

In a comprehensive and comparative study by Pérez-Palacios et al. (2008), the efficiency of six extraction methods for the analysis of total fat content in meat and meat products, including the Soxhlet and Folch methods, were evaluated. Fat content was analyzed in meat products with different fat levels. It was concluded that both the Folch and the Soxhlet methods with previous acid hydrolysis, are suitable for meat and meat products having low, intermediate and high fat content. For analyzing meat and meat products with a very high fat content, either the Folch or Soxhlet method without previous acid hydrolysis could be used. In general, it has been reported that the Soxhlet method with hydrolysis gives a higher fat content estimate compared with the Folch method (Prevolnik et al., 2005; Gallina-Toschi et al., 2003). A comparison between methods is also presented in Chapter 2 on intramuscular fat.

1.5 References

1.5.1 Methodological references

Protein:

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ISO 5983-1:2005. Feeding stuffs – Determination of nitrogen content and calculation of crude protein content – Part 1: Kjeldahl (N x 6.25).

§ 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin.

AOAC. 976.05, 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Washington, DC.

Schormüller J 1968. Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1203.

Moisture:

Drying at 100-105°C to constant weight.

Schormüller J 1968. Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1200-1201.

ISO 6496:1999, Animal feeding stuffs – Determination of moisture and other volatile matter content.

AOAC Official method 950.46B(a) 18th Edition 2005.

Freeze drying
AOAC Official method 950.46B(a) 18th Edition, 2005.

Microwave
§ 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin.

Ash:

Ashing at 500-600°C.

AOAC. 920.153, 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Washington, DC.

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ISO 5984:2002 Animal feeding stuffs – Determination of crude ash.

Microwave
§ 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin.

Fat:

Folch method
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Nikmaram P, Yarmand MS and Emamjomeh Z 2011. Effect of cooking methods on chemical composition, quality and cook loss of camel muscle (*Longissimus dorsi*) in comparison with veal. African Journal of Biotechnology 10, 10478-10487.

Pérez-Palacios T, Ruiz J, Martín D, Muriel E and Antequera T 2008. Comparison of different methods for total lipid quantification in meat and meat products. Food Chemistry 110, 1025-1029.

Prevolnik M, Čandek-Potokar M, Škorjanc D, Velikonja-Bolta Š, Škrlep M, Žnidaršič T and Babnik D 2005. Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. Journal of Near Infrared Spectroscopy 13, 77-85.

Zomeño C, Gispert M, Carabús A, Brun A and Font-i-Furnols 2015. Predicting the carcass chemical composition and describing its growth in live pigs of different sexes using computed tomography (accepted for publication). doi:10.1017/S1751731115001780

Chapter 2

Intramuscular fat and marbling

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2.1 Description of meat quality parameter

2.1.1 Intramuscular fat

Fat tissue is formed by adipogenesis, which can be stimulated by insulin and glucocorticoids hormones and by insulin like growth factor I (IGF-I). In the carcass, different types of adipose tissue can be found: subcutaneous fat, intermuscular fat, flare fat and intramuscular fat (IMF). In live animals, there are other fat depots in the visceral and intra-abdominal area. IMF is deposited between fascial or muscle fibre bundles mainly as adipocytes but in lesser amounts also within the cytoplasm of the myofibres. IMF develops later than other adipose tissues and has different characteristics compared to subcutaneous fat in terms of development of cellularity and metabolic capacity. Two types of lipids can be found in the muscular tissue: depot lipids and structural lipids. Depot lipids are composed mainly of triglycerides although small amounts of monoglycerides, diglycerides and fatty acids can also be present. When these lipid depots increase in size and number, the droplets can be visible in the muscle surface, showing white flecks or streaks which is known as marbling. Structural lipids are found in the cell membranes and comprise phospholipids and cholesterol. These membrane lipids are important for muscle structure and function. Thus, IMF is mainly composed of triglycerides, phospholipids and cholesterol.

IMF is the last adipose tissue to be deposited, because in young animals it deposits at a lower rate than muscle tissue, while in older animals it deposits at a higher rate than muscle tissue. IMF is accumulated during growth, because of the increase of both the number and the size of adipocytes, and although it is related with the amount of other fat depots, it is not dependent of them (Yan et al., 2006). Metabolic routes for IMF synthesis depend on the species. In ovine, bovine and porcine species IMF is synthesised in the muscle while in poultry it is synthesised in the liver and is then transported by blood stream. Synthesis of fat in the muscle comes from the uptake of blood fatty acids by muscle, and endogenous synthesis and degradation of triacylglycerols. Synthesis of fat in the liver comes from the dietary fat supply, synthesis *de novo*, uptake by muscle of blood non esterified fatty acids and partitioning of fatty acids towards oxidation. Thus, IMF content depends on the variation of adipocytes in the muscle in terms of quantity and metabolic activity and also it depends on the muscle growth rate and the metabolic activity of other organs such as liver (Hocquette et al., 2010).

Some studies in pigs have shown that IMF is related to the tenderness and other palatability traits of the meat, which can affect consumer acceptability (Fortin et al., 2005; Font-i-Furnols et al., 2012). Apart from tenderness, IMF impacts more importantly on both juiciness and flavour because of lubrication during chewing (Thompson, 2004) although this effect is not clear in some other studies (O'Mahoney et al., 1991-1992; Channon et al., 2004).

2.1.2 Marbling

When the intramuscular fat is visible between the bundle of muscle fibres and close to capillary beds in the muscle surface is known as marbling (Harper and Pethick, 2004). Thus, marbling refers to the appearance of evenly distributed white flecks or streaks of fatty tissue between bundles of muscle fibres (Tume, 2004). The distribution of the flecks and streaks as well as its size and shape can be very variable between and within muscles which makes its evaluation difficult. Marbling is also dependent on the species and, for instance in beef is usually more visible than in pork (except some very marbled genotypes). In poultry marbling is less visible due to the low levels of marbling (<1% in breast muscle – Hocquette et al., 2010) and the light colour of the meat. Since premiums are paid for marbled meat, marbling is included as a quality parameter in some quality standards around the world.

2.2 Factors of variation

The IMF variation and consequently the marbling can be explained by a large number of factors such as species, age, maturity, breed, diet, slaughter weight, gender, muscle localization and myofibre type. These factors interact with each other resulting in a complex relation with the IMF development (Gao and Zhao, 2009; Hocquette et al., 2010). Often this relationship presents conflicting results between studies (Gao and Zhao, 2009). This section will present and discuss some of the most important factors related with IMF and marbling.

Species is one of the factors that most influences IMF content. It is well established that cattle, pigs, and sheep can deposit large quantities of IMF (Kauffman, 2012) whereas others such as rabbit, horse or goat deposit very little IMF (Culioli et al., 2003). Within species, several studies show that some **breeds** have greater tendencies to deposit IMF; e.g. Duroc pigs appears to contain more IMF for a given degree of maturity or age than other breeds (Hocquette et al., 2010). Also for cattle, at the same level of maturity, Angus presents higher values of IMF than Hereford or Charolais (Kauffman, 2012). However, for cattle the best example is presented by the Japanese Black breed in which an IMF range between 13 and 34% was reported (Albretch et al., 2011; Shirouchi et al., 2014). Additionally, differences exist among breeds not only in the amount of IMF but also in the structure and distribution of the marbling flecks in muscles (Yang et al., 2006; Albrecht et al., 2011). The distribution of marbling flecks is assessed as coarseness and fineness texture features for the evaluation of meat quality of Japanese Black breed and crosses (Osawa et al., 2008; Maeda et al., 2013).

The **chronological age** and **maturity** of the animals interact with IMF (Kauffman, 2012). In fact, the relative growth rates of the various body tissues are very different and fat is a late-developing tissue, the relative content of which increases at a slower rate than bone or muscle. In general, IMF is the last tissue deposited in finishing meat animals although adipose tissue starts to accumulate earlier (Harper and Pethick, 2004; Pugh et al., 2005). For cattle, growth coefficients in a log-log regression of subcutaneous fat, intermuscular fat and IMF weight in total fat weight were 1.01, 0.97 and 0.91, respectively (Wood, 1990). For lambs, early IMF relative growth was discussed by Pethick et al. (2007a) and Mcphee et al. (2008). The analysis revealed that the proportion of IMF in the loin relative to total carcass fat decreases as animals mature, thus indicating that IMF deposition occurs early in the maturation of sheep. The number and distribution of marbling flecks within the muscle also evolves along with the animal age. For example, Albrecht et al. (2006) studied four cattle breeds and found a 40-fold increase in the number of marbling flecks and a 4-fold enlargement in the marbling flecks from 2 to 24 months of age.

In meat producing species, IMF and subcutaneous fat thickness are genetically positively correlated (Bindon, 2004; Suzuki et al., 2005), thus the continued selection for increased lean growth leads to a reduction in associated carcass fatness and consequently in a decrease in IMF content (Clelland et al., 2014). In addition, excluding some breeds like the

Japanese Black cattle, the IMF (%) increases relatively slowly until a carcass fatness of about 30-35% is reached (Pethick et al., 2007a). Therefore, it is necessary reach a heavy carcass weight to meet consumer demands for IMF content, which ranges from 2.0 to 5.0% (Verbeke et al., 1999; Hopkins et al., 2006). The minimum amount of IMF to achieve acceptable consumer satisfaction is about 3% to 4% for beef (Savell and Cross, 1988), 5% for sheep meat (Hopkins et al., 2006) and 2.5% for pork (Enser and Wood, 1991; Fernandez et al., 1999). Moreover, fatness levels around 30% are irreconcilable for profitable production systems and also for consumer expectations for a low fat level surrounding retail cuts (Pethick et al., 2007a). The major industry challenge is to produce meat with enough IMF to satisfy eating experiences, but without any excess of fat so as to satisfy health concerns and to provide meat products with a good appearance (Hocquette et al., 2010).

Feeding is a factor that greatly influences the percentage of IMF. Good examples related to cattle are presented by several authors (Crouse et al., 1984; French et al., 2000). In these studies cattle were fed high-energy concentrate versus forage-based diets which increased levels of IMF. Net energy available results in a higher IMF content (Pethick et al., 2004). Much evidence has been gathered confirming that in pork, a subtle protein deficiency will increase marbling (Pethick et al. 2007b) whereas for cattle it is necessary to increase the days on high energy density feed to increase marbling (Brethour, 2000).

Muscle **location** and **myofibre type** also account for IMF variation. Differences were found between muscles but also within the same muscle. For example, in young bulls differences in IMF percentage between *longissimus dorsi* and *semitendinosus* muscles were found (Costa et al., 2013) and in pigs, differences were reported between *trapezius* (5-6%), *rhomboideus* (3.5%), *longissimus dorsi* and *semitendinosus* (1.5-2%) (Gondret and Hocquette, 2006). Faucitano et al. (2004) studied 14 locations on the *longissimus dorsi* muscle from 50 crossbred pigs and reported that the highest IMF values were obtained in the middle section of the thoracic region (T5-T8) and in the middle-caudal section (L2-L4) of the lumbar area. On the other hand, Huang et al. (2014) in cattle found differences along the *longissimus dorsi* with high IMF content recorded in L6 and L7. From a practical point of view, this variation is important for sampling site choice. The difference of IMF content between muscles is related with the myofibre type (Gotoh, 2003). This author studied several muscles of fattened Japanese Black steers and found correlation between the percentage amount of intramuscular fat and the percentage distributions of type I ($r = 0.88$) and type IIB ($r = -0.72$) myofibres respectively.

2.3 Reference methods

2.3.1 Reference methods for intramuscular fat

Reference methods are methods published by supranational organizations, and used and recognized by the scientific community, but do not necessarily represent the official national standardised methods of the different countries. AOAC International (Professional Association Dedicated to Analytical Excellence) and NMKL, Nordic Committee on Food Analysis are examples of organizations that developed official methods for food analysis. The Food and Agriculture Organization of the United Nations (FAO) has also published food standard codes in *Codex Alimentarius*. In recent years, the ISO (International Organization for Standardization) has developed and published standards of methods of chemical analysis, some of them are applicable also to meat and meat products.

Triacylglycerol is the most important chemical component in intramuscular fat. Phospholipids, free fatty acids, sterols, mono and diacylglycerols and fat-soluble vitamins are present in smaller amounts. The choice of a method to determine fat content depends on the components we want to assess and be included in the analytical result. The Soxhlet

extraction (Soxhlet, 1879) using a non-polar solvent such as petroleum ether is the classical method, which extracts the major part of triacylglycerol and cholesterol, but only a fraction of phospholipids and lipoproteins. If phospholipids are to be included in the analysis, then a previous acid hydrolysis should be performed with hydrochloric acid (Figure 1). All the different steps of the soxhlet extraction were done manually. However, nowadays there are some devices in the market that allow a more automatic extraction of fat (Figure 2). Moreover it is possible to obtain the total fat, phospholipids included, without a previous hydrolysis once some devices have optimized applications, using a hot extraction. The sample is placed directly in the beaker containing the boiling solvent (petroleum ether) that is refluxed at the end of each cycle. The Soxhlet extraction chamber is emptied when the set level (containing meat sample) is reached, with the solvent flowing to the heated beaker. During each cycle a portion of the fat dissolves with the solvent. At the end of the process the fat is concentrated in the beaker. This is an automated process, which increases the turn over (reducing solvent consumption) and determines the crude fat directly without the time consuming hydrolysis prior to extraction. For fresh meat, a program with 60 cycles with approximately 7 h is enough for this purpose.

Figure 1: Acid hydrolysis process.

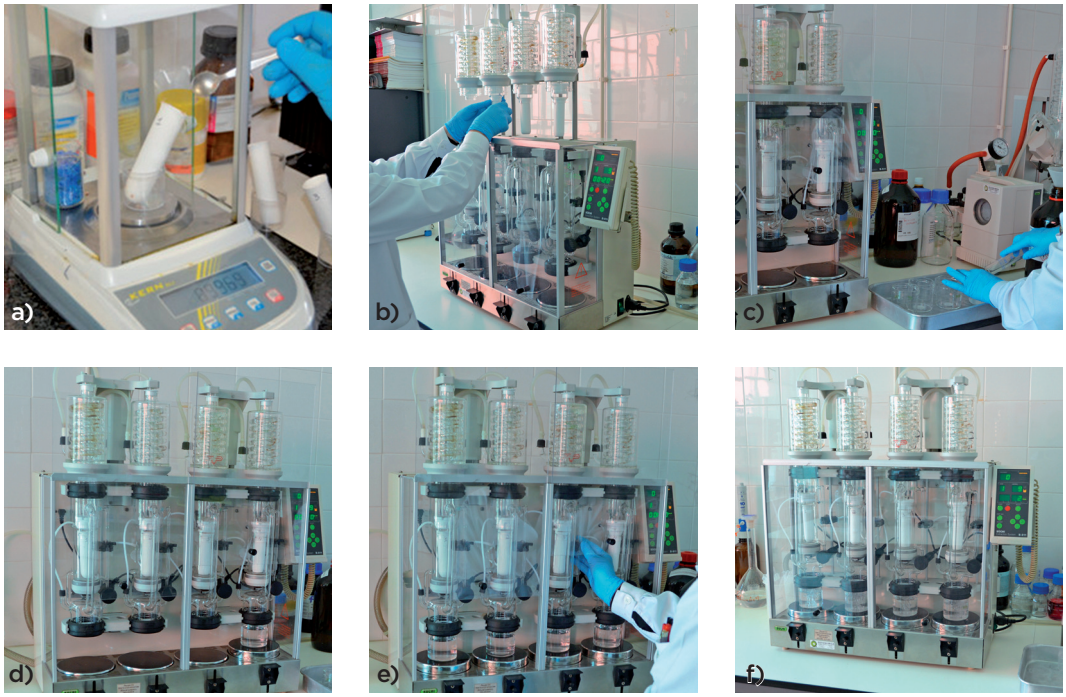

Figure 2: Procedure to analyse fat content using the automatic equipment Soxhlet: (a) sample weighting, (b) placement of the thimble with the sample in the equipment, (c) introduction of the thimble in the tubes and preparation of the petroleum ether, (d) introduction of the extraction cups with petroleum ether to the equipment, (e) opening of the connection to allow recirculate the petroleum ether, (f) extraction process. At the end, the extraction cups have to be dried and weighed and the fat content calculated.

When analysts want to extract all the simple and complex lipids from a tissue they usually use the "Folch method" (Folch et al., 1957; see more details in Chapter 6) or its variant the "Bligh & Dyer method" (Bligh and Dyer, 1959) using a mixture of a non polar solvent, chloroform and a polar solvent, methanol. The three methods mentioned (Soxhlet, Folch and Bligh & Dyer) are the most commonly used for lipid extraction in meat and meat products.

The standard methods by Folch et al. (1957) and Bligh and Dyer (1959) based on chloroform ($CHCl_3$)/methanol (CH_3OH) mixtures added directly to the meat were used for several years. Some adaptations have been developed to improve the accuracy of the standard and reference methods:

- Morrison and Smith (1964) used boron fluoride-methanol as methanolysis reagent in a preparation of methyl esters and dimethylacetals from the major classes of lipids;
- Marmer and Maxwell (1981) developed a dry column method for the determination of the total fat content of meat and meat products as an alternative to he traditional chloroform/methanol extraction methods, allowing the separation in neutral (mostly cholesterol and triacylglycerols) and polar lipids (mostly phospholipids);
- King et al. (1996) made an extraction of fat from ground beef for nutrient analysis using analytical supercritical fluid extraction. Eller and King (2001) found that the method can be used to accurately determine fat gravimetrically for ground beef;
- Philips (1997) used a simplified gravimetric method after the chloroform-methanol extraction for determination of total fat. Even though the method involves less analyst time and less solvent loss, the chloroform-methanol extraction may overestimate fat content on the other hand and underestimate fat content because low molecular fatty acids might not be extracted;
- Pendl et al. (1998) used the caviezel method. A homogenized sample and an internal standard (IS, tridecanoic acid) was added to the n-butyl alcohol solvent. Potassium hydroxide was used to saponify and extract the fats simultaneously. An acidic aqueous solution was added to convert the fatty acids salts to fatty acids, producing a two phase system where the fats and internal standard are contained in the top layer;
- Dionisi et al. (1999) developed a Supercritical CO_2 extraction (SFE) as an alternative to solvent extraction for the measurement of total fat in food. The method was not used for fat extraction in raw meat or meat products;
- Ruiz et al. (2004) improved the Marmer and Maxwell method using a solid phase extraction minicolumns and Pérez-Palacios (2007) for separation of animal muscle phospholipid classes. All these conventional protocols are time consuming and require a large amount of sample and solvent, which makes them frequently not suitable for routine analysis (Segura and Lopez-Bote, 2014);
- Segura and Lopez-Bote (2014) and Segura et al. (2015) developed a new procedure to extract IMF fat minimizing the sample amount, the solvent used and the time of analysis using lyophilised samples.

Apart of differences in the determination of IMF due to the methodology applied, other methodological factors influence in its determination such as:

- Muscle and anatomical part of the muscle used in the determination since IMF varied between and within muscles.
- Homogenization of the sample that can affect at the amount of IMF in the samples analyzed.
- Level of accuracy of trimming: all epimysium and external fat must be removed.
- Amount of sample.
- Time of extraction.

Thus all these aspects have to be considered when IMF is analyzed because they can modify the results and the accuracy of the measurement.

2.3.2 Reference methods for marbling

Determination of marbling is usually done visually by means of different reference standards such as those presented in Figure 3. The National Pork Producers Council (NPPC, 1999) proposes a reference standard from 1 (devoid of marbling) to 10 (abundantly marbled). For cattle, the USDA Quality Grades (USDA, 1996; Smith et al., 2008), the Japanese Meat Grading (JMGA, 1988), the Canadian Grading System (Anon, 2009) and the Meat Standards Australia (MSA (Anon, 2014)) are all systems aiming to standardized meat-grading. All these systems include marbling as a quality grading factor, and assess marbling in the meat by comparison with a visual standard. These systems also include other carcass or meat attributes combined with marbling. For example the USDA combines marbling with physiological maturity, meat colour, meat texture, rib fat, *longissimus dorsi* area and kidney and perirenal fat (Smith et al., 2008) whereas MSA includes animal traits and technological factors with consumer sensory testing to predict beef eating quality (for a review, see Polkinghorne and Thompson, 2010). In Europe, although some advanced carcass grading systems have been set up, reliable systems guaranteeing eating quality are still lacking and are perceived as a major need. Indeed, a recent European study using beef consumers in four different European countries indicated good opportunities for the development of a beef eating-quality guarantee system (Verbeke et al., 2010). Since all these standards depend on the operator, work is being done in the use of objective assessments of marbling mainly using computer vision (Jackman et al., 2011; Yang et al., 2006).

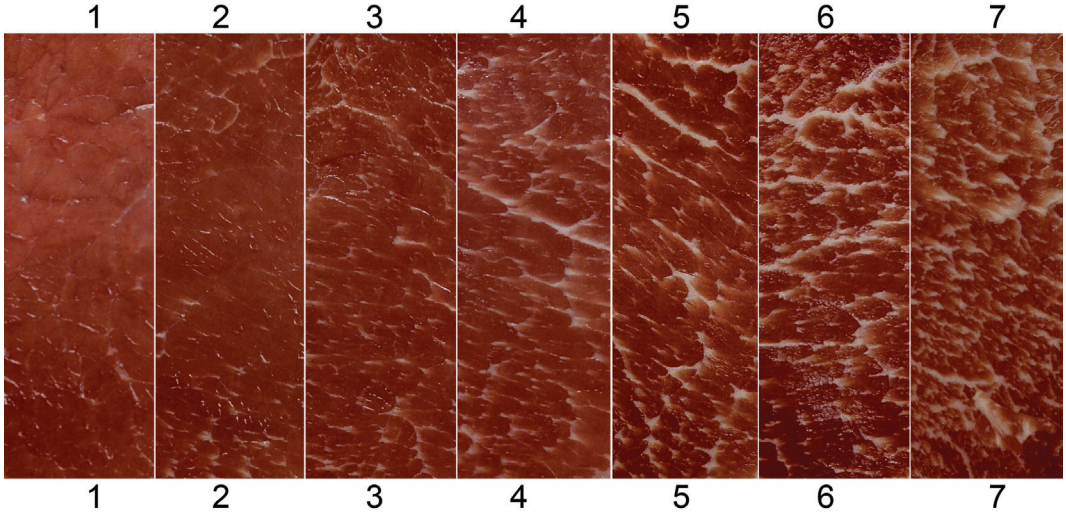

Figure 3: An example of marbling scale for pork prepared for internal use at the Agricultural Institute of Slovenia (Šegula et al., 2010).

Regardless the type of standard used for marbling measurement, the results obtained using the same standard are dependent on several factors such as:

- the operator is a key factor and grading accuracy and precision are depend of the training and skills of the operator;
- the size of the sample, which if too small can make it difficult to perceive the marbling correctly;

- the size and shape of the flecks, which can modify the perception of the marbling, especially if they look different than those of the reference scale used;
- the light used for the evaluation because it can affect at the visualization of the flecks;
- the anatomical position of the cut which can influence the measurements because marbling varies between and within muscles and also depends on the direction of the cut.

Thus, as far as it is possible it is necessary to control these factors during the evaluation of the marbling.

2.4 Comparison of reference methods

The relationship between marbling and IMF is not very strong because some of the IMF is not visible, and also it depends on the size and shape of the flecks. Some studies in pork show correlations between marbling and IMF of between 0.34 and 0.87 depending on the breed, muscle and area of the muscle. In beef loin Yang et al. (2006) found correlations between IMF and the intramuscular adipocyte area (r = 0.71), number of marbling flecks (r = 0.58), proportion of marbling fleck areas (r = 0.70) and total length of marbling flecks (r = 0.64).

Regarding comparison between methodologies, Prevolnik et al. (2005) studied the repeatability of the Soxhlet and Folch methods and compared them. The repeatability of the methods was studied by means of the standard deviation of the difference between two replicates. For the Soxhlet method, it was 0.09% in the *longissimus dorsi* and 0.23% in the *semimembranosus*. For the Folch method it was 0.11% and 0.20%, respectively. Also the Folch method was evaluated in beef *longissimus dorsi* and it was reported to be 0.20%. Thus, repeatability was similar for both methods, since overall it was 0.18% for the Soxhlet with hydrolysis and 0.17% for the Folch method. The comparison between both methods show that the means of IMF content were overestimated in the Soxhlet method with hydrolysis compared with the Folch method with an average difference of -0.32±0.50 in both pork muscles. This overestimation is higher in samples with more than 2% of IMF content. However, the regression between both methods is very good (r = 0.99). These results are not aligned with those of Dow et al. (2011). In this study the Folch, Soxhlet and Nuclear Magnetic Resonance (CEM SMART Trac system) methods were compared in beef meat samples. The Folch and Soxhlet methods extracted similar amounts of fat and the Soxhlet method was slightly more accurate than the Folch method (R^2 = 0.859 vs. R^2= 0.816). Gallina-Toschi et al. (2003) also found higher IMF values using the Soxhlet method with hydrolysis than using a modified version of the Folch method in chicken muscle. Differences between the two methods varied between 1.1 and 2.4%.

2.5 References

2.5.1 Methodological references

Intramuscular fat:

Several chemical standard and reference methods available to determine the intramuscular fat are the following:

AOAC 991.36: Fat (Crude) in Meat and Meat Products.

AOAC 985.15: Fat (Crude) in Meat and Poultry Products.

AOAC 976.21: Fat (Crude) in Meat.

AOAC 960.39: Fat (Crude) or Ether Extract in Meat.

ISO 1443:1973 Meat and Meat Products – Determination of Total Fat content.

ISO 1444:1996 Meat and Meat Products – Determination of Free Fat Content.

NMKL No. 38, 2001, 4th Ed.: Acid Value/Free Fatty Acids, Determination in Fats.

NMKL No. 131, 1989: Fat. Determination According to SBR in Meat and Meat Products.

Some of these standards have also been translated to several national standards and they are based on: Soxhlet F 1879. Die gewichtsanalytische Bestimmung des Milchfettes. Dingler's Polytechnisches Journal 232, 461-465.

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Chapter 3

pH value and water-holding capacity

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pH is an important quality attribute in meat from all the species considered here (beef, pork, lamb and chicken); it is related to the nature of *post-mortem* conversion of muscle to meat and is crucial for meat properties. pH affects the water holding capacity (WHC) of meat and consequently affects the technological suitability of the meat for further processing and manipulation. WHC is a quality trait which is mostly studied in pig meat because, in contrast to meat from other species, a large proportion of pig meat is not consumed in its fresh form, but instead is processed into a great variety of products. For this reason, emphasis in this chapter is placed on pork, although the methodologies have general relevance for meat from other species.

3.1 Description of pH and WHC

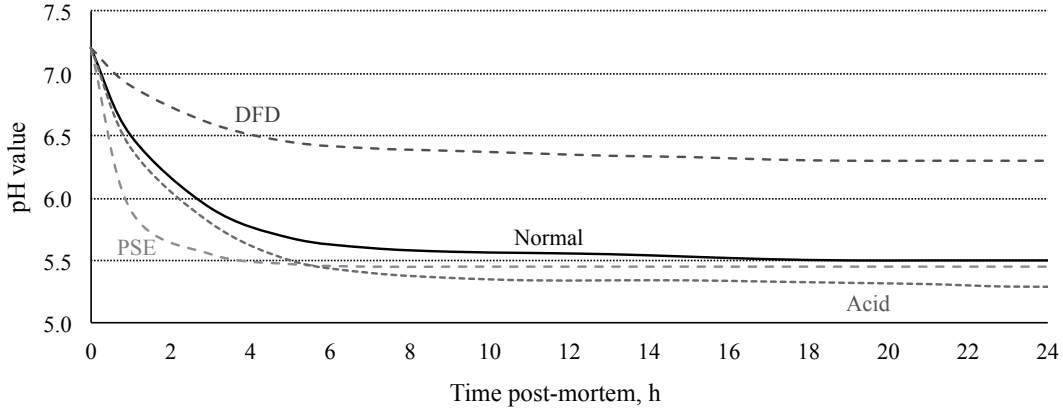
3.1.1 pH value

The pH value is one of the most important meat characteristics. In a muscle of a live animal, the values are in a neutral zone (≈7.2). After slaughter, muscle metabolism is strictly anaerobic and pH decreases due to the *post-mortem* conversion of glycogen to lactate. *In vivo*, muscles differ according to the prevailing metabolism (oxidative, glycolytic), and thus in the nature (rate and extent) of the pH decline *post-mortem* (Warriss, 2010). The rate of *post-mortem* pH decline is proportional to the activity of mATPase i.e. the speed of ATP degradation in muscles which, activated by increase of concentration of released Ca^{2+}, stimulates glycolysis (Krischek et al., 2011).

A normal rate corresponds to pH of 5.8-6.0 measured within 45-60 min *post-mortem* (also denoted as pH1). Fast glycogen degradation induced by acute (short-term) stress prior to slaughter, or by genetic predisposition triggers rapid pH decline (less than pH 5.5 1 h after slaughter in the extreme cases), which coupled with high body temperature, leads to the development of pale, soft and exudative (PSE) meat (Figure 1). The glycogen content of the muscle at the moment of slaughter determines the extent of the *post-mortem* pH decline (denoted as ultimate pH or pHu). Oxidative muscles have less glycogen and thus higher pHu than glycolytic muscles.

Normal pHu is situated in the range of 5.5-5.8 in pork and beef, while it is a bit higher in poultry meat (5.8-6.0). Values of pH1 and pHu are closely related to other meat characteristics, especially WHC and colour (for additional information on colour see Chapter 4), lower pH1 or pHu values being associated with lower WHC and paler colour. Higher pHu values in meat are related to darker colour, better capacity to bind water, but the meat also has lower shelf life (higher tendency to spoil) and is less suitable for drying. In extreme cases, the anomaly is called DFD (dark, firm, dry) and such meat is not suitable for processing into dry-cured products. It can develop when muscle glycogen reserves at slaughter are depleted (e.g. in stressed animals, long transport, etc.) and is more often encountered in beef than pork. In contrast, high glycogen stores (genetic predisposition, cf. 3.2.) cause an increased extent (but normal rate) of *post-mortem* pH decline which results in a very low pHu, known as acid meat.

Besides PSE and DFD quality, there are three other distinct quality categories of pork (NPPC, 1999): RFN (red, firm, non-exudative; most desirable), RSE (red, soft, exudative) and PFN (pale, firm, non-exudative) which are based on pH and colour. The pH1 and pHu values are thus very important indicators of meat quality.



DFD – dry, firm and dark meat, PSE – pale, soft an exudative meat

Figure 1: The rate and amplitude of *post-mortem* pH decline (the example of pig meat).

3.1.2 Water-holding capacity

WHC is the ability of meat to retain its moisture when exposed to external forces (e.g. gravity, heating, pressing, etc.). In meat, water can be found in different states, i.e. chemically bound to proteins, immobilized or entrapped within the myofibrilar structure of the muscle or as free water. Bound water (water molecules bound to non-aqueous constituents such as proteins) represents less than 10% of total water and changes very little in post rigor muscle (Offer and Knight, 1988). The majority of water in muscle is immobilized. It is held either by steric (space) effects and/or by attraction to the bound water (but not directly to protein). In early *post-mortem* tissue, this water does not flow freely from the tissue, yet it can be removed by drying, and can be easily converted to ice during freezing. Due to *post-mortem* changes in muscle structure and pH, this entrapped water is released. Free water can be easily mobilised from the muscle tissue. Weak surface forces mainly hold this fraction of water in meat. Free water is not readily seen in pre-rigor meat, but can develop as conditions change and allow the entrapped water to move from the structures where it is found (Huff-Lonergan, 2002).

The mechanism of WHC relies on the proteins and structures that bind and entrap water, among them the myofibrillar proteins play the most important role. The solubility of proteins is the lowest in isoelectric point (pH=5.1-5.3), where WHC reaches the lowest values. Deviation from isoelectric point causes an increase in muscle WHC. According to Huff-Lonergan and Lonergan (2005) pH, ionic strength, and oxidation of proteins have an effect on the ability of myofibrillar proteins to entrap water. Degradation of cytoskeletal proteins in *post-mortem* muscle may also contribute to capacity to retain moisture.

The quality of fresh meat, especially pork and poultry (particularly in turkey), relates largely to WHC which is technologically and financially important for food-processing industry. WHC in form of excessive purge is also unattractive for consumers when purchasing meat. Excessive purge results in economic losses, i.e. reduction in saleable product weight and, along with moisture, the loss of valuable water-soluble proteins and vitamins. The WHC of meat also influences processing characteristics, because meat with low WHC tends to produce inferior yields and lower quality of processed meat.

3.2 Factors influencing pH and WHC

Meat WHC is affected by various factors along the production chain. Based on reviews discussing this topic (Cheng and Sun, 2008; Den Hertog-Meischkel, 1997) the most influential factors can be distinguished into inherent (muscle type, genetics) and external (rearing, factors related to pre- and post-slaughter handling and the process of conversion of muscle to meat).

The **conversion of muscle to meat** is probably the key factor influencing WHC. After exsanguination, metabolism shifts from aerobic biochemical pathways to anaerobic processes. Two critical events occur with this metabolic shift. Due to insufficient energy supply, the muscle proteins start to form cross-bridges (irreversible muscle contraction) and the onset phase of *rigor mortis* occurs. Secondly, lactic acid is produced by the anaerobic metabolism of glycogen and the pH starts to decline. The change of pH during *post-mortem* conversion of muscle to meat and its effect on WHC is described in paragraph 3.1.1 and Figure 1.

With respect to **genetics**, there are two major genes related to pH and WHC, the Halothane or *RYR1*, and the *RN*$^{-}$ gene. An inherited mutation C1843T on *RYR1* gene (ryanodine receptor/calcium release channel in the sarcoplasmic reticulum) is responsible for malignant hyperthermia syndrome in pigs (Fuji et al., 1991). In relation to the *RYR1* gene, meta-analysis (Salmi et al. 2010) showed: ***i)*** the lowest pH1 in *nn* genotype (=PSE defect), followed by than *Nn* and *NN* genotypes, ***ii)*** lower pHu in *nn* and *Nn* compared to *NN* genotype and ***iii)*** higher drip loss in *nn* than the two other genotypes. Another gene with great effect on pork quality is the *RN*$^{-}$ or *PRKAG3* gene (with different polymorphisms such as R200Q, T30N, G52S and V199I; Milan et al., 2000; Ryan et al., 2012). It plays a key role in glycogen storage and is known to cause acid meat (or Hampshire effect). The *RN*$^{-}$ carriers have considerably greater muscle glycogen concentration (in the glycolytic fibres/muscles) leading to low pHu values, and exhibit impaired WHC than non-carriers (*rn*$^{+}$) (Le Roy et al., 2000). There are also well-known difference in WHC and pH among **species** and **breeds**. As mentioned, WHC is more important in pork and poultry meat than in beef. Differences between breeds are mainly associated with the incidence of specific major genes. As regards the comparison between autochthonous and modern breeds, selection for leanness resulted in lower meat quality (e.g. decreased WHC and intramuscular fat). In relation to **sex**, it seems it has a minor influence on pH and WHC, and as suggested by Pauly et al. (2012), no major influence on pH and WHC is anticipated if entire male pigs rather than castrates are reared.

Differences in pH and WHC between **muscles** of the same carcass are related to the metabolic muscle type. According to the prevailing metabolism, the muscles are generally divided into glycolytic/white (e.g. *longissimus or semimembranosus*) and oxidative/red (*masseter*), however, most muscles are actually metabolically mixed. There seems to be no effect of muscle type on the rate of pH decline (Lefaucheur, 2010). On the other hand, the lower pHu in glycolytic muscles is due to their higher glycogen content at slaughter (as compared to red muscles). Therefore, red muscles have a better WHC than white muscles. Differences in WHC and pH occur also **within muscle**. They can mainly be explained by different muscle fibre type proportions, but also by the effect of refrigeration, the outer parts being more rapidly chilled than the inner parts.

Rearing conditions comprise very different aspects of feeding/supplementing strategies and housing conditions (e.g. type of production systems – e.g. organic, conventional, free-range, release, enrichment of the environment, etc.). Nutrition is important for meat quality (e.g. intramuscular fat content, fatty acid composition), but less so for WHC and pH. Overall, housing conditions do not alter pH and WHC in a consistent manner, even though alternative outdoor systems and low ambient temperature may lead to higher muscle glycogen stores and lower pHu values than conventional housing conditions (Lebret 2008; Millet et al. 2005).

With respect to pH and WHC of meat, the physical activity and animal handling immediately prior to slaughter (such as transport, stunning, etc.) seem to be much more influential compared to rearing issues.

Pre-slaughter handling (fasting, mixing, transport, loading/unloading, lairage, stunning, electrical stimulation, etc.) is, even when optimally performed, stressful for the animals due to increased physical activity, encountering new environmental conditions, mixing of unfamiliar animals, etc. Animals respond to stressful situations by increasing their heart rate, respiratory rate, and muscle metabolic rate, which have negative effects on the onset of rigor mortis and evolution of pH in muscle *post-mortem*, protein denaturation, WHC, and meat functionality. Acute stress (related to any of the pre-slaughter stages) can cause PSE in animals with no genetic predisposition and contribute a lot to its development in PSE-prone animals. Each stressful situation causes changes in the secretion of hormones (adrenaline), which activate the degradation of glycogen. Chronic stress related to pre-slaughter handling such as long fasting time, long transport or fighting due to mixing of unfamiliar animals results in exhaustion of glycogen reserves and DFD meat (Terlouw et al, 2008; Warriss, 2010). Gentle, calm handling of livestock in the last few minutes before stunning is critically important for the prevention of an undesirable increase in the rate of pH decline (which decreases WHC) early *post-mortem*.

Post-slaughter handling (e.g. chilling, ageing, injecting non-meat ingredients, cooking) can also considerably influence WHC of meat. Chilling temperature has a crucial effect on pH and WHC. Muscles with normal rate of glycolysis must not be chilled too fast i.e. not below 10-15°C before rigor development (important to prevent cold shortening, mainly in lamb, beef). The way of chilling is especially important in PSE prone muscles, which must be chilled very rapidly and very early after slaughter in order to reduce drip loss (Honikel, 2004). Heating causes intense structural changes of myofibrillar proteins and of membrane structures in muscle tissue, which results in shrinkage, hardening and release of cooking juice. The cooking loss increases with progressing heating time and temperature and could reach values up to 45% of the raw muscle weight on heating to 95°C (Honikel, 2004).

3.3 Reference methods for measuring pH and WHC

3.3.1 pH value

Measurements of pH value are carried out with a pH-meter (different devices, different producers). Before the measurement, the pH meter needs to be calibrated using buffer solutions, usually with pH of 7.0 and 4.0. When measuring, it is very important to adjust/calibrate the device (buffers) to the temperature of meat. The pH is most often measured directly in meat with an electrode. It is also possible to measure pH in a muscle homogenate prepared in distilled water or iodoacetate. The latter is advisable for early *post-mortem* pH determination when glycolysis needs to be stopped (Le Roy et al., 2000). This method also allows a more accurate determination of pH due to better contact between pH electrode and muscle homogenate instead of pre-rigor muscle tissue and is thus highly recommended for early *post-mortem* pH determination. Pre-rigor pH is most often measured at a given time around 30 to 60 minutes after slaughter (e.g. pH1, pH_{30}, pH_{45}). Final pH is usually measured 24 h to 48 h after slaughter (pH_U, pH_{24}, pH_{48}) directly in the carcass. Between laboratories, there are differences in regard to the muscle in which pH measurements are taken. Most often the pH is measured in *longissimus dorsi* and *semimembranosus*. Accurate description of anatomical location and measurement position within muscle is very important. As pH can vary considerably even within the same muscle, it is advisable to standardise the measurements in terms of location and repetitions (take the average of duplicate or triplicate pH measurements). Repeated measurements of pH can lead to a decrease in accuracy of the equipment over time, thus it is important to clean and re-calibrate instruments regularly (Roehe et al., 2013).

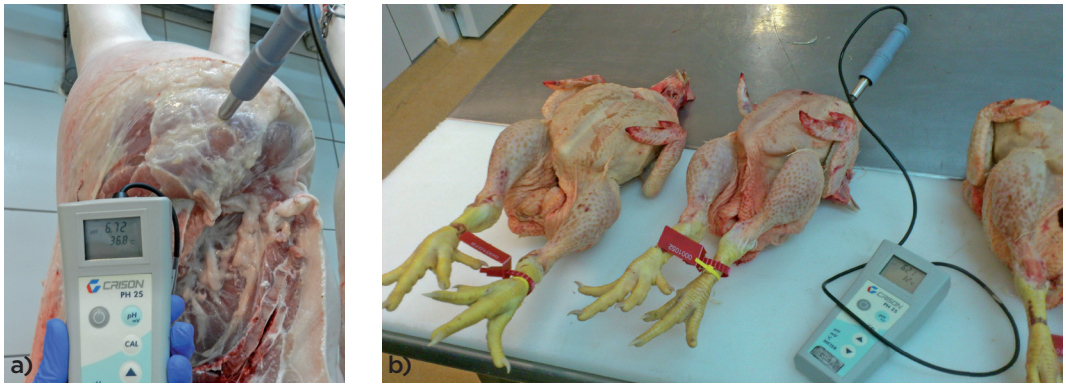


Figure 2: Measurement of pH a) in pig muscle *semimembranosus* and b) in chicken breast (*pectoralis major*).

Information that needs to be provided when describing the measurement of pH should include:

- equipment (brand, model),
- probe or electrode (brand, model),
- calibration: temperature, pH of buffer solutions (30.01),
- measurement: temperature (of carcass/meat),
- anatomical location (as precise as possible, muscle, region),
- time *post-mortem*,
- if measured in muscle homogenate, the description of medium and procedure.

3.3.2 Water-holding capacity

In practice, many diverse methods have been used to measure WHC, including drip loss, cooking loss, thawing loss, centrifuge force, etc., and within the each methodological approach, several modifications exist. In spite of methodological heterogeneity, the principles of the various methods are generally the same, i.e. a piece of meat is weighed before and after a certain treatment (centrifugation, cooking, freezing, etc.) and then the result (loss of water) is expressed as the weight difference with regard to initial sample weight (in percentage). The most influential factor when measuring WHC is a type of treatment/methodology applied (force applied, time *post-mortem*, treatment duration, etc.). Irrespective of the method used, several other methodological factors affect WHC measurement:

- anatomical location (muscle, part of a muscle),
- sample weight and geometry (size, shape, thickness, diameter),
- fibre orientation/direction,
- external conditions (temperature, etc.),
- intact or minced sample,
- it is valuable to provide complete information on the animals (such as species, breed, sex, age, feeding regime, transport and pre-slaughter handling, slaughter conditions, chilling and ageing conditions) and the rate and extent of *post-mortem* pH decline.

In general, WHC methods can be divided into three basic groups (Honikel, 2004).

The first group consists of WHC methods where **no external force** is applied; only 'free' drip due to gravity is measured. These methods are often called drip loss methods and

measure fluid that is lost from fresh, non-cooked meat *via* passive exudation. Within this methodological approach, bag method (Honikel 1997, 1998; Figure 3a, b) is most often applied and often referred to as a reference. In principle, a standardised piece of meat is suspended in bag, jar, cube, etc. without touching the walls and bottom, sealed and left for a certain period (generally between 1 and 3 days). Modifications of the bag method concern the 'chamber', period of suspension, size/shape of the sample, the use of special meat containers (method called EZ drip loss; described in Rasmussen and Anderson, 1996 and Christensen, 2003) or polystyrene trays covered with semi-permeable polyvinyl chloride film (known as tray drip loss or retail display; method is described in Allison et al., 2002 and Merour et al., 2007; Figure 3c). According to Otto et al. (2004), EZ drip loss method (Figure 3d) is highly correlated to the bag method, but has greater sensitivity and is easier to perform in a reproducible way (simplicity and small standardized meat pieces). Tray drip loss has the advantage of directly imitating commercial conditions. A methodological factor which is important when measuring drip loss is the dabbing of samples before weighing. In general, the main disadvantage of drip loss methods is that they are time consuming (one to several days), so methods using external force have been developed in order to accelerate WHC evaluation.

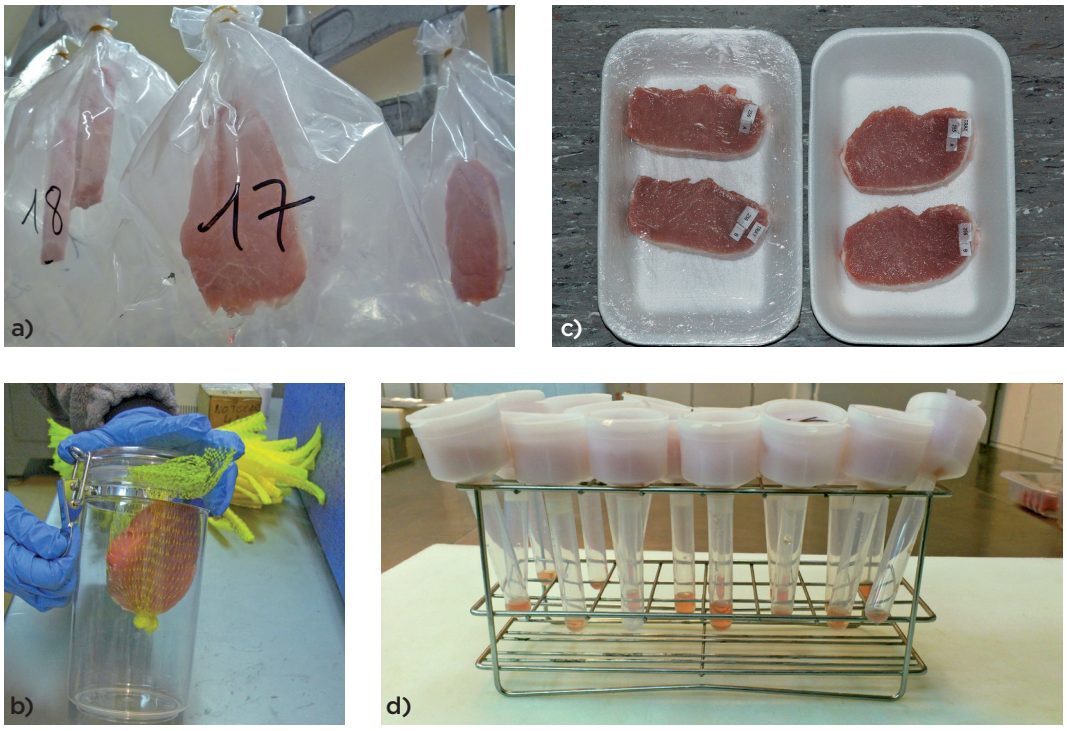


Figure 3: Measuring WHC in pork using different drip loss methods: bag method (a) and its adaptation (b), tray drip loss or retail display (c) and EZ drip loss method (d).

The second group of WHC methods involves the use of **mechanical pressure.** Using this approach the WHC of meat can be determined within few minutes or an hour (e.g. centrifugation methods or filter paper press methods). The filter paper press method as originally described in Grau and Hamm (1953) is simple and has been widely used for measuring WHC. In principle, water is pressed out of meat and absorbed by the filter paper. A small piece of meat sample (0.2-0.4 g) is covered by filter paper and placed between plexiglas plates which are then compressed. Due to the pressure, water is squeezed out and

absorbed by the filter paper forming a ring of expressed juice. The ratio area of this ring to the meat is an index of WHC.

Modifications of original method have often been applied and concern mainly the sample size, duration of force application and compression force. The main advantage of this method is that the operation is easy, requires a small sample and can be employed with ground or processed meat, whereas disadvantage is that area measurements made with the planimeter are laborious. Thus it has been suggested to weigh the filter paper before and after compression (Figure 4a). It may be mentioned that other methods using filter paper but without application of external force have been proposed: ***i)*** weighing pieces of filter paper before and after being placed on meat surface for a certain time period (Kauffman et al., 1986) and ***ii)*** measuring the time needed for filter paper of defined area to be soaked. In the case of centrifugal force applied as mechanical pressure, small and standardized pieces of meat are centrifuged, applying a defined speed (rpm) and time (as described in Allison et al., 2002; Figure 4b). Also in this case, many variations regarding abovementioned factors could be applied.

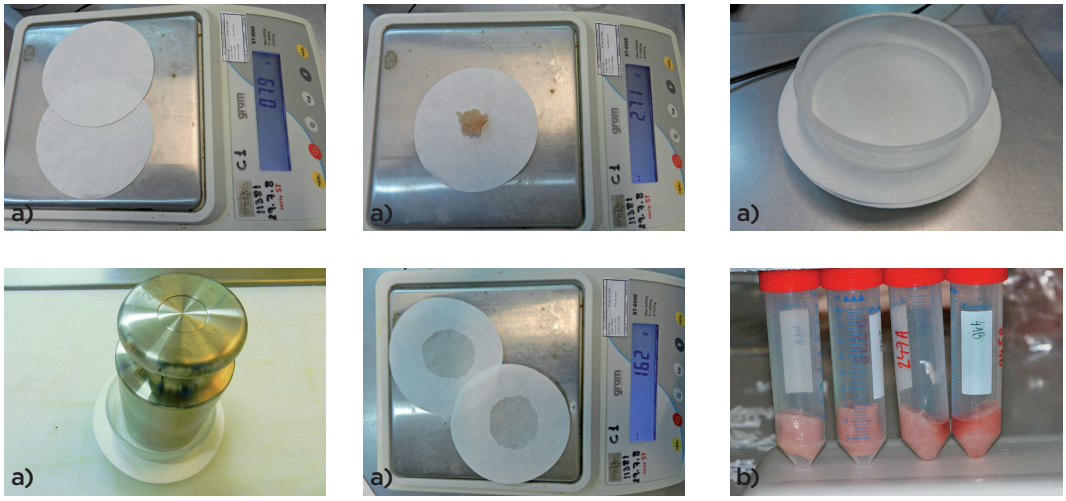


Figure 4: Determination of WHC using a) filter paper press methods (in chicken meat) and b) high-speed centrifugation (in pork loin).

Due to external mechanical force, the amount of purge is far bigger than in the 'free' drip approach because the pressure of applied forces induces greater release of water from the intra- and extracellular space of the muscle structure. Therefore, methods applying mechanical force indicate 'meat behaviour' but the absolute values are not comparable to measurements of 'free' drip loss achieved without the use of mechanical pressure.

The third group involves WHC methods with the application of **thermal force.** This approach, which measures 'cooking loss', has practical relevance (compared to the use of 'free' drip or mechanical force) as meat is usually consumed after heating. Cooking loss (Figure 5) is most often carried out as described in Wheeler et al. (2005) or Honikel (1997; 1998). In principle, a piece of meat is heated to a desired temperature (e.g. 72°C for pork). In case of thermal force, the state of meat, sample weight and shape, method of heating (wet/dry or cooking/roasting) and its duration, end temperature of meat are important for the resulting WHC expressed as cooking loss. During heating, the meat proteins denature, the cellular structures are disrupted, which leads to release of water and decreases the WHC of meat.

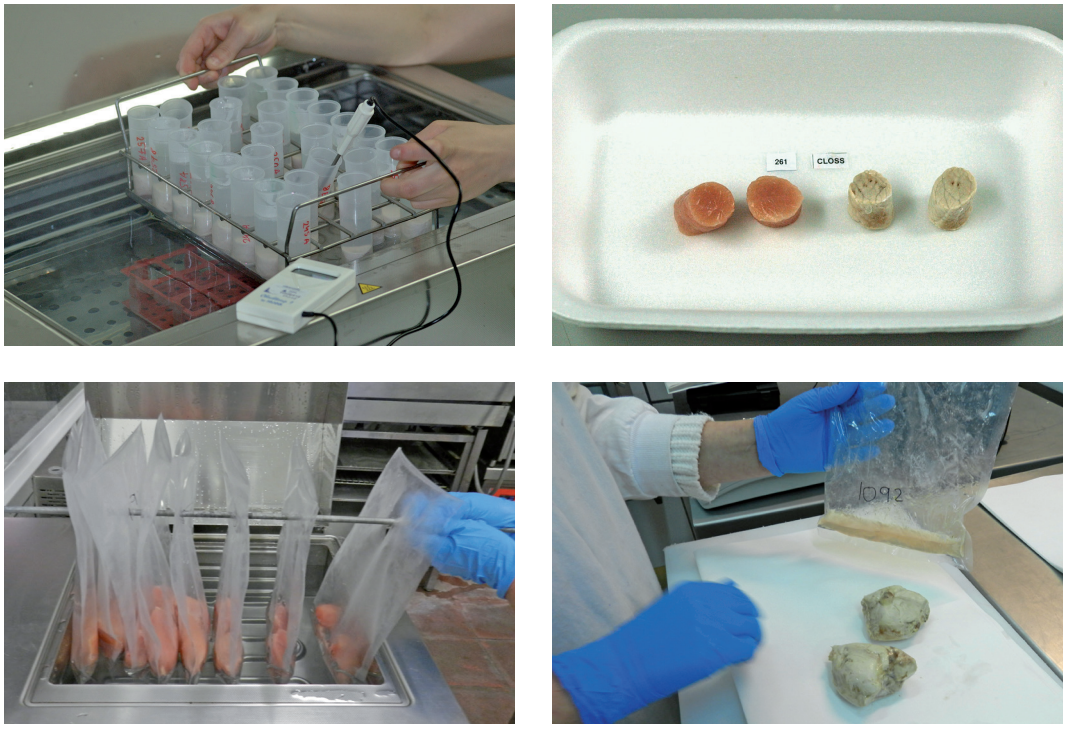


Figure 5: Determination of WHC using cooking loss method.

There are also some other methods used for assessing WHC of meat, such as loss of juice when meat thaws and the juice produced during vacuum packaging of meat. In this case again the most important methodological factors are environmental conditions, such as temperature (of storage, thawing, measurements, etc.) and sample geometry. In particular in relation to thawing, it is important to be aware of the important effect of conditions at freezing (size of water crystals), storage (growth of water crystals) and defrosting (capacity of proteins to bind free water), all of which should be considered.

3.4 Comparison of reference methods for measuring pH and water-holding capacity

Regarding reference methodology for pH determination the following influential factors should be considered: the electrode and apparatus, temperature, calibration to buffers and measurement location. Presentation and comparison of different pH meters can be found in BPEX (2010) or Roehe et al. (2013). The repeatability of pH measurement (replicates made in the neighbouring positions of a *longissimus dorsi* muscle slice) was assessed to 0.05 (Čandek-Potokar et al., 2006).

Comparisons of different WHC methods have often been studied. Published studies report very variable results. In general, due to differences in physical principles applied, there are poor to moderate correlations among different WHC methods (Prevolnik et al., 2010; Škrlep et al., 2013). Comparison of drip loss-tray method, drip loss-EZ method, centrifuge force and cooking loss published in Prevolnik et al. (2010) showed correlations between 0.46 and 0.68 (Table 1).

Table 1: Correlation coefficients among WHC methods (published in Prevolnik et al., 2010).

N=228	EZ drip loss, %	Cooking loss, %	Centrifuge force, %	Tray drip loss, %
EZ drip loss, %		0.46	0.67	0.68
Cooking loss, %			0.47	0.66
Centrifuge force, %				0.68

Other literature data (correlation coefficients – *r*) on the comparison of WHC methods:

- tray drip loss and EZ drip loss → *r* = 0.93 (Otto et al., 2006);
- bag method and EZ drip loss → *r* = 0.86 (Otto et al., 2004);
- bag method and the EZ drip loss → *r* = 0.85 (Cristensen et al., 2003);
- EZ drip loss and bag method → *r* = 0.49-0.84 (Merour et al., 2007);
- retail display and cooking loss → *r* = 0.64 (Allison et al., 2002);
- retail display and centrifuge force method → *r* = 0.47 (Allison et al., 2002);
- centrifuge force method and cooking loss → *r* = 0.19 (Huff-Lonergan et al., 2002);
- bag method and cooking loss → *r* = 0.02 (Huff-Lonergan et al., 2002);
- bag and EZ drip loss → *r* = 0.83-0.93 (Barbe and Westphal, 2011);
- EZ drip loss measured 24 and 48 h *post-mortem* → *r* = 0.96 (Čandek-Potokar et al., 2006);
- EZ drip loss measured 24 and 48 h *post-mortem* → *r* =0.85 (Otto et al., 2004);
- bag method measured 24 and 48 h *post-mortem* → *r* = 0.96 (Otto et al., 2004).

Besides moderate associations among WHC methods, there are also distinctive differences among them in terms of absolute values (Allisson et al., 2002; Prevolnik et al., 2010), which primarily depend on the method/force used. The highest water loss occurs when thermal treatment is used, followed by centrifugal force and the lowest in drip loss methods or losses due to gravity (Table 2). All WHC methods are highly affected by different environmental factors and sample geometry, which is probably the reason for poor repeatability (Table 2, Prevolnik et al., 2010; Čandek-Potokar et al., 2006). Similarly low repeatability has recently been obtained by Gispert (unpublished data) for cooking loss in poultry meat (mean 3 sd = 23.1 3 2.61%) with 1.42% and 0.53% for repeatability standard deviation and repeatability standard deviation calculated on standardised value, respectively. In the literature, data on repeatability of WHC methods is scarce and also not uniformly reported. Barbe and Westphal (2011) assessed repeatability as deviation of duplicate determinations from the mean and obtained 12 vs. 21%, repeatability for bag and EZ drip loss, respectively. Allison et al. (2002) calculated repeatability using variance component procedure, and reported repeatability values of 0.90, 0.91, 0.81, 0.50 and 0.59% for centrifugation loss, bag drip loss, filter paper, tray drip loss and cooking loss, respectively.

Table 2: Univariate statistics and repeatability for different WHC methods (published in Prevolnik et al., 2010).

N=228	Mean ± sd	CV, %	s_r	Ns_r
EZ drip loss, %	4.4 ± 1.6	36.4	1.1	0.62
Cooking loss, %	31.3 ± 3.1	9.9	1.8	0.55
Centrifuge force, %	11.9 ± 2.9	24.4	2.0	0.65
Tray drip loss, %	3.2 ± 1.3	40.6	0.4	0.30

sd – standard deviation; CV – coefficient of variation;
s_r – repeatability standard deviation (SD of the difference);
Ns_r – repeatability standard deviation calculated on standardized values.

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Chapter 4

Muscle and fat colour

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4.1 Description of muscle and fat colour

The colour of meat influences meat purchasing decisions by consumers and is therefore a very important quality attribute. Indeed, discolouration is used as an indicator of product spoilage and unwholesomeness.

Meat colour results from complex interactions between several *ante* and *post-mortem (p.m.)* factors, through their effects on the concentration and chemical state of pigments and muscle micro-structure that determines the light-scattering properties of meat. Fresh meat colour is defined by the total amount in myoglobin, which is associated with the muscle oxidative capacity, and the amounts of the three derivatives of myoglobin (Figure 1): i) **reduced myoglobin (Mb or deoxymyoglobin)** which is the purple pigment of deep muscle and of meat under vacuum, ii) **oxymyoglobin (MbO_2)** which is bright red and considered as freshness indicator and attractive and results from oxygenation occurring when Mb is exposed to oxygen, and iii) **metmyoglobin (MetMb)**, the oxidized form of myoglobin which is brown and unattractive and results from low oxygen partial pressure. A layer of MetMb can be found between the meat surface, where the oxygen level is high enough to produce MbO_2, and the internal part of the muscle where anaerobic conditions maintain myoglobin as the reduced form. However, MetMb will eventually shift towards meat surface, the rate of discolouration depending on many intrinsic and extrinsic factors (see below) (Renerre, 1990; Mancini, 2009).

The oxidation of Mb and MbO_2 into MetMb decreases meat shelf-life. Enhancing MetMb reduction is thus of interest, to improve meat quality. However, this process is not straightforward and depends on many muscle biochemical factors, including meat reducing capacity and reduction in oxygen tension. To counteract these problems, low levels of carbon monoxide can be added to meat packages, leading to formation of the bright red pigment carboxymyoglobin (MbCO) (Renerre, 1990; Mancini, 2009).

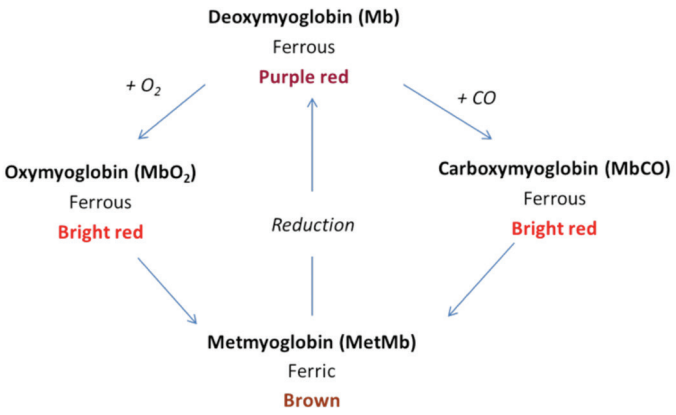

Figure 1: Myoglobin redox states and colour changes on meat surface (adapted from Renerre, 1990 and Mancini, 2009).

Besides pigment concentration, muscle structural properties influence meat colour by determining the reflectance of light from the surface and therefore the perceived meat paleness. The structural properties are highly dependent on the rate and extent of *p.m.* pH decline: both a high rate and extent of pH decline lead to high differences in refractive indices between sarcoplasm and myofibrils, giving rise to high light scattering and pale meat (Warriss, 2010).

The colour of fat is also a determinant of meat colour and appearance. Subcutaneous fat colour results from the redox state of residual hemoglobin, and from carotenoid level (Mancini, 2009). In lambs, a white fat colour is considered as normal. Yellow fat colour can result from high levels of carotenoids originated from feeding (grazing), or excess of bilirubin (product of hemoglobin degradation) in pathological cases. Brown/red colour of adipose tissues can result from excessive heme pigment concentration, and peroxidation of unsaturated fatty acids (Prache et al., 1990). Orange fat colour resulting from accumulation of lipid oxidation products can also occur in cured pork products after long storage time.

4.2 Factors influencing muscle and fat colour

4.2.1 Ante mortem factors

Ante mortem factors influence muscle and fat colour through their effects on the concentration of the pigments. As a general rule, muscle myoglobin content is positively associated with the level of physical activity of animals. Other factors also modulate muscle myoglobin level or chemical state and thereby influence meat colour. In addition, genetics can strongly affect meat colour parameters including lightness, especially in pigs with the effects of major genes Hal and RN.

Species greatly influences the concentration of the muscle pigments. For example myoglobin content varies from less than 0.1 mg/g in the breast muscle of broilers chickens to approximately 2 mg/g in the *longissimus* muscle (LM) of pigs, and up to 5 mg/g in the LM of cattle (Warriss, 2010). Furthermore, in *p.m.* muscle, the balance between myoglobin redox states differs between species: on freshly cut LM surface, the rate of myoglobin oxygenation is fastest in pork, intermediate in lamb and slowest in beef, enhancing species differences in terms of meat colour (Lawrie, 2006).

Within species, **breed** can affect pigment concentration; a particular effect is found in horses where thoroughbreds exhibit much higher levels of myoglobin than draught horses in the

LM involved in running, whereas only few breed differences are found in the *psoas* muscle (Lawrie, 2006). In cattle, meat from dairy breeds is usually redder than meat from beef breeds when compared at similar age, due to the higher proportion of oxidative muscle fibers (see below) in the earlier maturing dairy breeds (Lebret et al., 2015). In pigs, the most important **genetic effect** on pork colour is stress susceptibility and *p.m.* muscle metabolism. The colour of pork especially from white muscles like LM is dramatically affected by the presence of n halothane allele (RYR1 locus), which induces an acceleration in *p.m.* pH decline and leads to Pale, Soft and Exsudative (PSE) meat, with Nn being intermediate between NN (normal) and nn genotypes (Sellier, 1998). The genotype of the other major gene, RN, also affects meat colour, with carriers of the RN^- allele exhibiting higher muscle glycogen content and lower ultimate pH (acid meat), and lighter but also redder meat (higher a* value) than the non-carriers (Le Roy et al., 2000; see also Chapter 3). Indeed, higher pigment content and redder meat (higher a* and lower hue angle) have been found in the LM of Hampshire breed which exhibit a high frequency of the RN^- allele compared to Swedish Landrace or Swedish Yorkshire breeds (Lindahl et al., 2001).

The influence of the **sex** of an animal on meat colour may be related to pigment concentration. In cattle, the age related increase in muscle pigment concentration is faster in females than males; however the darker and less acceptable colour of meat from bulls compared with steers would be mostly explained by the higher ultimate pH often found in bulls (Seideman et al., 1982). Nevertheless, meat colour is highly dependent on animal **age**. In all animal species, muscle myoglobin content increases with age, in a two-phase manner: an initial swift rise that lasts about 1 year in pigs and 2 to 3 years in cattle, followed by a more gradual phase (Lawrie, 2006). Therefore, meat redness as determined by physical measurement or visual assessment increases with animal age at slaughter in cattle and lambs. Improvement in meat redness with animal age also occurs in pigs when considering great differences in slaughter age (Lebret et al., 2015). However, in beef meat from older animals, the bright red layer of MbO_2 is thinner and consequently, meat colour stability is weaker than in beef from younger animals (Renerre, 1990).

Irrespective of animal species, breed and age, meat colour mainly depends on the **anatomical location and physiological function of muscles** (locomotive, support, etc.) which determines their muscle fibre composition. Muscle fibres are typically classified according to their contractile and metabolic properties. Briefly, three major fibre types are generally considered: slow-twitch oxidative (SO), fast-twitch glycolytic (FG) and fast-twitch oxidative-glycolytic (FOG). SO fibres have a slow contraction speed but high oxidative metabolism, are rich in myoglobin and exhibit a good fatigue resistance, whereas FG exhibit high contraction speed and high glycolytic metabolism but have low myoglobin content and low fatigue resistance, the FOG are intermediate between SO and FG (Lefaucheur, 2010). Therefore, the myofiber composition, and especially the metabolic profile, directly influences pigment content (which can double between muscles from the same beef carcass such as between *semitendinosus* and diaphragm muscles), and thereby determines meat colour (Talmant et al., 1986). In pigs, higher pigment content and a* value and lower lightness and hue angle have also been reported in *biceps femoris* (BF) compared with LM, indicating the darker and redder meat of the BF (Lindahl et al., 2001). In addition, colour stability varies highly according to muscle metabolic type. Formation of MetMb depends on many factors such as the rate of spread and consumption of oxygen and the rate of MbO_2 formation, and is enhanced with increasing intensity of oxidative metabolism. Therefore, muscles with a predominant SO fibre type exhibit low colour stability, e.g. the diaphragm muscle in beef (Renerre, 1990).

Animal **diet,** through both feeding allowance and composition, can affect several muscle traits that influence meat colour including glycogen storage, chilling rate, pH, or antioxidant accumulation. In bulls, forage-based diets fed in restricted quantities might promote muscle

oxidative metabolism and decrease muscle glycogen content, leading to higher pH and darker meat colour, than *ad libitum* feeding of concentrates (Mancini, 2009). In pigs, finishing diets with a low digestible carbohydrate content reduced muscle glycolytic potential, thus decreasing pork paleness and improving meat darkness (Rosenvold et al., 2001). Diet can also impact meat colour through the influence of feeding level on fat thickness and subsequent chilling rate. In fat carcasses, the high muscle temperature shortly after slaughter promotes anaerobic metabolism and pH decline, increasing protein denaturation and muscle lightness (Mancini, 2009). Moreover, a diet low in iron level leads to low muscle myoglobin concentration, as encountered in calves fed maternal milk (Lawrie, 2006).

Stability of meat colour can be influenced by the animal diet, through intake of antioxidants and their accumulation in tissues, which modulates lipid and pigment oxidation. For example, colour stability of meat from cattle fed on high-herbage diets is greater than that from cattle fed *ad libitum* concentrate, probably due to the increased lipid stability of the former (O'Sullivan et al., 2003b). In pigs, dietary supplementation of vitamin E has also a positive effect on meat colour stability (Lebret et al., 2015).

Diet also affects fat colour: grazing increases the yellowness of the subcutaneous fat in steers and lambs, due to the accumulation of β-carotene within the lipid tissues. This has led to the use of carotenoid pigments as diet markers in lamb carcasses (Prache et al., 2003).

Housing and rearing conditions of animals can also influence meat colour through their effects on physical activity and muscle metabolism. In steers and lambs, grazing leads to darker meat due to increases in both muscle myoglobin content and oxidative metabolism resulting from higher physical exercise (Lebret et al., 2015). Similar results have been found in outdoor reared pigs, even though these effects are not systematic but depend on the overall influence of housing conditions on *ante* and *p.m.*, muscle metabolism characteristics including glycolytic potential and rate and extent of *p.m.* pH decline. In poultry, outdoor rearing has been shown to increase meat yellowness (Lebret et al., 2015). Fat colour can also be influenced by animal rearing conditions especially grazing in ruminants, as mentioned above.

4.2.2 Peri and post-mortem factors

Pre-slaughter handling of animals can strongly affect the development of meat colour through its influence on the **kinetics of** *p.m.* **muscle pH fall**. It is well known that in all animal species, an important stress during pre-slaughter handling or a high physical activity level during transport or due to fighting after mixing of animals for example, leads to low muscle glycogen content at time of slaughter and therefore low *p.m.* glycogenolysis and high ultimate pH. This leads to dark coloured meat and the DFD (Dark, Firm, Dry) or "dark cutting" defect in extreme cases, this defect being more frequent in cattle than in pigs or poultry (Lawrie, 2006; Terlouw et al., 2008; see also Chapter 3). It is also muscle dependent, the locomotive muscles located at the back of the carcass in cattle being more susceptible to "dark cutting" meat than those in the forequarters. By contrast, stress immediately before slaughter increases peri slaughter and *p.m.* muscle metabolism while muscle temperature is still high, leading to rapid *p.m.* pH decline and PSE meat exhibiting high paleness (cf above). This can occur especially in poultry and pigs as a consequence of inadequate conditions immediately pre-slaughter and independently of the genetic halothane defect in pigs (Terlouw et al., 2008). In particular, the **high muscle temperature** early during the *p.m.* period, induced by pre-slaughter stress, plays an important role in colour development and colour stability of pork (Rosenvold and Andersen, 2003).

Besides meat colour, the kinetics of *p.m.* pH fall can influence meat discolouration. Indeed, the rate of autoxidation of myoglobin increases with decreasing pH whilst its enzymatic reduction is less effective at low pH. Consequently in general, muscles of low ultimate pH

discolour more rapidly than those of high ultimate pH (Renerre, 1990). This phenomenon contributes to the very pale colour of PSE meat, together with its high light scattering due to the 'open' muscle structure. Since meat colour can be markedly affected by the kinetics of *p.m.* pH fall, the **chilling rate of carcasses** that plays an important role in *p.m.* muscle metabolic activity is a critical point. Apart from reducing growth of micro-organisms, enhancing chilling rate allows the level of muscle anaerobic activity and the occurrence of excessive paleness in subsequent meat, especially in 'white' muscles of pigs and poultry to be limited. Overall, proper **cold chain management** and refrigerated temperatures during storage and display are key points to maximize shelf-life and colour of meat and meat products. Indeed, myoglobin oxidation and lipid oxidation are accelerated with increased temperature. Moreover, lighting intensity during retail display influences meat discolouration rate (Mancini, 2009).

Heating greatly influences meat colour because heat denatures proteins including myoglobin. The principal pigment of cooked meat is the brown globin haemichromagen. The brown colour of cooked meat is a desirable attribute, in contrast to the situation in fresh meat (Lawrie, 2006). Cooking temperature affects the degree of conversion of pigments and therefore meat colour. In beef, after 30 min of heating at 60°C, myoglobin denaturation is less than 30%, whereas it is about 60% at 65°C and almost 100% at 80°C. Therefore, the meat interior is bright red, pink, or greyish brown respectively, depending on the internal temperature reached during cooking (Lawrie, 2006). The brown colour of cooked meat is also determined by other factors, including the 'caramelization' of carbohydrates and the Maillard-type reactions between reducing sugar and amino groups. This phenomenon is important in particular in pork where high levels of reducing sugars are produced *p.m.* but pig muscle contains relatively low level of myoglobin.

The oxidation of Mb into the brown pigment MetMb is enhanced by heat but also by salt concentration, and depends on oxygen partial pressure. Prolonged meat **storage** at chill temperatures leads to meat surface desiccation which increases salt concentration thereby enhancing MetMb formation, even though low temperature delays Mb oxidation. Furthermore, the rate of discolouration (MetMb formation) of fresh meat is maximal at low oxygen partial pressure (4 to 7 mmHg, depending on pH and temperature) and is inhibited as oxygen pressure increases (Renerre, 1990, Lawrie, 2006). Therefore, **meat packaging** has a significant effect on raw meat colour. Traditional packaging (PVC overwrap) exposes meat to the atmosphere, thus allowing the development of the bright cherry-red MbO_2 on meat surface, although Mb oxidation will eventually occur. Vacuum packaging extends the colour stability compared with traditional packaging and prevents oxidative rancidity; however the purple colour of Mb (reduced state) in vacuum-packed meat, especially in beef, may be unattractive for consumers, this drawback is probably less important in white meats (Renerre, 1990; Mancini, 2009).

Modified atmosphere packaging has been developed, based either high or ultra-low oxygen atmospheres, or carbon monoxide atmospheres. High-oxygen atmospheres promote both the formation of MbO_2 and its penetration into meat, thus delaying the migration of MetMb to the surface. This enhances colour stabilization during storage, but can also increase lipid oxidation and rancidity flavour. By contrast, ultra-low oxygen atmospheres reduce lipid oxidation and aerobic microbial development. However residual oxygen must remain very low to limit MetMb formation, especially in beef (Mancini, 2009). To avoid this disadvantage of ultra-low oxygen atmospheres, carbon monoxide can be added in meat packages to a final concentration of around 0.5%, its high affinity for Mb and high ability to form the bright red MbCO pigment leads to an attractive meat colour and enhances colour stability (Mancini, 2009).

4.3 Reference methods used to evaluate muscle and fat colour

Meat colour is generally assessed by visual or instrumental methods. Some methods are also aimed at determining the metmyoglobin reducing activity and the oxygen consumption of meat. Colour is generally evaluated 24 h after slaughter or at determined times (days) *p.m.* to assess meat discolouration during ageing/retail, and is assessed after exposing the fresh cut meat surface to blooming, i.e. oxygenation of surface myoglobin.

4.3.1 Visual evaluation

To overcome difficulties associated with the subjective evaluation of meat colour, reference scales have been proposed (Figure 2). Colour cards are available which include a series of colour 'tiles' to which meat samples are compared. For example, the Japanese Colour Standard (JCS) (Nakai et al., 1975) comprises 6 tiles ranging from 1=pale to 6=dark colour and is widely used to evaluate pork colour. Some plastic resin models of the Japanese scale have been produced for easy use in pork industry. In general, values of 3 to 5 are considered favourable, whereas values of 1 and 2 are associated with pale and exudative meat, and a value of 6 with DFD meat. In the US, the National Pork Producers Council (NPPC) has also developed a set of visual reference standards to be used in evaluating fresh pork colour (NPPC 2000, National Pork Board 2010). The standards attempt to represent the full range of colour encountered, from 1.0 = pale pinkish gray to white, which corresponds to lightness (L*) Minolta value of = 61 (L* determined using D65 daylight source, cf below), 2.0 = grayish pink (L* = 55), 3 = reddish pink (L* = 49), 4.0 = dark reddish pink (L* = 43), 5.0 = purplish red (L* = 37) to 6.0 = dark purplish red (L* = 31). Since both the type and intensity of illumination of the samples have a major influence on the colour score assigned, NPPC recommends a D65 light source, and especially to keep illumination consistent within sample series.

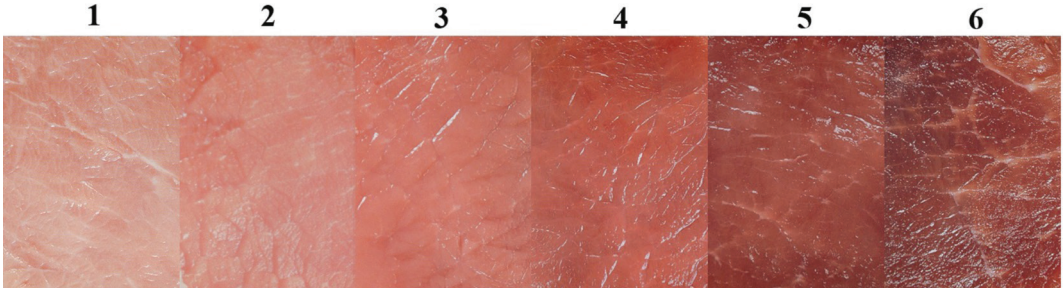

Figure 2: An example of colour scale (for internal use at Agricultural Institute of Slovenia).

In other animal species, colour scales have also been produced for visual colour assessment of meat, for example the Japanese beef lean and fat colour standards (7 levels). Colour guides based on photographic scales are also available in beef (8 levels; Meat laboratory, Iowa State University, IA), lamb (6 levels; Kansas State University, Manhattan, KS) and for processed meats. Regarding fish flesh, a colour card is available for the assessment of the pink colour of the salmonid flesh (DSM SalmoFan, Hoffman-la Roche, DSM, Switzerland).

Visual colour evaluation is cheap and seems quite easy using colour or photographic scales, but it is important that to ensure its accuracy; a visual determination must be standardized using only trained assessors. Meat colour can also be assessed by a trained panel which scores some precise colour traits, such as the intensity of redness or the homogeneity of redness on a meat sample. These evaluations must be undertaken according to ISO standards for sensory analysis, detailing guidance for the selection, training of monitoring of selected assessors and experts (ISO, 2009).

4.3.2 Instrumental evaluation

Visual assessment of meat colour is cheap but subjective, and these systems are based on discontinuous colour scales. Therefore instrumental evaluation using continuous colour scales has been developed. Indeed, any colour can be specified as a combination of different amounts of the primary colours pure red, pure green and pure blue: a colour can thus be defined as a point in a three-dimensional space. In 1931 the Commission Internationale de l'Eclairage (CIE) specified a colour space or 'chromaticity diagram' that encompasses all the colours that can be perceived by 'normal' human eyes. In this colour space, each colour that corresponds to three effective stimulus values is represented by a point with three coordinates X, Y, Z. Subsequently, Lab colour spaces have been proposed: Hunter Lab (1948) and CIELAB (1976). They include dimension L for lightness and a and b for the colour-opponent dimensions, based on nonlinearly compressed CIE XYZ colour coordinates (square root in Hunter and cube root in CIE). The CIELAB colour space has thus the shape of a sphere that includes all perceivable colours. It is mapped on a three-dimensional integer space in which the L*a*b* values are absolute and vary within a pre-defined range. This allows device-independent digital representation of colours which is the most important advantage of the L*a*b* model (Warriss, 2010; AMSA, 2012; Konica Minolta, 2015).

The **lightness L*** is usually represented along the vertical axis and varies from 0 (dark) to 100 (white). The **a* value** is represented on the X axis; it corresponds to the **green/red opponent colours** with green at negative and red at positive a* values (scale from - 60: green to + 60: red). The **b* value** is represented on the Y axis and corresponds to the **blue/yellow opponent colours** with blue at negative and yellow at positive b* values (scale from - 60: blue to + 60: yellow). In the center of the colour space (values of a* = 0 and b* = 0) is neutral gray. The L*a*b* colour space is the most commonly used for colour assessment of a wide range of things including food products.

The L*C*h° colour space is based on the same diagram as L*a*b* colour space, but with polar instead of Cartesian coordinates. In this space, L* is lightness and corresponds to the L* value in the L*a*b* space. **C* value is the chroma (or saturation)** and varies from 0 at the centre which is completely unsaturated (i.e. a neutral grey) to maximum value (60; or similar maximum value as for a* and b*) at edge of the circle for very high chroma or 'colour purity'. The **h° value is the hue** and corresponds to the angle formed between the a* and b* axes, starting from +a* axis and expressed in degrees. An h° value of 0° corresponds to +a* (red), 90° to +b* (yellow), 180° to –a* (green) and 270° to –b* (blue). C* and h° values can be calculated from a* and b* values with C* as square root of ($a^{*2} + b^{*2}$) and h° as $\tan^{-1}(b^*/a^*)$ (corrections needed if a*<0 : h°= 180 + $\tan^{-1}(b^*/a^*)$; if b*<0 : h°= 360 + $\tan^{-1}(b^*/a^*)$) (Warriss, 2010; Konica Minolta, 2015).

L*, a* and b* values are conveniently and very commonly measured to **evaluate meat colour** using portable tristimulus colour analysers such as the Minolta chromameter (http://konicaminolta.com/instruments; Figure 3). This device automatically calculates C* and h° values, and can also determine colour coordinates in other colour spaces such as CIE XYZ (Yxy coordinates). Colour coordinates (L*a*b*) for **subcutaneous fat** can be assessed using the same methodology. In scientific publications, meat colour is very often evaluated using L*a*b* values; C* and h° values are mentioned less often, even though they are very good indicators of the colour perceived by human eyes; especially h° which has been found highly (negatively) correlated to the red colour intensity of pork assessed by a trained panel (personal data).

Scientists and experts have agreed for a long time on the importance of reference methods for the determination of meat quality traits, including colour. Important publications have been published in order to circulate the agreed recommendations of the experts and to propose a common method for instrumental evaluation of **fresh meat colour** (Boccard et al., 1981; Honikel, 1997; Honikel, 1998; AMSA, 1991; Škrlep and Čandek-Potokar, 2006; AMSA, 2012).

These recommendations include:

- **History and specifications of meat sample**: animal (breed, genetics, nutrition, age, sex), as well as transport and slaughter conditions, and especially chilling, and pH must be reported.
- **Sampling time and location**: at least 24h *post-mortem*, with muscle name clearly specified and location within muscle described; samples (usually *longissimus*) should be a cross-section taken perpendicular to the long axis and a minimum of 15 mm thickness is recommended.
- **Storage of samples**: if storage occurs prior to colour measurement, samples must be refrigerated (≤ 4°C) and conditions (temperature, light, overwrap) must be specified.
- **Blooming**: time for blooming (oxygenation of surface myoglobin) is important prior to measurement; it depends of species and is shorter for white meat such as poultry and pork than red meat like beef and lamb. For pork, a blooming time of 20-30 minutes may be sufficient even though 1 hour blooming is usually practiced (in any case time of blooming must be consistent) and at a maximum temperature of 3°C. However, in some laboratories blooming is often very short and in some cases no blooming is allowed before colour measurement. Surface drying must be avoided by use of an oxygen-permeable but water-impermeable film, or humidity control. Conditions of illumination of the samples must be given.
- **Testing**: at least triplicate measurements are recommended, made on different sites of the meat surface (without blotting before measurement). In some muscles, considerable colour differences exist between lateral/medial sites, and high levels of marbling or connective tissue can also induce colour variability.
- **Equipment:** the model and brand of the equipment must be given. Both chromameters (e.g. Konica Minolta CR200, CR300, CR400) and spectrophotometers (e.g. HunterLab Miniscan XE Plus; Konica Minolta CM 2300d or CM 2500d) are commonly used. Chromomameters only measure tristimulus values (CIE L*a*b*) whereas spectrophotometers are more complex instruments that supply spectral analysis, thereby allowing the estimation of the percentage of surface myoglobin forms (cf below).
- **Procedure**: the recommended parameters are a **light source** of D 65 with the illumination/viewing system as 45/0 or 0/45 or diffuse 8 (d/8). The recommended **standard observer angle is 10°** and the **colour scale is L*a*b*** (CIE 1976). The **diameter aperture** should be as large as possible (1 cm diameter aperture is commonly used, 2.5 cm diameter is sometimes used for beef). **Specular reflectance** should be excluded if within the capabilities of the instrument. Information on room temperature should be given.
- **Calibration:** should be based upon a black standard as L*=0 and white as L*=100. A white tile is usually provided with each chromameter equipment for its calibration.

When providing data on colour parameters of meat /fat samples, all the information regarding the samples, storage and measurement conditions including blooming time, equipment used and procedure applied for determination of meat colour should be provided.

4.3.3 Myoglobin redox forms

In addition with L*a*b* or L*C*h° values of fresh meat, the **amounts of each myoglobin redox form** can be assessed using instrumental methods. These are based on the measurement of reflectance spectrophotometry at isobestic wavelengths (wavelength at which reflectance is equal for two or more of the three myoglobin forms). Data are converted to K/S values (K = absorbance coefficient, S = scattering coefficient) and ratios of K/S values at different

wavelengths allows estimating surface contents in deoxymyoglobin (K/S 474 nm / K/S 525 nm), oxymyoglobin (K/S 610 nm / K/S 525 nm), and metmyoglobin (K/S 572 nm / K/S 525 nm) (AMSA, 1991; Mancini, 2009). Redox state of myoglobin can also be assessed *in vitro* by determining absorbance at 503, 557 and 582 nm for metmyoglobin, deoxymyoglobin and oxymyoglobin, respectively. Absorbance at 525 nm is used to determine myoglobin concentration (Mancini, 2009). Contrary to surface measurement techniques, extraction techniques primarily characterize interior rather than surface meat pigments. They are also more time consuming.

Figure 3: Determination of L*, a* and b* colour parameters with a Minolta chromameter.

4.3.4 Metmyogobin reducing activity and oxygen consumption

Two other traits related to meat colour are metmyoglobin reducing activity and oxygen consumption.

Several techniques exist to assess these traits. Regarding reducing activity, assays generally include a two-steps procedure, the initial step being oxidation of myoglobin to induce MetMb formation, and the second step promoting MetMb reduction. Changes in redox state during the second phase (i.e. difference in MetMb between the initial oxidized and final (reduced) levels) allow calculation of the MetMb reducing activity which corresponds to the **ability of muscle to reduce oxidized pigments** (Mancini, 2009; King et al., 2011). Oxygen consumption can be assessed by placing a meat sample in a pouch or bottle flushed with oxygen, and determining difference in gas composition before and after incubation time. Another method consists of determining the percentage of oxymyoglobin on the bloomed surface of a meat sample using a spectrophotometric method as described above. Then the meat sample is vacuum-packed and incubated to induce pigment deoxygenation and reduction, and percentage of oxymyoglobin assessed again using the same method. The **oxygen consumption** is then calculated as the difference in percentage of oxymyoglobin prior to and after incubation (Mancini, 2009; King et al., 2011).

4.4 Comparison of methods

An intensive study (gathering over 1000 articles) of instrumental colour measurement information in the field of meat science showed that the majority of researchers use Minolta or Hunter machines (Tapp et al., 2011). It was interesting to notice that a large percentage of the papers failed to include pertinent information (which is discussed also in the above paragraphs) such as type of illuminant, aperture size, observations angle, bloom time and observations per sample, despite the fact that these data are necessary to replicate and accurately interpret/compare instrumental colour results.

As sensory colour of meat can be a deciding factor when purchasing meat, it is interesting to see the association between visually and instrumentally assessed colour. Some aspects of comparison of different methods (also visual and instrumental) can be found in the review of Mancini and Hunt (2005). Khliji et al. (2010) studied the relationship between consumer ranking of colour and objective measures of colour (Minolta L*a*b*) in lamb. It was demonstrated that a* values explained more of the variation in consumer scores than L* values. Analysis suggests that when the a* value exceeds 9.4 consumers will, on average, consider the meat colour acceptable. The corresponding value for L* is 34. Respecting 95% confidence interval that randomly selected consumers would score randomly selected sample as acceptable, these thresholds for L* and a* are indeed much higher (44 and 14.5 for L* and a*, respectively). This highlights the large variability between individuals regarding perception of fresh meat colour. The literature shows low correlations between the L* value and acceptability scores (r = 0.18 for lamb, r = 0.32 for beef; Hopkins, 1996; Shorthose et al., 1988). For pork, Brewer et al. (2001) reported that L* was most correlated to visual determinations of pinkness (r = -0.67 to -0.80). It was thus suggested that L* may be the best indicator of PSE and/or DFD pork (Brewer et al., 2001). Recent results showed that pork redness score assessed by a trained panel was highly correlated to L* (r = -0.70) and even better correlated to h° value (r = -0.73), than to a* (r = 0.37) or b* (r = -0.31) values (Lebret, unpublished data). For the panellists it is more difficult to assess b* (yellow-blue colour) than L* (lightness) or a* (red to green colour; O'Sullivan et al., 2003a). It was shown that haem pigment and metmyoglobin contents are only moderately correlated with pork L* values (r = 0.35 to 0.45), a* and b* values (r= 0.50 and 0.40, respectively; Lindahl et al., 2001). In veal, visual colour scores of carcasses were moderately correlated with L* (r = -0.68) and a* (r = 0.69; Hulsegge et al., 2001). O'Sullivan et al. (2002) reported that panellists who visually assessed colour were able to differentiate between four experimental groups (control, Fe, vitamin E, Fe + vitamin E) on different experimental days and were more effective in evaluating the colour quality of samples than instrumental assessment, in their case the Hunter L*a*b* technique. O'Sullivan et al. (2003a,b) demonstrated that trained colour panellists can be objective and discriminating. Despite these findings, researchers often ignore the relevance of visual panels and are more willing to accept instrumental data, probably because the use of colour devices is simpler than conducting sensory analysis.

There are a few articles comparing different instrumental techniques to measure colour. Holman et al. (2015) recommended the use of Hunter Lab for measuring lamb meat colour. Hunter Lab proved to be the best-suited technique also to measure colour of dry-cured ham. Hunter Lab and CIE L*a*b* provided more reproducible lightness data than L*u*v*and XYZ systems (Garcia-Esteban et al., 2003).

4.5 References

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Chapter 5

Instrumental tenderness – shear force

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5.1 Texture analysis for the assessment of a physical quality characteristic of meat, tenderness

Tenderness, together with water holding capacity, is generally considered as one of the major traits of meat quality (Koohmaraie and Geesink, 2006; Miller et al., 2001; Moeller et al., 2010; Yancey et al., 2010). Although palatability is influenced by flavour and juiciness as well as tenderness, tenderness seems to prevail as shown by the highly valued retail cut of tenderloin, which is the least flavourful and the least juicy meat cut but at the same time is one of the most tender (Koohmaraie and Geesink, 2006). Noteworthy is the evidence reported in literature (Boleman et al., 1997, Lusk et al., 2001, Shackelford et al., 2001) on the willingness of consumers to pay a premium for a tender product; hence the necessity of a reliable and objective method capable of giving stable and comparable instrumental measurements of meat texture related to meat tenderness.

According to Solomon et al. (2010), meat texture comprises different characteristics such as hardness, springiness, chewiness, cohesiveness and even juiciness, whereas sensory tenderness refers to hardness. The perception of texture is generally associated with mechanical failure properties that are related to muscle structure. Indeed, variations in meat texture originate from inherent differences within the structure of raw meat/muscle tissue in relation to contractile protein structures, connective tissue framework, lipid and carbohydrate components as well as external factors like cooking and sample handling (Solomon et al., 2010).

Quite different methodologies have been proposed to assess meat tenderness instrumentally and even today no single method provides a complete tenderness profile (Honikel, 1998). Each method has its advantages and limitations, showing a high variability in the results as well as in their correlation with sensory rating of meat tenderness. Basically, instrumental texture analysis measures the resistance of muscle tissue to shearing, compression, and/or penetration.

Among the many devices that have been developed over years for texture analysis (García-Segovia et al., 2014, Solomon et al., 2010) are:

- texture profile analysis (TPA; a compression test where the sample is placed on a flat surface and a compression plate is lowered onto the sample; force, position or percentage of the original height of the sample may be used to interpret the compression test; Friedman et al., 1963),
- Volodkevich bite tenderometer (VBT, consisting of blunt wedges that pinch the sample during compression; Volodkevich, 1938),
- Kramer shear cell (Kramer shear press, KSP, Allo-Kramer, AK; consisting of a multi-bladed cell which drives at a constant speed through the sample, compressing, shearing and extruding it through a slotted base; Kramer et al., 1951),
- Ottawa texture measuring system (a compression-extrusion tester; Voisey, 1971),
- MIRINZ tenderometer (consisting of a blunt wedged-shaped “tooth” which is used to shear through the sample, McFarlane and Marer, 1966),

- NIP tenderometer (a shear device, Smith and Carpenter, 1973),
- the Meullenet-Owen razor blade for shear force (RB, RBS, MOR, MORBS, or MORS, Cavitt et al., 2004),
- the Warner-Bratzler shear force (WB) and the slice shear force (SSF) methods measure the amount of force required to shear across entire muscle fibres.

There are also instruments that force needles to penetrate into raw meat:

- Armour Meat Tenderometer (10 needle probe, Hansen, 1972),
- star probe (consisting of a cylindrical solid punch with four, five or six tapered points, probably an adaptation of cherry, date, or olive pitter),
- tensipresser (with a round, hollow cylinder).

With tensile tests the samples are subjected to extension at a given strain rate; problems with gripping the samples are a major cause of failure with this kind of test, especially with raw meat.

Finally, non-destructive techniques have also been studied in relation with meat texture analysis, such as:

- spectroscopy (NIR, Raman),
- ultrasonics,
- sonoelasticity,
- image analysis,
- dual energy x-ray (DEXA),
- electromyography and electrognathography (consisting of several electrodes placed over facial muscles and the mandibular joint of a sensory panelist to measure muscle activity and forces during chewing),
- elastography (consisting of an ultrasound equipment measuring axial strain elastograms before and during external compression of the sample), Solomon et al. (2010).

5.2 Reference methods

Although it was one of the first methods developed and despite criticism regarding the repeatability and variability of the results and the lack of straightforward link with sensory perception of meat tenderness, Warner-Bratzler shear force is still the most widely accepted method for meat texture analysis (Wheeler et al., 1997), followed by slice shear force method. Hence only WB and SSF will be described in detail here.

5.2.1 The Warner-Bratzler shear force (WB) standardized protocol

The WB shear force method was developed back in the 1930's (Warner, 1928, Bratzler, 1932) as one of the first instrumental methods to evaluate meat tenderness by mimicking the forces produced during biting and mastication. Extensive studies to decrease variability (Wheeler et al., 1995; 1997) have resulted in a standardized protocol to measure WB shear force in meat from all species (AMSA. 2015). This protocol is described in detail below. Since modifications to the protocol given below potentially lead to different shear force results, the denomination WB should be restricted to the specifications given hereafter. Hence, any modification to this protocol should be clearly stated; for example, WBsq specifying the use of a square blade instead of a V shaped blade (protocol otherwise the same as the one

below) or WBsc specifying the use of square-section cores instead of round-section cores, etc.

- **Shear machine**: A Warner-Bratzler shear machine or an electric universal testing machine fitted with a Warner-Bratzler shear blade (Figure 4). Crosshead speed of 200 or 250 mm/min.
- **WB shear blade**: – Blade thickness: 1.1684 mm (0.046 inches)
  Cutting blade V shaped (60° angle)
  Cutting edge beveled to a half-round
  Corner of the V should be rounded to a quarter-round of a 2.363 mm diameter circle
- **Spacers**: the spacers providing the gap for the cutting blade to slide through should be 2.0828 mm thick.
- **Sample preparation**: Steaks (2.54 cm thick) should be cooked to a final internal temperature of 70-71°C (see also Cookery method below), Figure 1. The steaks (sample slices) are easier to obtain by cutting a frozen muscle with a band saw.
- After cooking and recording the maximum steak cooking temperature and weight, chilling the cooked steaks overnight at 2 to 5°C before coring is recommended. The rationale behind this procedure is that chilling firms the steak making it easier to obtain uniform diameter cores. However, if chilling is not used after cooking, some protocol to obtain consistent steak temperature before coring should be followed, such as allowing steaks to reach room temperature or using running tap water to cool the samples.
- **Coring devices**: Cores can be obtained using a hand-held coring device (cork borer) or an automated coring device (drill press with cork borer attached, Figure 2). Coring devices must be in good condition and sharp or the core diameters will not be consistent and will result in spurious increased variation in shear values.
- **Cores**: Round cores should be 1.27 cm (0.5 inches) in diameter and removed parallel to the longitudinal orientation of the muscle fibres so that the shearing action is perpendicular to the longitudinal orientation of the muscle fibres (Figure 3). Cores that are not uniform in diameter, have obvious connective tissue defects or otherwise would not be representative of the sample should be discarded. Cores should be kept refrigerated until sheared to maintain consistent temperature
- **Number of cores**: A minimum of six cores should be obtained from each sample (this may require 1 or more steaks or chops depending on the muscle and species). The number of cores should be reported.
- **Shearing**: Each core should be sheared once in the center to avoid the hardening that occurs toward the outside cooked edge of the sample (Figure 5).

Figure 1: Cooking pork steaks to 70°C internal temperature.

Figure 2: Electric drill press with cork borer attached.

Figure 3: Steak and cores.

- **Evaluation**: From the force deformation curve the maximum is recorded as the peak force (Figure 6). The mean of six measurements per sample is given as the WB shear force in N, alternatively in kg_f. Sometimes kPa, or N/cm^2 can be found in literature. The force deformation curve enables also the determination of the total energy as the surface under the curve, but this parameter is not commonly used. Some authors also report the initial yield as the first maxima, but this is not always apparent.

Additional information: Some additional information is necessary to consistently describe the texture analysis measurement: i) Steak temperature before-cooking and maximum cooking temperature. ii) A detailed description of the thawing and cooking methods. iii) Muscle type and sample location within muscle. iv) Animal breed, age and sex.

5.2.2 Slice shear force (SSF)

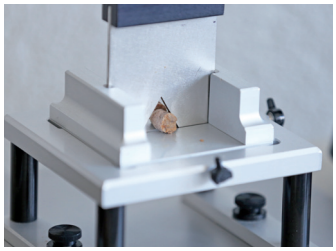
A simplified shear force protocol applicable to commercial processing conditions has been proposed by Shackelford et al. (1999a and b) and Wheeler et al. (2007). This protocol is described by AMSA (2015) and Shackelford and Wheeler (2009) as following:

- 2.54 cm thick steaks are cooked with a belt grill to a final internal temperature of 70°C (Figure 7).
- Immediately after cooking, a 1 cm thick, 5 cm long slice is removed from each steak parallel to the muscle fibres. The slice is acquired by first cutting across the width of the muscle at a point approximately 2 cm from the lateral end of the muscle.
- Using a sample sizer, a cut is made across the muscle parallel to the first cut at a distance 5 cm from the first cut.
- Using a knife that consists of two parallel blades spaced 1 cm apart, two parallel cuts are simultaneously made through the length of the 5 cm long steak portion at 45° angle to the long axis of the muscle and parallel with the muscle fibres (or 90° angle depending on the fibre orientation of the muscle so that the shearing action would be across the muscle fibres, Shackelford and Wheeler, 2009), Figures 8 to 11. For example, the *longissimus* muscle of either beef, pork or lamb is cut with a 45° angle (Figure 10 and 11) whereas the *semimembranosus* muscle is cut with a 90° angle. Different species or different muscle types may require more than one slice per meat sample (Shackelford and Wheeler, 2009).
- The 5 cm long, 1 cm thick slice is sheared perpendicular to the muscle fibres. With small muscles, which cannot give 5 cm long slices, as is the case with lamb chops, two slices 2.5 cm long are used together making up a total length of 5 cm (Shackelford et al., 2004a). The shear action is made using an electronic testing machine equipped with a flat, blunt-end blade, with a gap of 2.0828 mm for the blade to pass through (Figure 12). The SSF blade has the same thickness (1.1684 mm) and degree of bevel (half-round) on the shearing edge as WB blades. Usually the cross head speed is set at 500 mm/min to minimize the time required for SSF measurement.

5.2.3 Relationship between shear force methods and tenderness

Shackelford et al. (1999b) reported that SSF measurements in beef were more strongly correlated ($r = -0.82$) with tenderness rating by a sensory panel than WB shear force ($r = -0.77$). Whereas the following correlation coefficients between SSF and meat tenderness ratings were reported: $r = -0.81$ ($P<0.001$) for beef (Shackelford et al., 1999a), and $r = -0.72$ ($P<0.001$) for pork (Shackelford et al., 2004b). On the other hand, Solomon et al. (2010) reported correlation coefficients ranging from 0.92 to 0.7 between WB and sensory panel tenderness scores. Finally, a correlation coefficient of $r = 0.84$ ($P<0.001$) between SSF and WB in beef was reported by Shackelford et al. (1999a).

Figure 4: Electric testing machine fitted with a Warner-Bratzler shear blade.



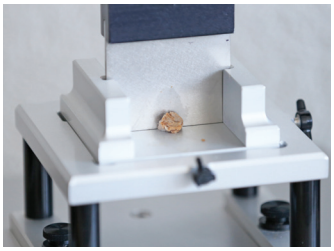


Figure 5: Performing a WB shear force measurement with an electric testing machine fitted with a Warner-Bratzler shear blade.

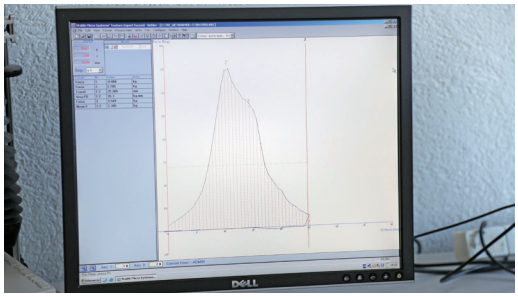


Figure 6: Force deformation curve. The peak force (maximum peak, number 3) is recorded here as kgf.

Figure 7: Belt grill used for steak cooking. From Shackelford et al. (2009).

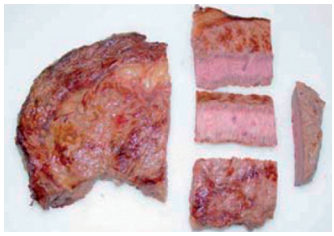


Figure 8: SSF slice prepared for shearing. From Shackelford et al. (2009).

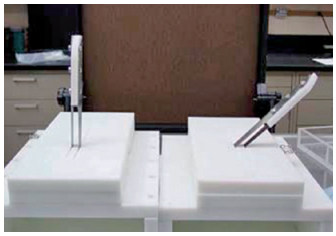


Figure 9: 90° and 45° slice boxes. From Shackelford et al. (2009).

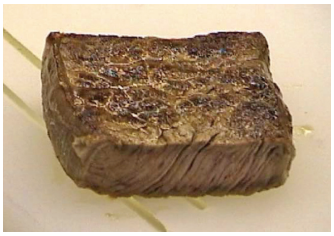


Figure 10: 5 cm section with 45° fibre angle.
From Shackelford et al.: www.ars.usda.gov/SP2UserFiles/Place/54380530/protocols/SSFProtocolforsmallvolume.pdf

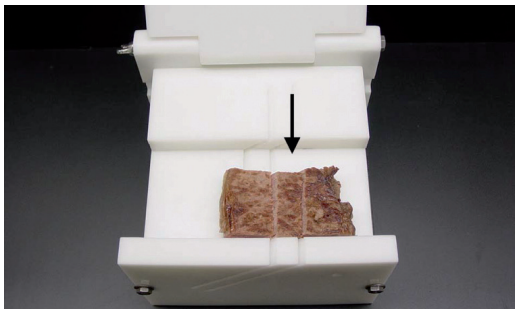


Figure 11: A slice 5 cm long, 1 cm wide cut at 45° angle from a 5 cm meat section.
From Shackelford et al.: www.ars.usda.gov/SP2UserFiles/Place/54380530/protocols/SSFProtocolforsmallvolume.pdf

Figure 12: SSF shearing on a universal testing machine. From Shackelford et al. (2009).

5.3 Sources of variation

It is important to note that textural analysis is defined by the instrument and the protocol of measurement. Furthermore, there is some evidence on the influence of intrinsic parameters such as breed, sex and age on tenderness and shear force. Hence, it is recommended that animal characteristics such as breed, gender, age, muscle type, etc. together with husbandry and slaughtering procedures are reported.

5.3.1 Common modifications of the WB shear force standardized protocol

The following are the most common modifications of the WB standardized protocol reported in literature. All these modifications are prone to give variable results as compared to standardized protocols.

- **Square blade (WBsq):** Chrystall et al. (1994) and Honikel (1998) recommend the use of a blade, 1.2 mm thick, with a rectangular hole 11 mm wide and at least 15 mm high. The hole should have square edges but the edges should not be sharp.
- **Square section core (WBsc):** Honikel (1998) recommends the use of sample strips cut from a block of cooked meat, with 100 mm^2 (10 x 10) cross-section and at least 30 mm long, with the fibre direction parallel to the long dimension. Some authors report the use of square cross-section cores because it allows easier recognition of the muscle fibre orientation and easier removal of cores. However, higher values ($P<0.05$, paired t-test) for square than for round cross-section cores have been reported for beef and pork samples (Silva et al., 2015). Furthermore, greater ($P<0.01$, F test) standard deviations of 5 replicates per steak were reported for square than for round cross-section cores (Silva et al., 2015). These authors also reported $R^2 = 0.78$ and 0.70 for the coefficients of determination between WB and WBsq for beef and pork respectively.
- **Crosshead speed (WB_{100}):** Chrystall et al. (1994) and Honikel (1998) recommend that the blade should be drawn or be pushed at 50 to 100 mm/min to shear the sample.
- **WBho:** A combination of a, b and c modifications may be denoted as WBho (WB by Honikel).
- Other less common modifications are: a straight cutting blade; a blade without a beveled edge; a steak thickness of 3 cm instead of 2.54 cm; higher or lower end cooking temperatures (different to 70-71°C).

5.3.2 Methodological factors affecting WB and SSF measurements

A number of parameters have been identified as affecting shear force measurements. Among these parameters the most important are described below. Not listed here are factors related to methods specially designed to modify tenderness such as stunning methods, carcass chilling rate, ageing methods, suspension techniques, electrical stimulation, injection of brine solutions, etc.

Muscle fibre orientation and sample thickness: Farag et al. (2009) discussed the importance of muscle fibre orientation and of sample thickness on WB shear force. These two parameters are fixed in the WB standardized protocol; nevertheless, some authors report difficulties with muscles presenting irregular fibre orientation (Hildrum et al., 2009).

Cookery method and before-cooking temperature: Chrystall et al. (1994) recommend cooking samples inside a plastic bag in a water bath at 80°C for one hour.

Cooking with an open-hearth electric broiler is recommended by AMSA (1995). The temperature is monitored at the geometric center of the steak. The steak is turned once the internal temperature reaches 40°C (values ranging from 35 to 45°C are reported in literature) and removed from the broiler once it reaches 70°C (values ranging from 68 to

71°C are reported in literature). Cooking with an open-hearth broiler for a constant time (35 min) instead of to a constant internal temperature (70°C) has also been suggested. However, Wheeler et al. (1996) observed a reduced repeatability of duplicates with this method, despite similar mean shear force values.

Wheeler et al. (1997) reported improved repeatability among institutions for WB conducted with a standardized cooking protocol (open-hearth broilers, turning the steaks when the internal temperature reached 40°C and removing them from broiler when the internal temperature reached 70°C) as well as a standardized thawing before-cooking protocol (thawing the steaks at 2 to 5°C and cooking them once the internal temperature had reached 2 to 5°C).

Wheeler et al. (1998) reported the use of a belt grill in an attempt to eliminate sources of variation in the measurement of WB shear force. Additionally, the belt grill is an instrument adapted to commercial processing conditions. Rather than cooking the samples (with a broiler) to a constant endpoint temperature, when using a belt grill samples are cooked for a constant time designed to achieve 70-71°C of internal endpoint temperature: 5.7 min (beef, Shackelford et al., 1999b), 5.8 min (pork, Shackelford et al., 2004b) and 5.3 min (lamb, Veiseth et al., 2004).

WB measurements differ between raw and cooked meat (Tornberg, 1996). The effect of cooking temperature and time on WB in rabbit meat is reported among others by Combes et al. (2003).

Endpoint temperature: AMSA (1995) recommends 71°C as endpoint temperature for WB measurements in meat from all species. However, it is important to keep in mind that the degree of doneness to which meat is cooked varies considerably among consumers and that tenderness is closely related to the degree of doneness. Indeed, it is well established that tenderness of the *longissimus* muscle generally declines as degree of doneness increases (Wheeler et al., 1999). Ultimately, doneness depends on parameters related to muscle type and maturity such as the extent of collagen shrinkage (up to 60°C), myofibrillar protein hardening (main temperature effect from 60 to 74°C), and collagen solubilization (from 74 to 90°C).

Storage mode (freezing and thawing methods: rates and freezing length and temperature): Leygonie et al. (2012) discussed the influence of freezing and thawing on the textural properties of meat. Among other authors Grayson et al. (2014) and Lagerstedt et al. (2008) reported a decrease in shear force after freezing and thawing beef samples without an additional aging period, probably as a result of structural damage of muscle fibres due to ice crystal formation. Munro (1983) suggested that at low freezing rates ice crystals in lean meat grow between the fibres and the fibre bundles, leading to no ice growth within the myofibrils; ice crystals tending to be aligned in the meat fibre direction. Accordingly, Grujić et al. (1993) and Petrović et al. (1993) reported that with low freezing rates large ice crystals preferentially formed in the extracellular region, whereas in rapid freezing, smaller ice crystals were more evenly distributed in both intra- and extracellular regions. Finally, these authors reported higher sensory tenderness scores after fast freezing below –70°C, probably favored by formation of small intracellular ice crystals.

Farag et al. (2009) found no influence of the thawing method (rapid radio frequency vs. slower conventional air tempering) on WB shear force.

Sample location within muscle: Lindahl et al. (2010) and Wheeler et al. (2007) reported variation of shear force in beef depending on sample location within muscle. Furthermore, Hildrum et al. (2009) describe the variability in tenderness within the muscles as both of random and systematic character.

Variation of WB values within steak (core location): Devine et al. (2006) discussed the WB variability among individual cores: WB values of individual cores from the same steak show a large variability. Moreover, the WB mean values do not always reflect this variability and the variability itself does not follow a normal distribution. Hence, the question arises whether the mean WB values are fully representative of the sample. The individual WB shear force values seem to be less variable with fully aged samples.

5.3.3 Intrinsic factors affecting textural analysis

Although there is evidence of the role played by intrinsic factors such as sex, age, breed and muscle location in literature (Huff-Lonergan et al., 1995, Kuber et al., 2004, Veiseth et al., 2004), the variation in tenderness and tenderness related traits is, however, ultimately related to the complex interaction of various biochemical traits (Rhee et al., 2004, Melody et al., 2004, Lepetit, 2007; 2008, Stolowski et al., 2006). Hence, noteworthy are the highly variable tenderness and tenderness related traits observed within and among many beef muscles (Rhee et al., 2004), among individuals in lamb *longuissimus* (Koohmaraie et al., 2002) and the greater variation observed within breeds than among breeds for the same muscle in beef (Wheeler et al., 2005). Therefore, it is recommended not only to report precisely the origin and husbandry of the live animal (including breed, sex, age, feeding regime), but also the transport, pre-slaughter handling, slaughtering procedures, etc.

Animal breed, age and sex: The effect of breed, age and sex on WB was discussed among others by Huff-Lonergan et al. (1995), Campo et al. (2000) and Monsón et al. (2004) for beef and by Veiseth et al. (2004) for lamb. Correlations between WB and sensory tenderness scores are reported to be greatest in a given muscle between animals of the same age (provided cooked to >60°C) whereas correlations between sensory scores and WB are reported to be least when different muscles from animals of different ages are compared (Chrystall et al., 1994).

Muscle type: Shear force has been reported to be muscle dependent in beef (Rhee et al., 2004, Hildrum et al., 2009) and in pork Wheeler et al. (2000) reported the dependence of tenderness on muscle type.

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5.4.1 Methodological references

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Chapter 6

Fatty acid analysis in meat and meat products

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6.1 Introduction to the structure and occurrence of fatty acids in meat

The determination of the fatty acid profile as well as the fatty acid content of meat and adipose tissue is of importance in view of the implications of fatty acids to human health, the sensory quality of meat and meat products, as well as their influence on the processing of meat products.

Fatty acids consist of C, H, O, arranged as a carbon skeleton with a carboxyl group (-COOH) at the end. They are classified according to their chain length (number of C-atoms) and their degree of unsaturation (number and position of the double bonds and their configuration).

In meat and adipose tissue, most fatty acids have a chain length between 12 and 22 C-atoms, mainly having an even number of C-atoms and a linear structure, and accounting for approximately 85% of the muscle fatty acids. Typically for ruminant products, a small proportion of the fatty acids have an uneven number of C-atoms, the so called odd-chain fatty acids, which can have a linear or a branched structure.

Based on the number of double bonds, fatty acids can be classified into saturated fatty acids (SFA) and unsaturated fatty acids (UFA). SFA do not contain any double bonds along the carbon-chain, and have a general formula as $CH_3(CH_2)_nCOOH$. UFA have at least one double bond in the their chain, and are further classified as mono-unsaturated fatty acids (MUFA), having one double bond, and those with two or more double bonds as poly-unsaturated fatty acids (PUFA). Double bonds are normally in the cis-configuration, while in ruminant products a small proportion of the double bonds are in trans-configuration, mainly due to the rumen is mainly done from a nutritional perspective. Microbial activity. Also conjugated fatty acids may be present, again mainly in ruminant products, the most important of which is conjugated linoleic acid, CLAc9t11, an isomer of linoleic acid (C18:2) with a conjugated bond system at the 9 and 11 C-position. In addition, a distinction is also made for the PUFA on the first double bond counting from methyl end, to obtain two classes i.e. n-3 and n-6 fatty acids. This classification is mainly done from a nutritional perspective.

The most important MUFA in meat and adipose tissue is oleic acid (C18:1c9), while linoleic acid (LA) (C18:2n-6) and α-linolenic acid (LNA) (C18:3n-3) are the most important PUFA in terms of quantity. However related to nutritional health implications other PUFA (i.e. arachidonic acid (AA); C20:4n-6, eicosapentaenoic acid (EPA); C20:5n-3 and docosahexaenoic acid (DHA); C22:6n-3) are also often studied.

Fatty acids as such are generally not present in meat and adipose tissue, but are incorporated in triacylglycerols and phospholipids. Phospholipids are polar lipids, located in the cell membranes. The content of the phospholipids in meat is rather constant, and independent of the total amount of fat. It often varies between 0.2 and 1 % of muscle weight. Triacylglycerols are the main neutral lipids, and are located in the adipocytes. The content of the muscle triacylglycerols is highly dependent on the total fat content, and can vary in muscle between 0.2 up to 5% of muscle weight. Triacylglycerols consist mainly of SFA and MUFA, while the PUFA are almost exclusively deposited in the phospholipid fraction.

Meat products consist not only of fatty acids present as lipids, but also contain variable proportions of proteins and other ingredients. Therefore to analyze the fatty acid profile and content in meat and meat products, often a solvent extraction is performed. As fatty acids are not volatile, an esterification step is needed before further analysis with gas chromatography (GC) is possible. Some studies of direct transesterification, meaning without extracting the lipids from the matrix, were reported.

The following sections comprise an overview of the different factors influencing the fatty acid profile in meat and a discussion of the possible methods for the analysis of the profile and content of fatty acids in muscle tissue.

6.2 Factors influencing the fatty acid profile of muscle tissue

The profile and content of fatty acids in muscle tissue can vary considerably depending on several factors. In fresh muscle, animal species, genetics, muscle type and animal's diet are the main factors affecting the fatty acid profile. Some excellent reviews discuss the effects of species, genetics and dietary strategies on the muscle fatty acid composition (e.g. Dannenberger et al., 2013; De Smet et al., 2004; Raes et al., 2004; Scollan et al., 2014; Wood et al., 2008; Woods and Fearon, 2009).

It is clear that species is the major source of variation in the fatty acid composition. Compared to monogastric animals (pig, poultry), ruminants have a biohydrogenation step during the digestion of feed, resulting in a saturation of the dietary fatty acids. Therefore, ruminant products are characterized by a higher saturation content compared to non-ruminant products. Due to the microbial biohydrogenation, ruminant tissues are also characterized by small amounts of microbial lipids and microbial fatty acid metabolites such as odd and branched chain fatty acids, and trans isomers.

In addition to variations between species, within species differences in the fatty acid profile are also seen between genotypes and breeds, even when the animals received similar diets. This can be related to differences in fatness, and the capacity to deposit triacylglycerols. In general, it is observed that the higher the fatness of the muscle, the lower the PUFA/SFA ratio, as mainly triacylglycerols are deposited, containing predominantly SFA and MUFA. This effect is more obvious in beef, than in pork, because the influence of the dietary fat on the fatty acid profile of pork is much greater than in beef. The fat level will also influence the ratio n-6/n-3 PUFA, but here the effect of nutrition is significant. Not only is fatness responsible for the differences in fatty acid profile between genotypes and breeds, but genetic differences in the fatty acid metabolism are also observed. Breed differences in terms of fatty acid profile are small, but reflect differences in gene expression or the activities of enzyme involved in fatty acid synthesis.

Major changes in the fatty acid profile of muscle tissue can be achieved by dietary strategies, especially in non-ruminants. Many studies have been conducted which have focused on changing the fat content and fat sources in the animal's diet to obtain more (n-3) PUFA, less SFA and less trans fatty acids, the latter specific for ruminant meat. It is clear that the n-3 PUFA content of meat can be increased by the inclusion of n-3 rich fatty acid sources. To increase the long chain n-3 PUFA such as EPA and DHA, fish oil, fish meal or algae are of interest. If LNA rich sources, such as linseed (oil), grasses and forages are included in the diet, an increase in LNA is mainly observed, with a lower response towards EPA and C22:5n-3 (docosapentanenoic acid; DPA). This is due to the reliance on the enzymatic conversion of LNA to its long chain metabolites. The enzymes involved (desaturases and elongases) are not specific for n-3 FA but also act on the n-6 FA, so there is a competition between the n-6 and n-3 FA to be converted by these enzymes. Increasing the DHA content in the meat by adding dietary LNA rich sources is very limited. DHA increase is mainly

realized by including a DHA rich source in the feed such as algae or fish oils. Generally a lowered n-6 content is observed when increasing the n-3 content. It is clear that the impact of the diet is much larger in monogastric animals compared to ruminants. The success of altering the fatty acid composition in ruminant muscle is largely determined by the ruminal biohydrogenation of the dietary lipids. Due to this biohydrogenation step, meat of ruminants contains several 'odd' fatty acids, occurring in minor components, such as CLAs and conjugated linolenic acids (CLNA), and cis and trans C18:1 isomers, all originating as intermediates from LA, LNA and C18:1c9, as well as iso and ante-iso odd-chain fatty acids coming from the ruminal microflora. The main CLA isomers in ruminant muscle are c9t11CLA and t10c12CLA, accounting for respectively 80 and 3-5% of total CLA. Although these fatty acids are formed during biohydrogenation, they are also depending on the dietary fatty acids, as the precursors of these biohydrogenation isomers are dietary fatty acids. Also dietary fatty acids are influencing the microflora composition thus as well the odd and branch chain fatty acids.

The fatty acid composition also varies with the location in the carcass, and between and within the muscle tissue. Jiang et al. (2010) reported that the SFA concentration increased from external to internal sample location, which was accompanied with a decreased MUFA concentration. Similar observations have been made for the different fat depots in the carcass (Webb et al., 1998).

As well as animal factors, storage and preparation of muscle tissue can also affect the final fatty acid composition of the meat. After slaughtering, muscle tissues are packed using different packaging conditions, stored at 0-4°C or frozen before further processing, which often involve heating steps. The impact of these processing steps on the fatty acid profile, and changes in the fatty acids are less often studied and reported. In particular, the introduction of heating steps will have an impact on the fatty acid amounts and profile, and during heating, lipid derived products are formed e.g. oxidation products, flavour products. Nuernberg et al. (2006) showed a small decreased in PUFA content during storage of the meat. Generally, increasing the cooking time and internal meat temperature results in higher cooking losses, thus affecting the dry matter content of the meat. Therefore, care should be taken when comparing results from studies of the effect of heating on the fat content and fatty acid composition.

In general, when expressed on a fresh matter basis, higher fat contents are observed after heating meat. Taking into account the moisture losses, often no effect of the fat content on a dry matter basis is observed after heating compared to the raw tissue. As PUFA are most prone to oxidation, PUFA proportions are sometimes (slightly) decreased, resulting in a decreased PUFA/SFA ratio in heated meat compared to raw meat. The n-6/n-3 ratio is not affected, indicating that there is no difference in heat susceptibility between n-6 and n-3 PUFA. The effect of heating on individual fatty acids is often very small, as only low amounts of fatty acids need to be oxidized to obtain the characteristic heat flavour components. These very small changes are often not measured by the classical gas chromatography measurements. There are a number of interesting papers dealing with the effect of heating on meat and its effect on the fatty acid composition for example, Alfaia et al. (2010), Bou et al. (2006), Campo et al. (2013), He et al. (2012), Maranesi et al. (2005), Nuernberg et al. (2006) and Sarries et al. (2009). However when meat is cooked, often frying oils, margarines or other external fat sources are used during the preparation. Juarez et al. (2010) and Clerjon et al. (2012) showed that the influence of the cooking method on the fatty acid content and profile of the meat is mainly due to the fatty acid composition of the frying oil itself. These results are a confirmation of the results obtained by Haak et al. (2007).

6.3 Fatty acid analysis in muscle food

Fatty acid analysis in muscle food involves several process steps, before results are obtained. After preservation of the samples in a proper way to avoid losses of FA and especially unsaturated ones, extraction of the lipids is often done by different solvents. As the resultant fatty acids are not volatile, esterification procedures are performed to obtain more volatile derivatives which can easily being analyzed by gas chromatography (GC).

Below, more information is given, not only on the extraction but also on the other process steps to analyze the fatty acids in the muscle. An overview of the most important parameters for the fatty acid analysis in meat and the corresponding methods used by the members of the COST-Action FAIM, is given in Table 1.

6.3.1 Preservation of the samples

As muscle food is highly perishable due to its high water content, and as UFA are highly susceptible to oxidation processes, sampling and preservation of the samples is very important. During sampling, factors promoting the oxidation process should be limited by keeping the temperature as low as possible. Fresh meat samples should be stored vacuum-packed in the dark where possible, so that oxygen and light cannot induce lipid oxidation. Furthermore, samples should be stored at cold temperatures, -20°C or lower is preferable. However it should be noted that at even lower temperatures (e.g. -80°C), the lipid fraction is still prone to modifications such as lipolysis and oxidation. Indeed, although the rate of these processes is slowed down by low temperatures, the processes are not completely stopped. Nevertheless, samples can be kept several months before further analysis once they are stored appropriately (vacuum-packed, frozen). Although freeze-drying is sometimes used to preserve samples, it is not advised as water-soluble antioxidants, present in the muscle, are (partly) destroyed during freeze-drying. Also freeze-dried material should be rehydrated before further analysis, as otherwise the extraction efficiencies will be too low.

6.3.2 Extraction of the lipids from muscle tissue

Muscle is characterized by phospholipids (polar lipids) and triacylglycerols (neutral lipids). Both these groups should be extracted, meaning that proper solvent combinations should be used to obtain the correct polarity. In addition, the non-lipid compounds also need to be removed from the extracts. If the solvents mixtures are too polar, side reactions will occur and non-polar simple lipids and triacylglycerols will not well dissolve. Also interactions between lipids and other tissue compounds should be avoided during the solvent extraction (Christie, 1992).

Generally, the method of Folch et al. (1957), based on the use of chloroform/methanol (2/1; v:v), is used for the lipid extraction from muscle tissue (Table 1). This method takes into account the amount of endogenous water in the muscle tissue, as a third solvent, so in fact the final ratio of chloroform/methanol/water is 8/4/3 (v/v/v). A detailed description of the Folch procedure is given table 2. Due to the toxicity of the solvents used in the Folch method, some research laboratories have sought other extraction procedures. Sometimes the Bligh and Dyer method (1959) is performed; however the extraction efficiency of non-polar lipids is lower, and decreases with increasing total fat content. Other extraction solvents used are hexane/2-propanol (3/2; v/v) as described by Hara and Radin (1978). Commonly, the Folch method is used because the chloroform/methanol mixture is still the best extraction solvent for meat. After the extraction procedure, some clean-up steps are necessary to remove the non-lipid contaminating compounds and to remove water. The most appropriate method is to add a dilute aqueous salt solution (KCl or Na_2SO_4) or pure water as washing solvent.

After separation into two phases the lower phase, containing the lipids, has an optimal solvent ratio of chloroform/methanol/water of 86/14/1 (v/v/v). Then the solvents can be removed under reduced pressure to concentrate lipids, before further esterification steps are performed.

To protect the PUFA in the meat lipids from oxidation during the extraction, antioxidants such as butylated hydroxytoluene are often added. However it should be noted that these components also can elute with the fatty acids during the chromatographic analysis.

6.3.3 Fatty acid esterification

As fatty acids are not very volatile, so it is best to esterify them, so that ester derivatives with a higher volatility are obtained which, compared to the underivatized fatty acids, are more suitable for analysis by GC. Also the polarity of the ester derivatives is lower compared to the underivatized fatty acids, which avoids absorption to the column packing material and dimerization, and will result in better peak properties (less peak tailing, more symmetric peaks, fewer peak shoulders) (Eder, 1995; Shanta and Napolitano, 1992). Although any type of esters can be used, most often fatty acid methyl esters (FAME) are used (Table 1). The procedure to obtain the FAME is important as this step can influence the separation of the FA but can also result in unwanted isomerization reactions. In general, the esterification solutions can be divided in several groups which will be discussed more in detail below. The choice of the esterification procedure needs to be careful taken into consideration as it will influence not only the final fatty acid composition but also the profile and difficulties observed in separating the fatty acids during GC analysis.

Acid-catalysed esterification

A frequently used acid-catalyzed esterification solution is a methanolic hydrogen chloride solution. To this, an inert solvent (e.g. benzene) (Sukhija and Palmquist, 1988; Sattler et al., 1991), or less toxic alternatives as methylene chloride (Iverson et al.,1992), toluene (Ulberth and Henninger, 1992) and tetrahydrofuran (Christie, 1992), need to be added to make triacylglycerols and cholesterol soluble in the methanolic hydrogen solution. Another acid methylation solution, giving similar results to the methanolic hydrogen chloride (5%) solution, is 10% sulphuric acid in methanol. However with the latter, PUFA are oxidized and modified by this strong oxidizing acid solution. A proper alternative, to obtain in a reasonable time the esterification of most lipid classes, is the use of boron trifluoride in methanol (Morrison and Smith, 1964), although the formation of methoxy artefacts of UFA is an important drawback, especially when high concentrated boron trifluoride solutions are used.

An advantage of the acid-catalyzed reagents is their ability to esterify both free fatty acids and O-acyl lipids (Glass, 1971). Acidic esterification procedures are often quiet rapid to perform, but allylic methoxy ether artefacts (Kramer et al., 1997) and derivatives of BHT, when boron trifluoride is used (Moffat et al., 1991), can be formed. Dimethylacetals, derived from plasmalogens of meat, can be formed, resulting in peaks just ahead their corresponding esters in the chromatograms. A further drawback is the possible isomerization of conjugated double bonds during acid esterification. For studies in which the identification and quantification of the isomers of conjugated fatty acids (e.g. CLA, CLNA) are important, acid based methylation procedures are not the methods of choice. The preferred methods for these types of studies would be those using an alkaline based procedure such as a sodium methoxide based method (Kramer et al., 1997).

Base-catalyzed esterification

Base-catalyzed esterification solvents are often composed of potassium hydroxide in anhydrous methanol, methanolic sodium and potassium methoxide (0.5-2M) (Christie, 1992). The use of methanolic sodium methoxide is preferred over potassium containing solvents. This procedure is not time consuming, but care should be taken that lipids are in solution before the esterification starts. This can be done by adding some inert solvents. Sometimes tetramethylguanidine in methanol is reported as a base-catalyzed esterification procedure (Schuchardt and Lopes, 1988), although this method is not recommended for meat, as phospholipids are difficult to esterify (Park et al., 2002; Kramer et al., 1997).

Diazomethane

Diazomethane as esterification solvent is less used nowadays, because of its explosive character (Agrawal and Schulte, 1983; Rosenfeld, 2002). It is not commercially available and so has to be prepared in the lab which carries significant explosive risks and requires appropriate precautions. However, it very rapidly esterifies unesterified fatty acids under mild conditions. This suggests that esterification with diazomethane should be preceded by an alkaline esterification method, when dealing with complex lipids. As well as its explosive character, diazomethane reacts with double bonds in fatty acids leading to artefacts, and it is not capable of reacting with fatty acid esters (Rosenfeld, 2002).

In some cases, no extraction of the lipids from the meat matrix is performed, but instead a direct transesterification on the matrix is carried out. One major reason for choosing this procedure is to reduce the amount of (toxic) solvents needed for the extraction and to reduce the analysis time. There are some studies comparing the fatty acid profile and content after using the direct transesterification method (i.e. directly adding methylene chloride, 0.5 M sodium hydroxide, or 14% boron trifluoride to the muscle) with methods using first an extraction method (Folch method) followed by an esterification procedure on the extracted lipids using the same esterification solvents. Both methods showed similar results, except for arachidonic acid (C20:4n-6), and other fatty acids linked to the cell membranes (Rule et al., 1997; Park and Goins, 1994). So, direct esterification maybe an option, but it should be cautioned that the results obtained (i.e. the efficiency in conversion to methyl esters) can be highly variable, as muscle tissue contains high amounts of water (Christie, 1992). Therefore, it is advisable to use acid-based esterification solvents when applying direct trans esterification, as the presence of water is highly influencing the alkaline esterification reactions.

6.3.4 Fatty acid identification and quantification

The most commonly used and most convenient technique for fatty acid identification and quantification is GC. Different parameters and factors can affect the separation quality of the fatty acids and thus the obtained chromatogram (example as Figure 4). These factors will be briefly discussed.

First of all the type of column used is important. The most preferable columns for meat fatty acid analysis are capillary columns with polar stationary phases such as 100% cyanoethylsilicone oil, 100% cyanopropylsilicone, 68% biscyanopropyl-32% dimethylsiloxane, 70% cyanopropyl polysilphenylene-siloxane. This type of column has a much higher resolution capacity of the UFA compared to apolar stationary phases (Eder, 1995). Depending on the length of the column, a better separation can be obtained. Commonly columns between 30-60 m length are used, resulting in a fatty acid profile of 30 identified peaks and accounting for at least 85% of the total fatty acids (depending on the meat sample). However, it is possible to separate up to 96 peaks occurring in ruminant meat using a 100 m column (Rule et al., 2002). Nevertheless, it is important to note that minor

compounds can still overlap using columns up to 100-120 m (e.g. on a 100 m CP-Sil88 column overlap of C18:3n-3 isomers with C20:0 or C20:1 peaks occur). Also overlap can occur between trans C18:1 isomers and CLA and CLNA isomers, so a combination of methods is advisable in these cases (Chin et al., 1992; Kramer and Zhou, 2001). The separation of the different fatty acids is also determined by the oven temperatures and flow rates used. These parameters varying considerably and appear to be different in almost every lab performing fatty acids analysis.

Normally the identification of the peaks is based on the comparison of the retention times with commercially available pure standards. Problems can arise particularly for minor fatty acids, and some isomers (e.g. CLA, trans C18:1) when no pure commercially standards are available. This leads to difficulties in the identification of the minor compounds, which can partly be overruled when Relative Retention Times (RTT) and Equivalent Chain Length (ECL; based on the Kovats' retention index) are used (Christie, 1989).

FAME are normally detected using a Flame Ionisation Detector (FID) coupled on the GC. This type of detector is very useful as it shows a good linearity over a wide concentration range and it is very sensitive. The output obtained by the FID detector is a chromatogram containing several peaks, of which the area of the peak is directly linked to the concentration of the corresponding fatty acid. The fatty acids are then reported based on a proportional basis (% of total FAME) or on a quantitatively basis (mg/100g product).

To report on a proportional basis, the peak area of the individual fatty acid is divided by the total area of all detected fatty acids and multiplied by 100. Doing this, a relative response factor of 1 is assumed, which can be used for the higher fatty acids (chain length of 12 C-atoms minimum), as these response factors are very close to 1. For short chain fatty acids, this is not valid, but these fatty acids are less important in muscle tissues, and are not normally reported. To quantify the individual standards, internal standards are often used instead of external calibration curves for each fatty acid. Using the internal standard, a relative response factor of 1 is also assumed for the higher fatty acids. The internal standard should be carefully chosen, with the main prerequisite that the fatty acid used as standard is not present in the matrix itself. Regularly used internal standards are C19:0, C17:0 (not valid for ruminant tissues) and C23:0. It is preferable to add these internal standards to the meat lipids before the esterification process, so that they undergo the complete analysis (except extraction).

Table 1. Overview of the most used parameters for determining the fatty acid profile, applied in the research labs involved in the COST action.

Extraction	
Method	Folch et al., 1957 Bligh and Dyer, 1959 Hara and Radin, 1978 No extraction
Methylation	
Method	Acid based Alkaline based Diazomethane TMSH
Internal standards	
	C19:0; C23:0; C21:0; C11:0; C13:0; C17:0 or combinations and/or combined with triacylglycerols of C11:0; C13:0; C23:0
Capillary column	
Packing	50-90% cyanopropyl 50-10% methyl polysiloxane poly(90% biscyanopropyl/10% cyanopropylphenyl siloxane 1,9-di(3-vinylimidazolium)nonane bis(trifluoromethylsulfonyl)imide polyethylene glycol poly(6% cyanopropylphenyl/94% dimethylsiloxane) 70% cyanopropyl polysilphenylene-siloxane
Length	Varying between 30 and 100m
GC conditions	
Injection	Injection temperature varying between 250-250°C Most often split injection Injection volume between 1 and 2 µl
Detector	FID Detector Detector temperature varying between 250-300°C
Oven	Oven temperature programs are highly variable, without any uniform program Gas used: hydrogen or helium Gas flow rate between 1 and 2 ml/min
Identification standards	
	Supelco 37 Component FAME MIX Nu-Chek 68A, GLC463, GLC569B, GLC569C Matreya GC110

Table 2. Detailed procedure of the Folch extraction method (based on Folch et al., 1957).

Step	
1	Weigh an amount of muscle (supposing the density is 1g/cm^3).
2	Add chloroform/methanol (2/1; v/v) to the muscle so you obtain a 20 fold dilution (5 g muscle + 95 ml chloroform/methanol).
3	Homogenize this very well to obtain a complete homogenization (e.g. ultraturrax 13,000 rpm – 1-2min). (Figure 1)
4	Optional: Leave the mixture overnight in the dark at maximum room temperature to obtain a complete extraction.
5	Filter the homogenate through a fat-free filter and collect the filtrate in a vessel. (Figure 2)
6	Wash the obtained filtrate by adding water or a salt-solution, in a ratio filtrate/water of 1/0.2.
7	Allow the solvent mixture to separate into 2 phases by standing or by centrifugation. (Figure 3)
8	Remove as much as possible of the upper phase by siphoning. Remove the interfacial phase by rinsing it three times with small amounts of pure solvents without disturbing the lower phase.
9	The lower phase and the rinsing solvents are collected and diluted with chloroform/methanol to a desired volume.

Figure 1: Homogenization procedure.

Figure 2: Filtration procedure.

Figure 3: Two phases separation of the solvent mixture.

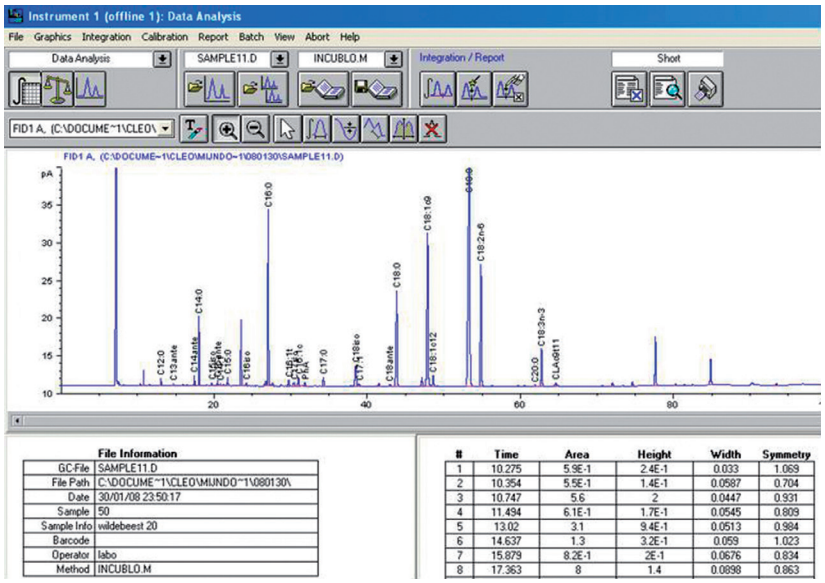

Figure 4: Chromatogram obtained in the analysis.

6.4 References

6.4.1 Methodological references

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Chapter 7

Reference measurement for sensory attributes: tenderness, juiciness, flavour and taint

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7.1 Description of sensory parameters

The sensory meat quality attributes that will be considered are **juiciness** and **flavour** mainly in beef, **tenderness** in all the species (beef, pork, lamb and poultry) and **taint** in pork and lamb.

Flavour is the mixture of sensations including taste, odour (via retronasal route), pressure and cutaneous sensations (such as warm, cold and pain) perceived during the evaluation of a product (Amerine et al., 1965), thus it is a very complex attribute. Raw meat has only a bloody taste and very little aroma but when meat is treated thermally, flavour is created. Precursors of meat flavour are water-soluble components, such as free sugar, sugar phosphates, nucleotide-bound sugar, free amino acids, peptides, nucleotides and other nitrogenous components (Mottram, 1998) and lipids. During cooking there is the formation of volatile compounds due to lipid degradation (for instance aldehydes, ketones and alcohols) and the Maillard reaction (for instance heterocyclic compounds, phenolic compounds and sulphur compounds) as well as the interaction between compounds from them. Fatty aromas and the flavour related with the different species are mainly due to the lipid degradation while a large amount of heterocyclic compounds are related with the Maillard reaction (Elmore and Mottram, 2009; Mottram, 1998). A lot of attributes have been used to define parts of the flavour of the meat such as pastoral, fatty, liver, sweet, sweat, sourish, rancid, herbs, muttony, floral, grassy, urine and boar taint, among others. For some of the descriptors references are available (for instance sweet), for some others there are not, and training sessions are necessary to prepare the taste panel for the evaluation of flavour.

Texture is defined as "the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics" (Szczesniak, 2002). Thus, texture is a multi-parameter sensory attribute, related to the structure of the meat and which involves several senses in its detection (Szczesniak, 2002). Juiciness and tenderness are textural properties of meat highly related to the meat eating quality, together with the flavour. In meat, **juiciness** is the amount of juice released on sequential chews. The overall sensation of juiciness is given by the combination of several perceptions related with the characteristics of the juice in terms of amount, flow and force to squirt out from the product as well as individual characteristics of the saliva and cell debris (Szczesniak, 2002). **Tenderness** is usually understood as the force necessary to bite a piece of meat. However, tenderness is much more than that. It is a multi-parameter attribute related to several texture parameters such as hardness, cohesiveness, firmness, ease of fragmentation and juiciness among others. Usually in fresh cooked meat tenderness is evaluated as the hardness or force to compress the meat during the bite and also the time needed to masticate the meat or its chewiness. Tenderness is the main contributor to eating quality of meat and it is related to its physical and chemical composition. The most

important factors related to the tenderness of the meat are the connective tissue, sarcomere length and the myofibrillar protein degradation.

Taint is a flavour and smell related to the sex of the animal and it can be found in pork and in lamb. Boar taint is an unpleasant off-odour and off-flavour of pork from some entire male pigs, characterised as urine-like, pig-like, sweat-like or faecal-like, which may result in consumer dissatisfaction (Annor-Frempong et al., 1997a,b; Font i Furnols et al., 2000, 2008; Dijksterhuis, et al., 2000; Lunde et al., 2009). The main compounds responsible for boar taint in pigs are androstenone (5a-Androst-16-ene-3-one; Patterson, 1968) and skatole (3-methylindole; Vold, 1970; Walstra and Maarse, 1970) which accumulate in the fat tissue. In lamb, taint is higher in meat from ram and ewes slaughtered at high age and weight (Jeremiah et al., 1998). All these attributes are sensory attributes and the reference methods to measure them are generally by means of sensory analysis.

Sometimes some physical or chemical analysis can also be done, since they are correlated to some of these sensory attributes, although they only can explain them in part. In the case of sensory tenderness, there is a relationship with shear force (see Chapter 5) which, based on the average of 14 beef muscles, has been reported to be r=-0.85 (Sullivan and Calkins, 2007). Since juiciness is related to the amount of water in the meat, there is a relationship between water holding capacity (see Chapter 3) and juiciness (McGee, 2004). Regarding flavour, sometimes a very specific flavour could be due to the presence of a particular chemical compound and the analysis of volatile compounds can also help as indicators of flavour attributes. For example, since the sexual taint is related with the amount of androstenone and skatole in the case of boars and with the amount of skatole in the case of lamb, the chemical measure of these compounds can be a good indicator of the sensory perception of these taints.

7.2 Factors of variation

Since meat **flavour** is formed by lipid degradation or the Maillard reaction, changes in the composition of the meat, can change the flavour. Thus, the flavour of meat and meat products depends on a lot of different factors, both intrinsic and extrinsic (species, genetics, feeding, management practices, aging, cooking, etc.). Each species has a specific flavour, mainly due to differences in the volatiles derived from the lipids of the meat, and some species, like lamb, have a very characteristic flavour and aroma, probably due to the higher levels of S containing compounds, which differentiates lamb from other species' meat (Rhee and Ziprin, 1996; Young et al., 1993). **The pre-slaughter management** of animals influences on the final pH of the meat and, consequently its carbohydrate glycogen content (particularly glycogen), so affects flavour formation. Young et al. (1993) showed that lamb with high pH enhanced foreign flavour intensity and that sheep meat flavour scores were higher around pH 6. In beef, Holdstock et al. (2014) found higher beef flavour intensity and flavour desirability and lower off-flavour intensity in meat with pH higher than 6.0, compared to meat with pH lower than 5.8. **Breed** also can influence flavour in pork (Wood et al., 2004), beef (Campo et al., 1999; Serra et al., 2008), lamb (Martínez-Cerezo et al., 2005) although in some of the studies a breed effect cannot be separated from the production system. **Feeding** has also an important influence on the flavour of meat, especially in ruminants with a lesser impact in pigs. In this sense, grass-fed ruminants have higher linolenic acid that produces a more intense flavour and lower linoleic and oleic acids (Elmore et al., 2004; Montossi et al., 2013). In lamb, pastoral flavour is associated with grass-fed animals. Also the **age** of the animals affects flavour because in boars, age increases the boar taint odour/flavour and in sheep, it increases the muttony flavour (McGee, 2004).

Juiciness is perceived as moisture released by a few chews; the initial impression of juiciness is due to the release of free water from the product and after that during chewing, sustained juiciness is perceived because of the stimulation of the saliva due to the meat fat and flavour (McGee, 2004). Thus, juiciness is affected by the amount of bound water lost, and the odour and flavour formed in the meat, so it also depends on the **cooking characteristics** (type of cooking and temperature). Juiciness is also related to the *ante-mortem* **treatment** of the animal since this in turn affects at the final pH and water holding capacity of the meat. It seems that diet plays an important role in the juiciness of the meat (Wood et al., 2004) whereas breed has little effect on juiciness (Campo et al., 1999).

Tenderness can be affected by characteristics of the animals such as **nutrition and breeding** as well as the *ante mortem* **treatment** of the animals. Overall, *post-mortem* factors (pH, proteolysis and sarcomere length) are the most important in the final tenderness of the product (Maltin et al., 2003). Tenderness is also related to the cooking method and temperature. Thus, all these parameters have to be considered if the objective of the sensory study is the evaluation of the tenderness.

Taint depends on the **sex** of the animal. In pigs, taint can be found in entire males and it is mainly due to two compounds: androstenone and skatole. Androstenone is a hormone found in entire male pigs. Its content depends mainly on the **maturity** of the animal, the **age** and the **weight at slaughter** and the **genotype.** More mature animals, with higher age and weight at slaughter and from fatter genotypes such as Duroc, have higher probabilities of having higher androstenone content. Skatole is the other main taint compounds which depends on the **production conditions, cleanliness of the pig housing** and **feeding**. Since skatole is produced in the gut of the animal, it can be found in both, male and female pigs. However, the levels of skatole are higher in boars because the male sex steroids inhibits the breakdown of skatole in the liver. Therefore, it also tends to be accumulated the fat of in male pigs as the testes start to produce more testosterone at puberty. In the case of lamb, skatole is related with the **sex,** and the **feeding**, being higher in pasture fed animals (McGee, 2004). Mass fractions between 0.50 µg and 1.00 µg for androstenone per g melted fat and between 0.20 µg and 0.25 µg for skatole per melted g fat are generally accepted as thresholds for discriminating between tainted and untainted pork samples (back fat) (Bonneau, 1998).

7.3 Reference methods

7.3.1 Sensory analysis as a reference method

Sensory analysis has been defined as a scientific discipline used to evoke, measure, analyze and interpret human sensations towards foods, beverages and materials by means of the sense of sight, smell, taste, touch and hearing (IFT Sensory Evaluation, 1975).

Sensory analysis can be carried out by means of consumers and trained panel. Since the objective of this handbook is to identify reference methods for the calibration of spectroscopic or imaging devices that are used to determine meat quality attributes (tenderness, juiciness, flavour and taint), the use of a trained panel should be considered to be the reference method for sensory analysis, and the evaluation should be done by means of descriptive methods rather than discriminating methods.

Usually descriptive methods are based on a sensory profiling test or QDA (Quantitative Descriptive Analysis). These tests require training of the assessors in the vocabulary used and in the use of the intensity scale. Meat panels have to be trained specifically for this product. Moreover, specific training is required for each type of meat (pork, beef, lamb, poultry, etc.) and their products (sausages, dry-cured ham, etc.), because some of the attributes can differ. Also specific training is required if a particular characteristic of the meat is going to be evaluated, for instance boar taint in pork meat. Several ISO guidelines

are available for the training as well as books on sensory analysis. For more specific training, some published papers can be used as a reference. At the end of this chapter a list of references has been included.

7.3.2 Practical aspects

Some practical issues to be considered when performing a meat sensory analysis are as follows:

Number of trained panellists

A minimum of 6 trained panellists is recommended to perform the sensory characterization of a product but it is better to have between 10 and 12 trained panellists. They can be trained specifically for the test, or re-trained (if it is a long time since they participated in any sensory analysis) or given additional training if they are not used to the sensory evaluation of a particular type of product.

Evaluation can be done in sensory booths, with or without red light to avoid influence of the visual appearance of the meat on the panellists answers (Figure 1).

Figure 1: Sensory room with 10 boots (left) and detail of a prepared booth with red light (right).

If the panel is going to evaluate boar taint, it is recommended that the panellists are sensitive to androstenone and skatole. However, if some panellists are anosmic to androstenone, this can help to evaluate the skatole effect on the meat characteristics. If a panellist has to evaluate colour, it is important to ensure they are not colour blind (Ishihara Test can be performed). In any case it is recommended that panellists have been selected to have good sensory aptitudes.

Cooking temperature

Sensory characteristics of the meat are influenced by thermal processing conditions because the myofibrilar shrinkage that occurrs during this treatment affects different proteins depending on the temperature. For instance, in myosin it happens between 38°C and 60°C and in actin between 66°C and 83°C (Bertazzon and Tsong, 1990; Kodjogan et al., 2014; Martens et al., 1982). Thus, the choice of the temperature is important and depends on the objective of the study. So, if the objective is to mimic the way consumers cook meat, temperature will depend on the country and cooking habits. Cooking temperature includes the temperature of the surface of the meat and its gradient to the inner part of the meat, especially the end-point core temperature. High surface temperatures (>110°C) would allow the flavour and odour formation by means of the Maillard reaction (Whitfield and Mottram, 1992). The temperature gradient will influence the characteristics of the changes

in meat protein structure. Higher end-point temperatures produce a decrease in flavour, tenderness and juiciness of meat as the end-point temperature increases (Parrish et al., 1973). The end-point temperature has also an important effect on the meat, especially on its juiciness and also to less extent on the odour, flavour and tenderness (Figure 2). According to this and from a practical point of view, low core temperatures (60-65°C) are better than higher temperatures to evaluate the juiciness of the meat and the flavour components. Lower temperatures not only decrease juiciness but also tenderness, and are better for the assessment of samples according to their overall sensory properties (Aaslyng et al., 2014; Bejerholm and Aaslyng, 2004). However, these effects are not always clear since some studies show that species and the cut of meat may play a role.

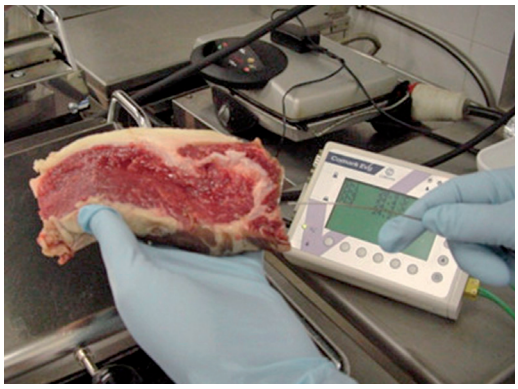

Figure 2: Control of meat temperature with a probe during cooking.

There are several studies of the effect of the temperature on the sensory attributes of the meat. For instance, Wood et al. (1995) found that pork loin had higher tenderness, juiciness and abnormal flavour scores and lower flavour intensity if cooked at lower endpoint temperatures. However, according to Prestat et al. (2002) pork loins cooked at 80°C lower higher off-flavour than those cooked until end point temperature of 70°C. Aguirre et al. (2015) found that increasing cooking temperature in grilled top loin steaks increases brown/roasted and burnt flavour while it decreases bloody/serumy and metallic flavours. Increasing the cooking temperature in an oven (up to 190°C) increase the roasted and toasted flavours in chicken (Byrne et al., 2002). With respect to boar taint, cooking and serving temperature is a critical point for the sensory evaluation of tainted meat and meat products. Although androstenone and skatole have different chemical properties (Table 1), both compounds can be volatilized quite easy during the cooking process and therefore, can be easily perceived.

Table 1: Chemical traits of the main compounds responsible for the boar taint in pig meat.

	Androstenone	Skatole
Boiling point at 760 mmHg	371.6 ± 42.0 °C	265.1 ± 9.0 °C
Vapour pressure (25°C)	0.0 ± 0.8 mmHg	0.0 ± 0.5 mmHg

Due to the cooking temperature and the chemical properties, skatole is the first compound that can be detected during the cooking process, followed by the androstenone. Boar taint in meat and meat products can be perceived during the cooking process and when meat or meat products are served warm to the consumers (Tørngren et al., 2012). For example,

when serving cooked smoked ham at different temperatures (65°C, 23°C and cold serving) it is observed that all odour attributes can be eliminated when serving ham cold, and it is not recommended to serve smoked streaky bacon hot, whereas if it is served cold, unpleasant boar odour can be eliminated (Kristensen et al., 2012).

Cooking method

The way the heat is transferred to the meat (cooking method) has also an important influence on its sensory characteristics. The method of cooking has to be chosen depending on the objectives of the study. Methods such as grilling or pan frying increase the extent of the Maillard reaction and allow the formation of roasted odour/favour amongst others in comparison with meat cooked in a pot or oven (Bejerholm and Aaslyng, 2014). However, some studies showed that in pork, grilling produces higher levels off-flavours than frying (Prestat et al., 2002). Furthermore, cooking beef *infraspinatus* to a high internal temperature of 95°C in steam or dry air has been shown to produce desirable juiciness (and tenderness) but these effects were not evident in *semimembranosus* (Modzelewska-Kapitula et al., 2012). Some cooking procedures can be seen in Figure 3.

Figure 3: Different types of cooking procedures: a) contact grill and b) oven.

Practical aspects in the sample preparation

Due to the effects of the cooking temperature and method, it is important to use exactly the same protocol during the study, trying to avoid variation between samples and testing days in the preparation of the meat. This means that as a minimum it is important to:

- check the device used for cooking,
- pre-heat the device used for cooking,
- control the internal temperature of the meat during cooking and during serving,
- keep the meat warm in pre-heated dishes or heaters, if it is not served immediately after cooking (Figure 4),
- control the size of the meat, its thickness and its shape,
- consider the sampling plan. If one slice is used by several panellists (for instance in most of the beef loin studies) it is recommended to block the sample site in the slice for each panellist for all the samples within a session. The sample site should be different in the different sessions to ensure that all panellists evaluate samples from the different sites. (Figure 5).

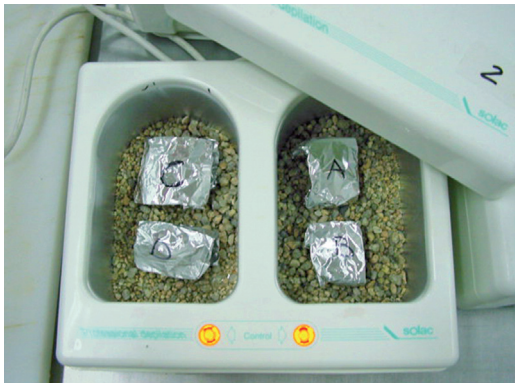

Figure 4: Example of a heater to keep meat warm.

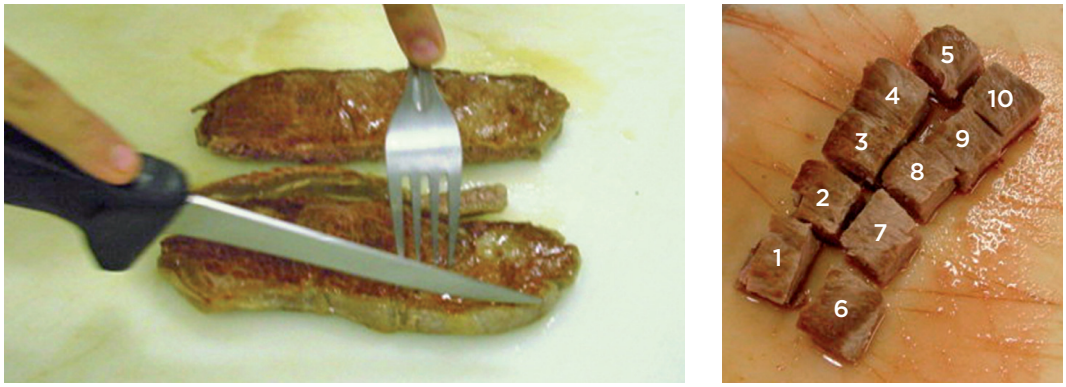

Figure 5: Preparation of the beef steak (left) and pieces prepared for each trained panellist (right).

The design of the allocation plan for the samples to be served to each panellist (Figure 5) as well as control of cooking and serving temperature will reduce random error due to sampling differences and expose the real sample differences due to treatment.

Practical aspects in the experimental design

The number and type of samples to be evaluated depends on the design of the experiment and the objective of the study. If possible, it is recommended to have a completed block design. If the number of treatments is too high (i.e. more than 10-12) then a break can be taken in the middle of the session. This would allow the evaluation of more samples in a session (i.e. around 20). Another option is to use an uncompleted block design to avoid panellist fatigue. If all the treatments of interest can be applied to meat from the same animal (for instance, cooking temperature, type of cooking, masking products, etc.), then 'animal' is the block and the number of animals can be reduced to 5-10. However, if the treatment is linked with the animal (feeding strategy, sex, genotype, etc.) then more animals (between 15 and 20) are needed per treatment. These numbers can vary depending on the type of study.

Samples can be served to panellists monadically (one after another) or all at the same time. In any case, it is important to design the order in which samples have to be evaluated by panellists, so as to avoid the effects of the first sample and the carry-over effect. This is especially important when one of the samples may have a very strong odour/flavour (for instance high boar taint) which could affect the odour/flavour of the following samples.

The intensity scale usually is a non-structured linear scale with or without initial and final anchor points. The length can be variable depending on the studies or the habits of the sensory laboratory. The preparation of the evaluation sheet and the reading of the scores from the scales can be performed manually or automatically using different specialized software.

Sensory attributes

The attributes to be evaluated depend on the objective of the study. If a sensory characterization of the meat is going to be performed, it is recommended that a sensory profile is developed. This can be done by different methodologies: checklist or literature descriptors, discussion sessions with panellists, pre-established protocol for a product, *etc.* Once the attributes are fixed, it is necessary to train the panellists in the use of these attributes using, if possible, references to ensure panellists evaluate the same attribute in the same way and to fix the utilization of the intensity scale, thus, achieving a consensus between panellists on the attributes and the intensity scale.

Tenderness and juiciness are attributes common in all the species, although with varying degrees of importance. In contrast, flavour and odour attributes are species dependent, but are dependent on the type of feeding, the sex and in some cases the genotype within species. Thus, attributes have to be adapted to the species and to the objective of the study.

Meat products

In meat products that are consumed cold, the serving temperature is less important but it still has to be considered to avoid important differences arising from temperature variation. The protocols and parameters to be considered will depend on the type of meat product. For instance in dry-cured ham evaluation, it is important to determine the anatomical position of the slice to be evaluated, the muscle or muscles within each slice, the thickness of the slice and the evaluation conditions, among other things. In this product, the evaluation of visual appreciation, odour and flavour attributes are recommended, however, the particular attributes to be evaluated can be varied depending on the objective of the evaluation. Figure 6 shows the evaluation of dry-cured ham by a trained panellist.

Figure 6: Sensory evaluation of dry-cured ham by a trained panellist.

7.3.3 Non-sensory references for sensory boar taint attribute

Since boar taint is mainly related to androstenone and skatole content, sometimes references for this attribute are not the sensory evaluation of boar taint but the chemical evaluation of these compounds. In the literature there are several analytical procedures to measure androstenone and/or skatole. They all cover various immunological and analytical methods and different sample clean-up procedures. Haugen et al. (2012) reviewed the analytical methods to measure boar taint compounds in porcine adipose tissue. The methods for measuring the concentration of androstenone in pork fat include immunoassays and various chromatographic methodologies. The chromatographic methods are used either in combination with mass spectrometry or with fluorescence detectors. For skatole, the methods that can be found in the literature are also based on gas chromatography, liquid chromatography and UV-Visible spectrophotometric assays.

The main handicap is that, despite the fact that these methods use different matrices (adipose tissue, melted fat or pure fat) and variations in every step (sample storage, sample treatment, separation and detection technique and quantification process, etc.), results are often reported without sufficient technical detail and do not specify fully the matrix or the methodology used (Ampuero et al., 2011; Haugen et al., 2012). This situation has complicated the interpretation of the results (i.e. Blanch et al., 2012), and demonstrates the need for further method harmonisation and standardisation for the quantification of boar taint compounds. In this regard, the Joint Research Centre (JRC, Retieseweg, 111, B-2440 Geel, Belgium) has worked on such validation via a collaborative trial in order to define a standardized reference method for the elaboration of rapid tests, and in the development of a harmonised understanding and expression of the analytical results (Buttinger and Wenzl, 2014). An in-house validation of this reference method has also been published (Buttinger et al., 2014).

7.4 References

7.4.1 Methodological references

To carry out sensory analysis, there are some references that can be used, published by ISO (International Organization for Standardization) and compiled also in other National Legislations. Some of the references are used for assessor training, while others are for different methodologies or type of test. Most of them can be used for all the attributes while other are attribute specific. A list of these references is given below:

Training of the assessors:

ISO 8586:2012, Sensory analysis. General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.

ISO 3972: 2011, Sensory analysis. Methodology. Method of investigating sensitivity of taste.

ISO 5496:2006, Sensory analysis. Methodology. Initiation and training of assessors in the detection and recognition of odours.

Methodology and type of tests:

ISO 11036:1994, Sensory analysis. Methodology. Texture profile.

ISO 4120:2008, Sensory analysis. Methodology. Triangular test.

ISO 5495: 2009, Sensory analysis. Methodology. Paired comparison test.

ISO 8587:2007, Sensory analysis. Methodology. Ranking.

ISO 10399:2004, Sensory analysis. Methodology. Duo-trio test.

ISO 4121:2003, Sensory analysis. Methodology. Duo-trio test.

ISO 13299:2003, Sensory analysis. Methodology. General guidance for establishing a sensory profile.

ISO 11035:1994, Sensory analysis. Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach.

ISO 11132:2012, Sensory analysis. Methodology. Guidelines for monitoring the performance of a quantitative sensory panel.

ISO 8588:1987, Sensory analysis. Methodology. "A" – "not A" test.

ISO 6658: 2005, Sensory analysis. Methodology. General guidance.

ISO 13301:2002, Sensory analysis. Methodology. General guidance for measuring odour, flavour and taste detection thresholds by a three-alternative forced-choice (3-AFC) procedure.

The reference method for the chemical determination of skatole, indole and androstenone and the in-house validation of this method are:

Buttinger G and Wenzl T 2014. Interlaboratory validation of a reference method for the determination of boar taint compounds by GC-MS and LC-MSMS. Publication office of the European Union, Luxembug, Brussels. Downloaded on March 2015 from URL: http://publications.jrc.ec.europa.eu/repository/handle/111111111/32433.

Buttinger G, Karasek L, Verlinde P and Wenzl T 2014. In house validation of a reference method for the determination of boar taint compounds by LC-MSMS. Publication office of the European Union, Luxembug, Brussels. Downloaded on March 2015 from URL: http://publications.jrc.ec.europa.eu/repository/handle/111111111/30428.

There are also some books that are very useful for training and preparing a sensory evaluation study:

Aaslyng MD, Meinert L and Bejerhom C 2014. Sensory assessment of meat. In: Encyclopedia of meat sciences 2e, vol.3. M. Dikeman, C. Devine (eds.). Elsevier. Oxford, UK, pp. 272-279.

Amerine MA, Pangborn RM and Roessler EB 1965. Principles of sensory evaluation of food. Academic Press, New York.

AMSA 2014. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. American Meat Science Association.

ASTM-MN13 1992. Manual on descriptive analyhsis. Testing for sensory evaluation.

Lawless HT and Heymann H 2010. Sensory evaluation of food, principles and practices. Second Ed. New Yori: Kluwer Academic/Plenum Publishers.

Meilgaard M, Civille GV and Carr BT 1987. Sensory evaluation techniques. CRC Press, Inc. Boca Raton, Florida, USA (pp. 281).

For specific evaluations, protocols may be different. For this purpose, references should be checked. For instance, for training of panels for evaluation of:

Boar taint:

Font i Furnols M, Guerrero L, Serra X, Rius MA and Oliver MA 2000. Sensory characterization of boar taint in entire male pigs. Journal of Sensory Studies 15(4), 393-410.

Meier-Dinkel L, Sharifi AR, Tholen E, Frieden L, Bücking M, Wicke M and Mörlein D 2013. Sensory evaluation of boar loins: trained assessors' olfactory acuity affects the perception of boar taint. Meat Science 94, 19-26.

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Guerrero L, Gou P and Arnau J 1999. The influence of meat pH on mechanical and sensory textural properties of dry-cured ham. Meat Science 52, 267-273.

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Chapter 8

General aspects of chemometrics for calibration and validation of spectroscopic technologies

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8.1 Introduction

The reference methods presented in the previous chapters (Chapter 1-7) for the determination of the quality characteristics of meat and meat products represent a basis for the calibration of different, novel spectroscopic and imaging technologies (presented in Chapter 9). The common aim when dealing with these technologies is to establish the relationship between spectroscopic/imaging data and the data obtained using extant methods known as reference methods (such as those in this handbook; for fat content, water-holding capacity, tenderness, etc.) with the purpose of being able to predict meat quality attributes or classify meat according to its quality characteristics. For this purpose, the following steps are necessary (according to Martens and Martens, 2000):

1. identify and define the objective of the calibration,
2. design the experiment in terms of cost, capacity limits and objectives, considering aspects such as variables of interest to be measured, variables that should be kept constant, number and type of samples (such as muscle, characteristics, anatomical place), statistical power, order of the measurements etc.),
3. carry out the experimental work following the design,
4. prepare and pre-process the data prior to the data analysis (to check for outliers and missing values, to correct mistakes, to adapt the data base to the software used for analysis, etc.),
5. analyse the data using the best model or analysis technique for the data available and the objectives, to estimate model parameters and to evaluate its accuracy and to interpret the results,
6. make conclusion from the work considering the objectives and the results obtained together with the limitations of the study.

This chapter deals with the development of calibration model (i.e. calibration, building of mathematical model to relate the signal from analytical instrument to the sample properties) and its validation/testing. Novel technologies (e.g. near infrared (NIR) spectroscopy, computed tomography (CT), image analysis, etc.) are known to produce enormous amounts of complex data and thus require advanced data treatment approaches which are described below together with different ways of the validation/testing of models. This chapter also provides the most common measures/indicators of the quality of the models, and considers the importance of the selection of the sample sets and the data pre-treatment which occur before the data treatment (i.e. calibration and validation).

8.2 General approach

8.2.1 Sample sets

Two data sets are necessary to construct the models, one for calibration and one for validation. These data sets can be obtained by means of an experimental design (producing the desired samples) or by means of selection of samples from an available data set (based on information on the spectra or other measured data).

When constructing and validating models the key rule is that the data used for the calibration and validation cover the whole expected distribution of variation generated by all the factors/components (variables) affecting the spectra. If a high number of samples are selected at random from the target population, it can be expected that samples from the whole distribution of variation will be present in the selected set of samples. However, if the target population has a non-uniform distribution (normal distribution is more usual than uniform distribution in food matrices), the proportion of samples will not be constant for the whole metric space (e.g., lower number of samples for extreme values in normal distributions). For small sample size, extreme values may not even appear. This can be a problem for the calibration set, because a concentration of samples in the central part of the distribution may hinder the fitting of predictive models. A uniform distribution of samples with respect the different variables is the most appropriate for fitting models.

When only one factor (the target variable to be predicted) is to be considered and a high number of samples with known reference values are available (quite unusual) a random uniform sampling can be applied.

If the reference values of the population are unknown (the most usual), but it is possible to control factors affecting them, a simple factorial design or fractional factorial designs (for high number of factors) can be applied in order to build a set of samples with a uniform distribution.

When no information about factors affecting reference values is available, and samples with unknown reference values but with spectral information are available, a possible approach could be the selection of samples (spectra) based on cluster analysis. Selection of samples from more distant clusters ensures that each selected sample has a spectrum not too close to any other spectra of the other selected samples. This subset of samples is the most informative with respect the spectral information. The goodness of this approach depends on the proportion of spectra variability due to the target variable to be predicted. If there are other variables (components) highly affecting the spectra, this selection approach will not ensure a uniform distribution of samples with respect the target variable.

8.2.2 Data pre-treatment

Before the spectral or imaging data analysis (prior to the development of the calibration models) a vital step called pre-processing is often needed to reduce noise, redundancy and useless variance, to improve prediction and robustness and to help in the interpretation of the spectra. It can be described as mathematical manipulation or transformation of raw data to enhance features of spectra and/or to reduce or sometimes remove unwanted sources of variation (noise).

The techniques are based on

(1) scaling/normalization, for instance 1-, 2- INF-Normalization, Normalization, Standard Normal Variate (SNV), multiplicative scattering correction (MSC), extended MSC and mean-centering;

(2) background correction, for instance 1st and 2nd derivatives, detrend, polynomial and wavelet transform;

and

(3) filtering, for instance smoothing, Fourier filter, General least squares weighting (GLSW) and external parameter orthogonalization (EPO).

The techniques can also be classified into two distinct categories of pre-processing tools:

i) manipulations of the single sample spectra such as normalization, smoothing (e.g. moving average smoothing and Savitzky-Golay smoothing, to reduce the noise without reducing the number of variables), baseline corrections (subtract a constant value – the minimum – from all the variables), first and second derivatives (e.g. in NIR spectroscopy it is quite typical to use derivatives for enhancing spectral features and SNV for minimizing baseline offset assisting, remove background due to scattering, etc.) and

ii) manipulations of the entire data set such as mean centering, variance scaling and autoscaling. Any mathematical transformation needs to be applied with caution to avoid introducing artefacts or loosing essential information.

8.3 Calibration methods

Chemometric data treatment signifies creation of the link between spectroscopic/imaging data and reference data. Spectroscopic/imaging data represent descriptors (input or independent variables) and reference data predictable variables (output or dependent variables). When an output variable is continuous or quantitative, the link between input and output variables is regression. In case when an output variable is a qualitative or class variable, the link is called classification/discrimination. The chemometric methods that are most often used comprise ordinary least squares regression (OLS), partial least squares regression (PLS), principal component analysis (PCA) and regression (PCR), discriminant analysis (DA) and artificial neural networks (ANN) and can be carried out using several of the chemometric softwares available (for more information see Statistical handbook for assessing pig classification methods; Causeur et al., 2003). In the next paragraphs, a short description of the methods is provided. Detailed descriptions of the methods can be found in several statistical publications (e.g. Eriksson et al., 2001; Höskuldsson, 1988; Martens and Naes, 1989; Osborne, 1991).

8.3.1 Regression methods

In general, **regression analysis** is a statistical process for estimating the relationships between variables and studying the type of relationship. It includes many techniques for modelling and analysing several variables, when the focus is on the relationship between a dependent variable and one or more independent variables. Modelling could be carried out using original (raw) data/variables (e.g. OLS) or, more often, using latent variables approach (e.g. PCR or PLS).

OLS allows the estimation of the relation between a dependent variable (output) and a set of explanatory variables (inputs). OLS minimizes the squared distances between the observed and the predicted dependent variable. It can be applied to linear models (e.g. multiple linear regression or **MLR**, i.e. linear relationship between one output and more than one input variables) or non-linear models. The method has limited ability to handle co-linear variables and huge amounts of data, both very common when using imaging and spectroscopic techniques. To handle large amounts of highly correlated variables, modelling data using the latent variables approaches (such as PCR and especially PLS) is more powerful.

PCR is based on principal components (derived in PCA) and MLR. **PCA** is linear transformation (rotation) of *m*-dimensional metric space which enables its examination

from different viewing angles. It enables to project the position of the data from *m*- into *2*-dimensional space, which could be graphically presented and thus easier to understand and handle. The first new axis (first principal component) represents the direction along which there is the greatest variation. This axis is not one of the old axis (original variables) with the highest variance, but is a linear combination of the original variables with the highest variance of the system. The second axis (second principal component) is orthogonal to the first and gathers the maximum variation left in the data. The procedure is then repeated until all dimensions (variation) is used up. At the end of rotations, there are *m* new axes, which are orthogonal to each other. Using described procedure the great majority of the system variance is gathered in a few new axes. Only those that are responsible for e.g. at least 90% of variation are kept, while the others can be neglected. The main aim of PCA is the visualisation of complex data and dimension reduction. However, PCA could be used as a prediction method (called PCR) in a combination with MLR. In this case, the scores of the most important principal components are used as the basis for the MLR with the target data.

Another (probably most often used and most successful) regression method using latent variables is **PLS**. In this case there are two sets of latent variables The central idea of PLS models is to extract orthogonal factors (latent variables) from original data for independent (descriptors) and the dependent data (output) and to set up a regression model between the latent variables. The number of factors is usually selected being as those that minimize the prediction error to avoid over fitting. PLS regression is particularly useful when there is a need to predict a set of dependent variables from a (very) large set of independent variables (i.e. descriptors); in particularly when the matrix of descriptors has more variables than observations, and in case is multi-collinearity among descriptors. By contrast, standard regression will fail in these cases.

8.3.2 Classification/discrimination methods

In discrimination models, dependent variable is a kind of class number (in contrast to regression models where output is a continuous variable). In the simplest case, the objects are the members of two classes (binary classification problem). The number of classes can be very different; however, it should not exceed the number of variables. Several methods exist to solve different classification problems (linear and non-linear) such as linear discriminant analysis (LDA), ***k***-nearest neighbours classification (kNN), logistic regression and neural network based classification. When solving classification problems a proper line (or curve) of separation between two or more groups of similar objects needs to be found (Figure 1). The type of separating line depends on the method used (e.g. a neural network could create a non-linear discriminating function, whereas the LDA creates only linear surfaces).

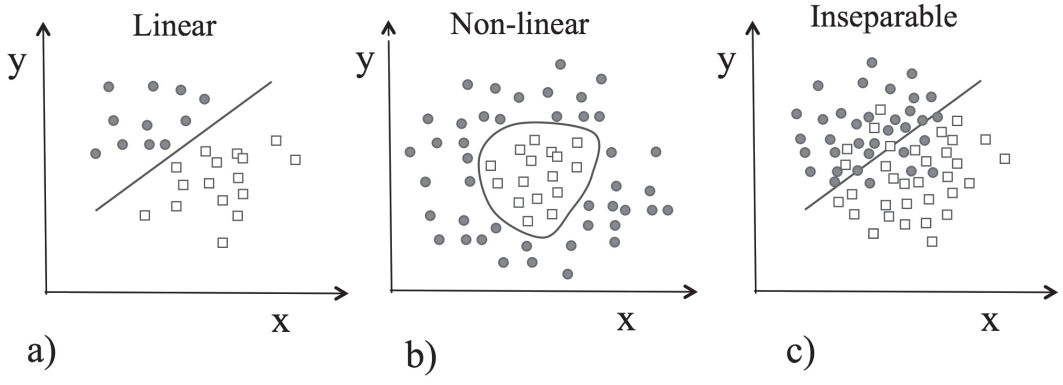

Figure 1: Most common classification issues (adapted from Lohninger, 1999).

The **kNN** is one of the most basic algorithms for classification. It predicts the desired output of a given sample by using information from the certain number (*k*) of the most similar samples. This similarity is measured with a distance function (e.g. Euclidean or Mahalanobis distance). The key idea behind kNN classification is that similar observations belong to similar classes. Thus, one simply has to look for the class designators of a certain number of the nearest neighbours and weigh their class numbers to assign a class number to the unknown. The number of the nearest neighbors, *k*, is recommended be odd in order to avoid ties, and it should be kept small, since large *k* tends to create misclassifications unless the individual classes are well-separated. The algorithm has several drawbacks including unsuitability in case of unbalanced data i.e. different number of samples per class (Lohninger, 1999).

LDA enables to discriminate two (most often) or more groups of samples in case of linear classification issues. LDA uses continuous independent variables (with assumption of their normal distributions) to explain a categorical dependent variable. It is closely related to principal component analysis (PCA) as they are both based upon the linear combinations of discriminating/predicting variables which best explain the output. LDA may be used as a linear classifier or for dimensionality reduction before later classification. Similarly, **logistic regression** also explains a categorical variable by the values of continuous independent variables, but does not require normally distributed independent variables (it could be any kind of variables).

8.3.3 Artificial neural networks (ANN)

The **ANN** is a machine learning method which evolved from the idea of simulating the human brain (Zou et al., 2008). The key characteristic of ANN is its ability to learn. Important assets of ANN are related to its ability to handle large data sets, to find out interesting relationships or behaviour between complex data. It is highly adaptable and has an excellent fault tolerance. When a data set is well explained by an appropriate mathematical model (e.g. linear regression), a neural network is unlikely to be needed. It becomes useful in the cases where the rules that underlie the data are not known, or are only partially known. The functional relationship between input and output is formed during the learning process (Prevolnik et al., 2008). A detailed information could be found in the literature specialized in description and mathematical concepts of ANN (Zou et al., 2008; Zupan, 1994; Zupan et al., 1994). Some applications of ANN in the field of meat production and technology are presented in Prevolnik et al. (2011).

ANN methods are difficult to explain in short as there are different types of ANN which differ considerable in the architecture (composition), learning strategy as well as regards the problem that are able to solve. In the widest sense, there are two types of ANN, supervised and unsupervised, which differ in the strategy of learning. In unsupervised learning, the input data is organised and processed without reference to the target, whereas in supervised learning, both the input and output are used. Among different kinds of ANN, Kohonen ANN is unsupervised and the other (counter-propagation (CP), back-propagation (BP) ANN, radial basis function ANN) are supervised. Like in the biological neural network, the ANN has an interconnection of neurons with three vital components:

i) node character which controls signals i.e. the number of inputs and outputs, the weights and activation function associated with the node,

ii) network topology defining how nodes are organized and connected, and

iii) learning rules for the initialization and adjustment of weights (Zupan, 1994; Zupan, 2009).

Kohonen ANN also known as **self-organising maps** (Figure 2a) belongs to the group of unsupervised neural networks due to the specific strategy of learning. The main goal of this method is to project or map objects from *m*-dimensional into *2*-dimensional space on the basis of input data (Zupan, 1994). After training the Kohonen ANN with all samples, the location of each sample is obtained in the Kohonen map, which is a rectangular grid of neurons. The algorithms used are excellent for establishing the relationship among complex sets of data (Zupan, 1994).

CP-ANN belongs to the supervised neural networks due to the target-dependent strategy of learning and represents an up-grade of unsupervised Kohonen ANN (Figure 2b). It is based on two-step learning procedure, unsupervised in the first step, and supervised in the second step Beside the input data ($X=(x_1, x_2,... x_{ii}, ... x_m)$) this type of ANN requires additional layer of neurons called the output layer or the property associated with individual sample ($Y=(y_1, y_2, ... y_i, ... y_p)$). These ($X, Y$) pairs are the input to the neural network, which is after being trained for certain amounts of epochs, capable of the prediction of the unknown samples. Every object excites one single neuron. The algorithm modifies the weight of the neuron with the weights the most similar to the input signal and smoothes the map by making modulated changes to neurons in a defined "neighbourhood" of that one. These corrections of weights are made around the neuron position in the Kohonen and output layer (Zupan, 1994).

BP-ANN are a type of supervised learning strategy based on an algorithm that corrects the weights within each neural network layer of neurons in proportion to the error obtained from the layer of neurons bellow that particular level, i.e. from the layer closer to the output layer (Figure 2c). The BP-ANN algorithm corrects the neural weights backwards, from the last (output) layer towards the first (input) layer of neurons (Zupan, 1994).

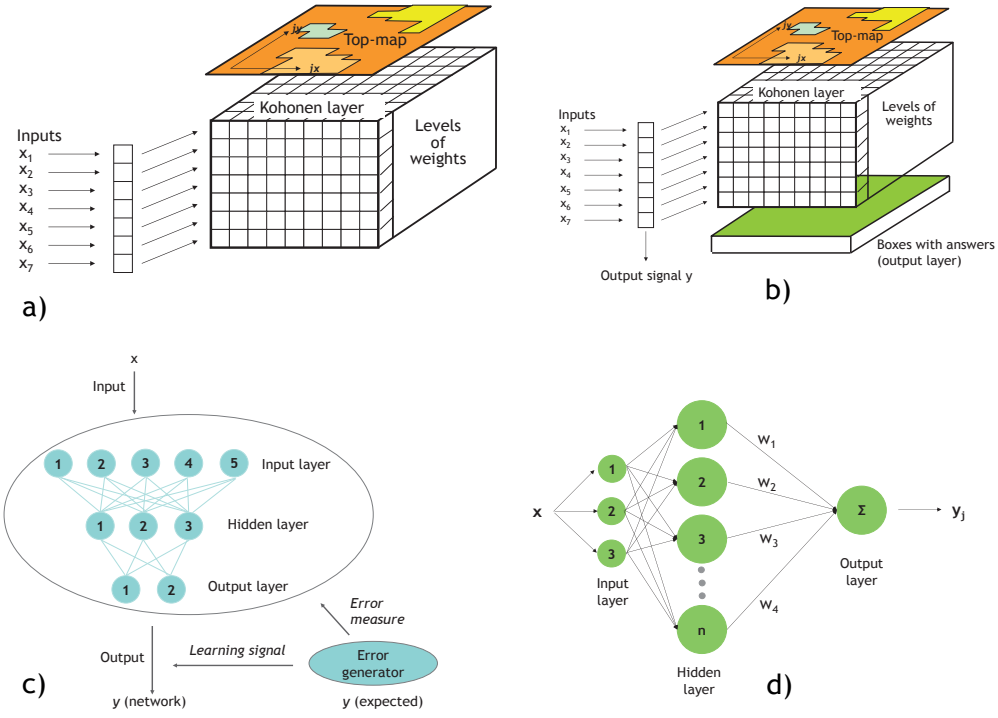

Figure 2: The structure of different types of ANN: Kohonen (a), CP-ANN (b), BP-ANN (c) and RBF networks (d).

Radial basis function networks (**RBF networks**) represent a special type of ANN which is considered as intermediate between regression models and nearest neighbour classification schemes, which can be looked upon as content-addressable memories (some workers in the field do not regard it as neural networks at all). The behaviour of a RBF network can be controlled by a single parameter which determines if the network behaves more like a multiple linear regression or a content-addressable memory. RBF networks (Figure 2d) have a special architecture, they have only three layers (input, hidden, output) and there is only one layer where the neurons show a nonlinear response (Lohninger, 1999). Some authors have suggested including some extra neurons which serve to calculate the reliability of the output signals (extrapolation flag). The input layer has, as in many other network models, no calculating power and serves only to distribute the input data among the hidden neurons. The hidden neurons show a non-linear transfer function which is derived from Gaussian bell curves. The output neurons in turn have a linear transfer function which makes it possible to simply calculate the optimum weights associated with these neurons (Zupan, 1994).

8.4 Validation of models

Performance or prediction ability of the calibration models is evaluated with a procedure of validation which can be internal i.e. using the same data set or subset(s) or external validation i.e. using independent data set(s) that have not been used to train the model.

Internal validation is performed using the same data set or subset(s) as in the calibration (training) step. *Cross-validation* is the most commonly used validation method; it is conceptually simple to understand, but very intensive in calculation. It attempts to imitate the prediction of "unknown" samples by using the calibration data set. The procedure consists of the extraction of one or more subsets of samples from the calibration set. The extracted subset is used to validate the model using calibration algorithm. The procedure is repeated several times with different subsets (bootstrapping). There are different ways of extracting subsets, but the most often is leave-one-out cross validation (also known as full cross validation or one-at-a-time cross validation). In this approach each subset is composed of one sample at a time and the procedure is repeated for all the samples of the calibration set. Alternatively, the extracted subset can also consist of a group or block of samples (e.g. every *n*-th sample selected, randomly selected samples, etc.) which is known as leave-*p*-out cross-validation. For instance, if the objective is to determine if the prediction ability is dependent or varies with the muscle evaluated, then each extracted subset can be composed by the samples of the same muscle.

Another type of internal validation, if there are enough amount of samples, is splitting the dataset in two, one used for calibration and another one for validation.

External validation is the most reliable method to assess the predictive ability of the model and its performance on "unknown" samples (condition: enough samples available). It is performed using an external i.e. independent set of data, different from those used in the calibration step. It allows an independent estimation of the accuracy and precision of the model to be made. As a rule, the external sample set should be similar to the calibration set. It is most often provided by the splitting of the original data set into calibration and validation sets. However, if the calibration model is developed on a different data set, the validation may become difficult, but in case of good results, the model can be considered as more general and robust. It is assumed and is advisable that the calibration set contains at least 70% more samples than validation set and not less than twice more than validation set.

8.5 Parameters used for prediction models' quality/adequacy

The quality of calibration/validation models can be assessed by means of different parameters, i.e. quality indicators. Their calculation involves some variables, which are referenced as follows:

y_i – observed (reference) value for the *i*-th sample,

$\hat{y}_i$ – predicted value for the *i*-th sample after apply the calibration equation,

n – number of samples.

The most common parameters used to evaluate the predictive ability of the models are coefficient of determination, coefficient of correlation, bias, different types of errors and residual predictive deviation.

The adequacy of calibration models should be evaluated combining several statistical indicators with regard to the purposes of the models (Tedeschi, 2006).

8.5.1 Coefficient of determination

Regression indicator **coefficient of determination** (R^2) and its counterpart **coefficient of correlation** (r) are the measures of precision, i.e. how close individual predicted values and true values are to each other, thus, how much of the total variation is described by the regression line. It can be calculated as:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \dot{y}_i)^2}$$

R^2 varies between 0 and 1, while r between -1 and +1. The closer the value of R^2 to 1 (or r to |1|), the better the precision. This measure is variable with the range used in the regression and it does not give an idea of the accuracy, only of the precision. It is important to note that values of R^2 or r close to 0 do not necessarily indicate the absence of correlation between observed and predicted values; this can happen in the case when the relationship is non-linear.

The representation of observed values against predicted and the regression line enable the accuracy and precision of the prediction. As shown in Figure 3, the predicted and observed values are correlated although when compared to the isoline ($y = x$) there is a slight overestimation at low values and an underestimation at high values.

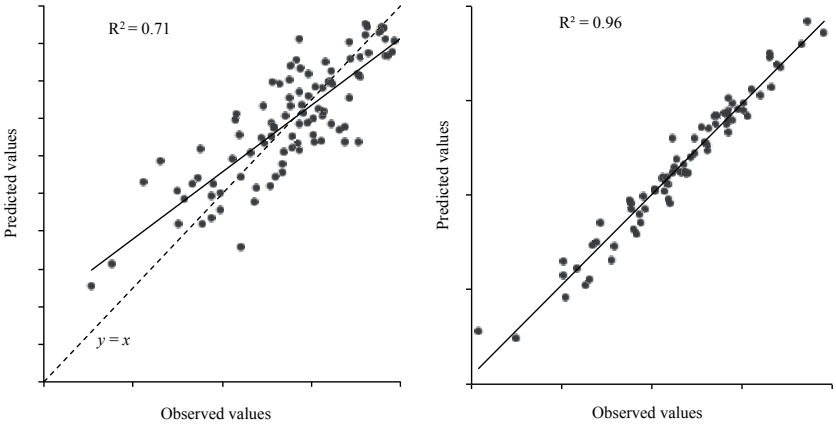

Figure 3: Presentation of the predicted and observed values and its coefficient of determination (R^2) with different degrees of deviation from the isoline ($y = x$).

8.5.2 Bias

Bias is the residual, i.e. the differences between the observed and the predicted value.

$bias = (y_i - \hat{y}_i)$

The sum of all the biases can also be calculated in the predicted values. In the estimated values, the sum of all the biases is zero since it is the rule of the least squared means methodology.

$sumbias = \sum_{i=1}^{n}(y_i - \hat{y}_i)$

The **mean bias** is a statistics/indicator used to assess model accuracy:

$$\overline{bias} = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)}{n}$$

The standard deviation of the bias can be used as a measure of repeatability.

It is recommended that the individual biases are represented graphically. This allows the detection of outliers and the observation of possible tendencies that make the prediction not good enough. Figure 4 presents different types of bias patterns: good distribution of the biases, without a clear pattern and outliers (a), overestimation of true value (b), overestimation of low and underestimation of high values (c) and the increasing biases with the increase in parameter values.

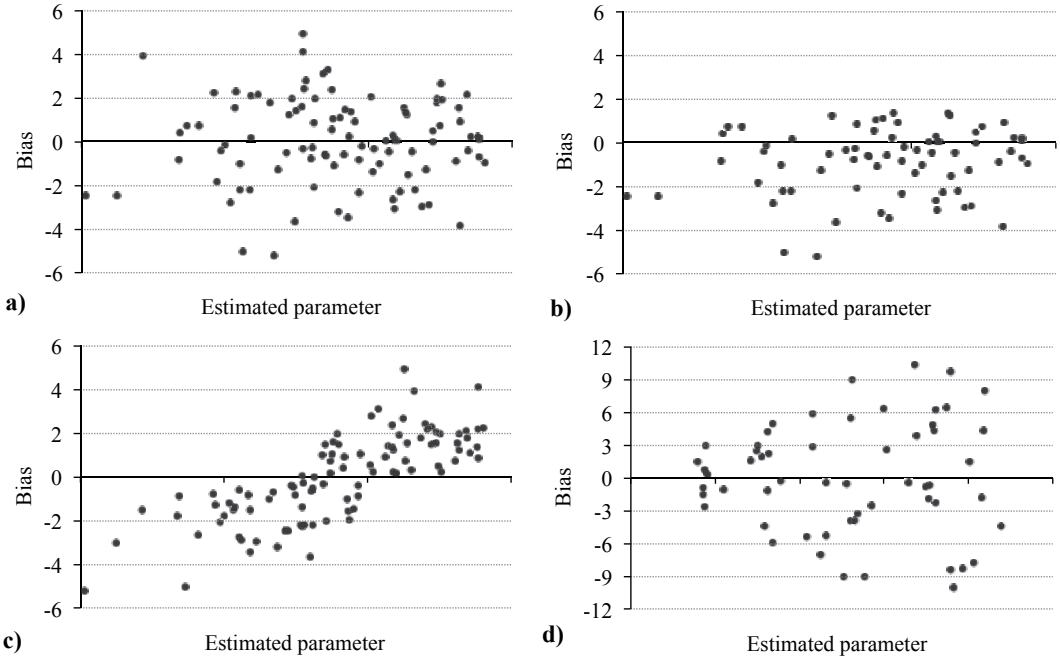

Figure 4: Different biases (true value minus predicted value) pattern of prediction equations.

8.5.3 Mean squared error and standard error

The **mean squared error** (MSE), also known as **residual mean square** or **standard error of the estimate** (SE), often presented as its root value, **root mean squared error** (RMSE) is an unbiased estimator of the variance of the random error. It thus measures the variability of the difference between the predicted and the reference values. The lower it is the better the prediction fits with the reference data. It can be calculated as follows:

$$MSE = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n} \qquad RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}}$$

The MSE can be split into three types of errors, error of central tendency, error due to regression and random error. The random error can indicate lack-of-fit if it is not high enough (Tedeschi, 2006).

The denominator of the equation is **n** in the validation dataset. In the calibration dataset, the denominator is *n-p*, being *p* the number of parameters of the equation.

The **standard error** (SE) is sometimes the same as the RMSE although there are other ways of calculation. One of them calculating it correcting for bias:

$$SE_b = \sqrt{\frac{\sum_{i=1}^{n}\left[(y_i - \hat{y}_i) - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)}{n}\right]^2}{n-1}} = \sqrt{\frac{\sum_{i=1}^{n}\left[(y_i - \hat{y}_i)^2 - bias\right]^2}{n-1}}$$

RMSE and SE_b are related as follows: $RMSE^2 = SE_b^2 + \overline{bias}^2$

The equation indicates that in case of small biases the RMSE and the SE_b are similar.

Other statistical parameters can be used such as **prediction sum of squares (PRESS)**, which is strongly related to RMSE and SEP.

8.5.4 Residual predictive deviation

The residual predictive deviation (RPD) is calculated as ratio between the standard deviation of the reference data (SD) and the error (RMSE):

$$RPD = \frac{SD}{RMSE}$$

RPD is used as a common measure to evaluate predictive ability of the models. In the literature, there are different recommendations as regards RPD. According to Williams (2001), RPD values higher than 3.0 indicate suitable calibration models. Williams (2008) specified the following RPD limits for practical application of prediction models:

RPD > 2 – applicable for rough screening,

RPD > 3 – applicable for screening purposes,

RPD > 5 – applicable for quality control,

RPD > 8 – applicable for analytical purposes.

8.5.5 Final remark

All statistical parameters can be calculated for different sample sets:

in case of self-prediction of samples from calibration data set they are marked as: R^2_C, RMSEC or $RMSE_C$,

in case of cross-validation on the calibration set of samples they are marked as: R^2_{CV}, $RMSE_{CV}$ or $RMSE_{CV}$, RPD_{CV},

in case of the external validation on the test set of samples they are marked as: R^2_P, RMSEP or $RMSE_P$, RPD_P, or as: R^2_V, RMSEV or $RMSE_V$, RPD_V.

Attention must be paid when comparing the results of different studies, as the signs/ symbols are not consistently used.

It is important to highlight that the interpretation of the parameters is different depending on if they are calculated in the calibration or in the validation data sets. In the calibration data set, they indicate how well the data fit the model while in the validation data set they indicate how well the model predicts new samples.

In general, prediction error is similar to or equal to the calibration error. If prediction error is much lower than calibration error, then data has to be studied to see the cause of this result (better fit with an external or incomplete -if cross validation- validation data set than with the calibration data set used for constructing the model) and see how to act (check both calibration and validation data sets, study the sampling effect on the goodness of fit of the calibration equation, etc).

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Chapter 9

Future trends in non-invasive technologies suitable for quality determinations

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9.1 Introduction

Emerging non-destructive technologies are of interest for food science and industry since they allow the characterization of food products and quality control throughout processing. They are based on different physical principles and they have their advantages and drawbacks.

Industry may take advantage of these technologies to control quality, improve their products and optimize processing since most of them may be implemented on-line. These technologies permit the determination of quality parameters in a non-destructive way and are thus suitable to be used under industrial conditions. However, to carry out these determinations it is important to perform a previous calibration and validation of the devices using the **reference analytical methods** described in the previous chapters of this handbook.

In this chapter, a review of spectroscopic and imaging non-invasive technologies is provided, including a description of the technology and its application in the determination of meat quality parameters. To evaluate the ability of these technologies to predict meat quality attributes, several statistical terms have been used. These terms vary between studies and sometimes it is difficult to standardise and harmonise both the meaning of the terms and their calculation. Some of the statistical terms used below are the following: correlation coefficient – r, coefficient of determination – R^2, several errors like root mean square error – RMSE of calibration, prediction – RMSEP or prediction by cross-validation – $RMSEP_{cv}$ as well as standard error of prediction – SEP and the residual predictive evaluation – RPD which is the relationship between the standard deviation and the error (see Chapter 8).

9.2 Non invasive technologies

There are several different technologies based on the use of acoustic or electromagnetic waves at different wavelengths which can be used to determine meat and meat products attributes.

9.2.1 X-ray technology

One of these uses **X-ray technologies** which are based on the differential attenuation of X-rays when going through a sample. The degree of X-ray attenuation is determined by the component densities of the product, which permits the quantification or differentiation of tissues of different density. Transmission radiography is the simplest approach to acquiring information at a single energy. Total fat content (Hansen et al., 2003; De Prados et al., 2015) and salt content (Fulladosa et al., 2015) in raw and processed hams can be determined using

this technology, obtaining predictive errors low enough to make this technology useful for the industry. For example RMSE of validations of 3.27% for fat in raw hams (De Prados et al., 2015) and of 0.43% for salt in dry-cured ham portions (Fulladosa et al., 2015) were obtained. In addition, there are advanced contrast modalities such as **dark field X-ray** which is sensitive to the different x-ray scattering, making this device also sensitive to structural differences. **Phase contrast X-ray** may increase contrast of the image by using the differential refraction of the X-rays. These modalities allow a superior contrast for soft tissues so are useful for foreign body detection in food (Nielsen et al., 2013) or frozen and thawed fruit identification (Nielsen et al., 2014), and are showing promise for application to meat quality. On the other hand, **dual X-ray absorptiometry** (DXA) (Figure 1) can couple the information acquired at two different energies, one at low (60 keV) and the other at higher energies (120 keV). This system allows meat tenderness evaluation with a R^2 of 0.69 (Kroger et al., 2006), meat fat content determination with R^2 values from 0.70 to 0.97 (Brienne et al., 2001), evaluation of carcass composition of different pig genetic lines with a $R^2 > 0.85$ for dissected lean prediction (Marcoux et al., 2003), or determination of carcass composition from live sheep also with high correlations (Pearce et al., 2009).

Unlike the above mentioned systems, **computed tomography** (CT) (Figure 2) is a system in which the X-ray source and detectors rotate together around the object, generating 3D images showing in detail, the internal structures of the products. Intramuscular fat content (Font-i-Furnols et al., 2013; 2014; Lambe et al., 2008; Prieto et al., 2010; Santos-Garcés et al., 2014) can be determined with R^2 values from 0.4 to 0.8 (RMSE or $RMSEP_{cv}$ between 0.42 and 0.75%) in both raw and processed meat and saturated and monounsaturated fatty acids with R^2 between 0.66 and 0.72 (Prieto et al., 2010). Salt content (Frøystein et al., 1989; Håseth et al., 2007; 2012; Vestegaard et al., 2004; Fulladosa et al., 2010) and salt diffusion (Picouet et al., 2013), water content (Fulladosa et al., 2010; Santos-Garcés et al., 2010) and water activity (partial vapour pressure of water a_w (Santos-Garcés et al., 2010)) can also be accurately determined. In contrast, colour, texture and sensory characteristics (Prieto et al., 2010) are more difficult to estimate using CT ($R^2 < 0.20$). Phase contrast and dark-field modalities can be also combined with this concept in order to increase resolution (Miklos et al. 2015; Jensen et al., 2011). **Microcomputed tomography** allows the improvement of spatial resolution from mm to µm allowing inspection of small samples. Assessment of intramuscular fat level and distribution in beef muscles (Frisullo et al., 2010), microstructure in fermented sausages (Santos-Garcés et al., 2013) or dry-cured ham (Santos-Garcés et al., 2014) using this technology has been reported.

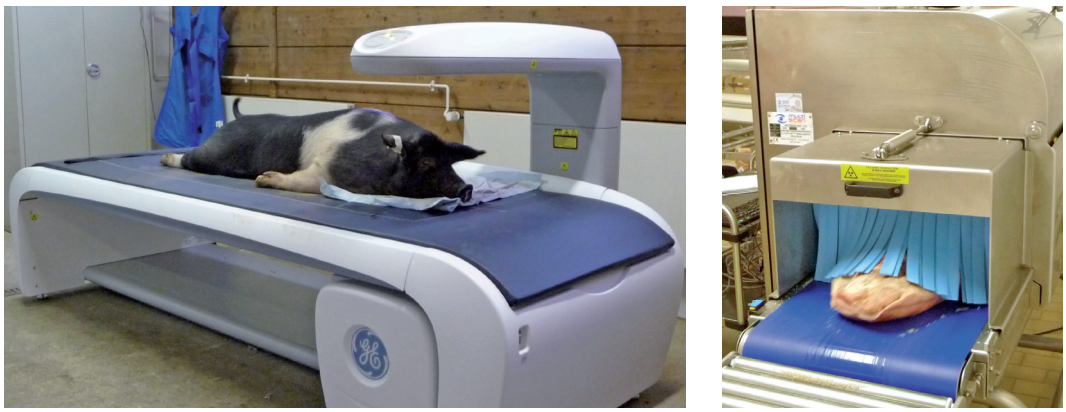


Figure 1: Pig carcass (left) (photo courtesy of Armin Scholz, Ludwig Maximilian University of Munich, Germany) and ham (right) evaluated with DXA devices.

Figure 2: Beef rib measured with a computed tomography equipment.

Figure 3: Pig carcass evaluated with a MRI equipment (photo courtesy of Armin Scholz, Ludwig Maximilian University of Munich).

9.2.2 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is a spectroscopic technique based on the spinning mechanism of specific nuclei present in the molecules of biological systems. The nuclei that contain odd numbers of protons or neutrons are aligned in a magnetic field and subsequently perturbed by a radiofrequency pulse putting some of the spins into a higher energy state. When the pulse is turned off, the spins return back into the lower energy state by a process described as relaxation. The relaxation phenomenon provides information about the physical/chemical nature of certain nuclei in terms of mobility and compartmentalization. Water holding capacity and cooking loss (Bertram et al., 2001) have been shown to be associated with several NMR responses with a maximum r between 0.72 and 0.77 and RMSEP between 1.83 and 2.75, as well as intramuscular fat (r = 0.57-0.77 and $RMSEP_{cv}$ = 1.06-1.20; Brøndum et al., 2000). Sensory characteristics (Bertram et al., 2005) have been found to be related to the NMR responses with the strongest relationships being obtained for final juiciness, tenderness and chewing time attributes (r > 0.80; $RMSEP_{cv}$ < 1.0) followed by hardness (r = 0.78, $RMSEP_{cv}$ = 1.02) and crumbliness (r = 0.64, $RMSEP_{cv}$ = 1.24). Relationships were not strong for fibrousness. **NMR relaxometry** is widely used in food research as well as **magnetic resonance imaging** (MRI) (Figure 3) which includes spatial coding to obtain images and then local information (Antequera et al., 2003; Antequera et al., 2007).

9.2.3 Computer image analysis

Computer image analysis (CIA) and **video image analysis** (VIA), which include capturing, processing and analyzing of images (Figure 4 and Figure 5), allow the rapid and objective assessment of visual/physical characteristics of the product, as well as quality features that cannot be visually differentiated by human inspection, i.e., structural and textural characteristics through the extraction of suitable features. Pattern recognition techniques which consist of extracting a set of features (geometric, texture, etc.) from the segmented image and assigning a category from a given set of categories to this data has been widely used in combination with CIA for classification purposes.

The meat grading systems normally focus on features such as colour, marbling and texture which can be easily measured by a CIA system with basic equipment (Jackman and Sun, 2012). Thus, determination of the intramuscular fat in chicken with a R^2 of 0.69-0.74 (Chmiel et al., 2011), marbling with R^2 of 0.73-0.92 (Osawa et al., 2008; Huang et al., 2013), tenderness with a R^2 of 0.17 to 0.72 (Li et al., 1999), and colour with a % of agreement of 77-95% (Tan et al., 2000) have been reported. Additionally to simulate the expert meat grading process the computer vision technology systems in meat will continue to be based around visible wavelength imaging (Jackman and Sun, 2012). Nonetheless more expensive non-visible wavelength imaging has been successfully used for meat features evaluations (Xiong et al., 2014).

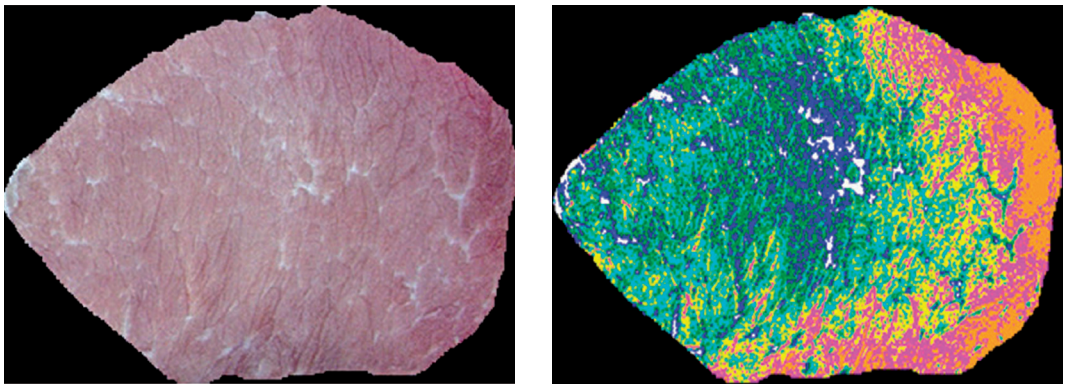


Figure 4: Pork loin image analysis to determine marbling (photo courtesy of Armin Scholz, Ludwig Maximilian University of Munich, Germany).

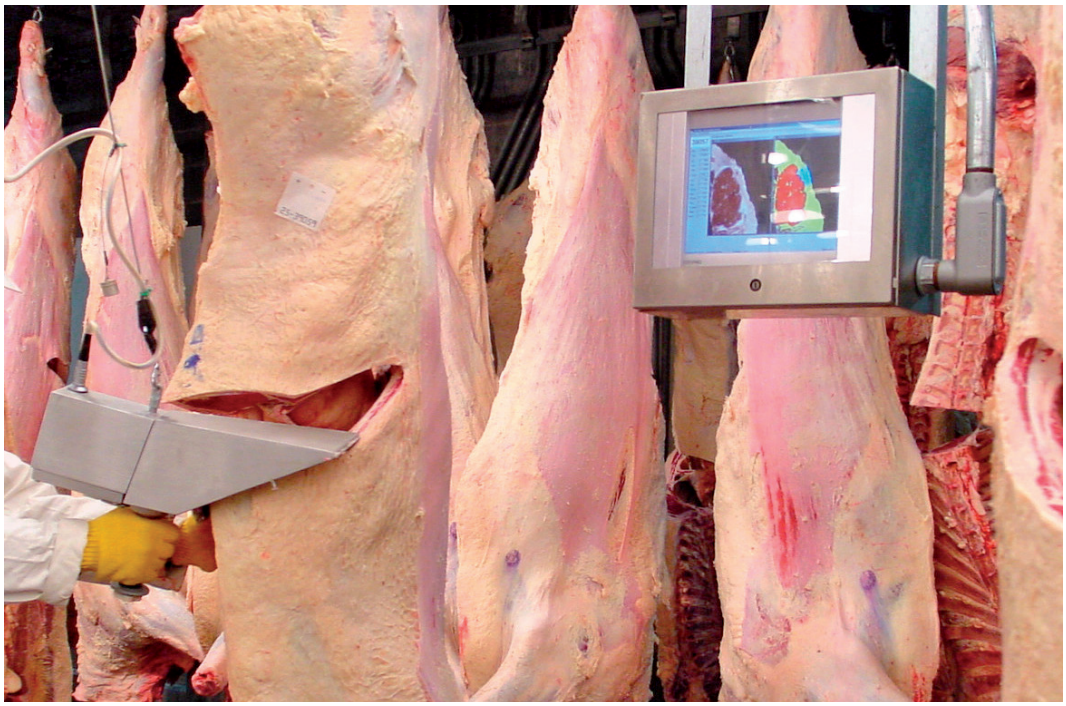


Figure 5: Beef loin image analysis to determine quality characteristics (photo courtesy of e+V Technology GmbH, Oranienburg, Germany).

9.2.4 Infrared spectroscopy

Infrared spectroscopy is based on the fact that organic molecules (bonds) absorb light of specific wavelengths depending on the characteristics of their structure. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-infrared (NIRS – Figure 6; wavelength range 700-2,500 nm), the mid-infrared (MIRS; wavelength range 2,500-15,000 nm) and the far-infrared (FIRS, wavelength range 15,000-100,000 nm), with NIRS and MIRS being the most suitable for the qualitative and quantitative analysis of foods. Measurements can be performed in transmittance, reflectance or transflectance mode on the intact, minced or homogenized samples.

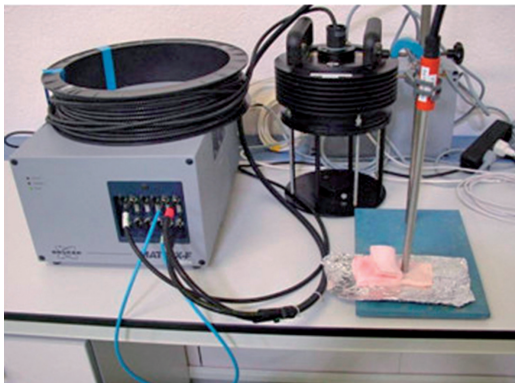
The technology has been demonstrated to have great ability to measure chemical composition (fat, protein, water, dry matter, etc.) in poultry (Berzaghi et al., 2005; Cozolino and Murray, 2002), lamb (Cozolino and Murray, 2002; Viljoen et al., 2007), beef (De Marchi et al., 2007; Prevolnik et al., 2005; Prieto et al., 2006) and pork (Collell et al., 2010; Gaitán-Jurado et al., 2008) and pork sausages (Ortiz-Somovilla et al., 2007). For instance, the maximum intramuscular fat prediction accuracy has been reported as an R^2 = 1.00 and RPD = 28.46 (Viljoen et al., 2007) with more typical accuracy values of R^2 between 0.34 and 0.94 and RPD between 1.09 and 4.1 depending on the species being given (Cozzolino and Murray, 2002; Prevolnik et al., 2005). The technology has also been shown to have a satisfactory ability to determine major fatty acids composition in all the species, e.g. in case of oleic acid R^2 between 0.77 and 0.963 and RPD higher than 2 were obtained (Pérez-Juan et al., 2010; Pla et al., 2007).

However, compared to the prediction of chemical constituents (where the accuracy often approaches the accuracy of analytical methods), the ability of infrared spectroscopic technologies to determine meat quality characteristics is, in general, lower. Namely, the predictive ability is limited by the accuracy of the reference methods which are, in the case of meat quality, subjected to many environmental factors and thus less accurate and reproducible.

Colour parameters L*a*b* have been estimated with different degrees of accuracy (Andrés et al., 2008; Čandek-Potokar et al., 2006; Prieto et al., 2008). Results showed low to moderate correlation with water holding capacity (e.g. drip loss, cooking loss, press loss) and pH (Andrés et al., 2007; Leroy et al., 2003; Prieto et al., 2008a; Prevolnik et al., 2010). Also in case of tenderness (assessed via WB shear force, texture, sensory evaluation, etc.) the accuracy is not very high (De Marchi et al., 2007; Liu et al., 2004; Ripoll et al., 2008).

In addition, the technology has been also used for classification (e.g. discriminating genotypes, feeding regimes, meat quality classes, etc.) and often showed high accuracy for such purposes (Josell et al., 2000; García-Rey et al., 2005; McDevitt et al., 2005; Prieto et al., 2008b). More information on this topics can be found in reviews of Prevolnik et al. (2004), Prieto et al. (2009) and Weeranantanaphan et al. (2011).

When used in combination with imaging technology, infrared spectroscopy gives rise to **NIRS imaging technologies**. As in the case of CT and NMR imaging, this technique allows the determination of the spatial distribution of the composition of the product although in this case lower penetration is obtained.



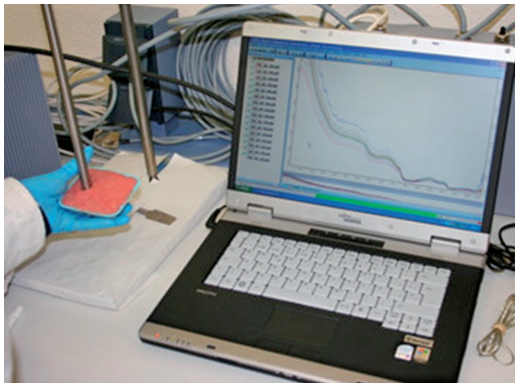


Figure 6: NIRS measuring subcutaneous fat (left) and minced meat (right).

9.2.5 Hyperspectral imaging

Hyperspectral imaging (HSI) (Figure 7) is based on the integration of image processing and spectroscopy techniques to attain both spatial and spectral information from a sample, which allows it to be used to obtain physical and chemical information from the sample. Hyperspectral images are made up of hundreds of contiguous wavebands for each pixel of an image providing the spatial distribution of biochemical constituents of a sample. If only a limited number of wavebands are obtained for each pixel it is called **multispectral imaging**. This technology has been used to determine moisture, fat, protein (Barbin et al., 2013) in minced meat with R^2 from 0.86 to 0.95 and SEP between 0.37 and 0.64. Less accurate predictions were obtained for chemical constituents in intact meat as well as for colour (L* with R^2 between 0.88 and 0.90 and $RMSEP_{cv}$ between 1.24 and 1.85) (Barbin et al., 2012a; El Masry et al., 2012) and drip loss (SEP = 2.61-2.34 and r = 0.77-0.78) (Qiou et al., 2007a). It has also been used to classify meat as PSE, DFD, RFN and other categories with results between 50 and 100% correct classifications (Barbin et al., 2012b; Liu et al., 2010; Qiou et al., 2007b). Classification of beef loin into 3 categories according to its tenderness was also performed with HSI with an accuracy of 96.4% (Naganathan et al., 2008). Also it has been used in the determination of water, salt and fat content with RPD between 2.5 and 3.7 in dry-cured ham slices (Gou et al., 2013).

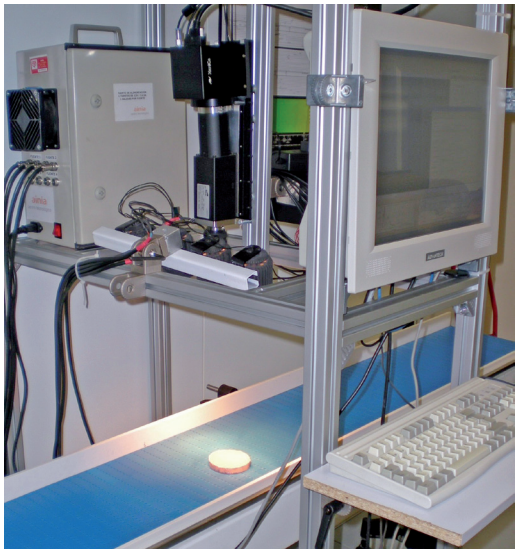


Figure 7: Hyperspectral analysis measuring a hamburguer (photo courtesy of Ricardo Diaz, AINIA, Spain).

9.2.6 Raman spectroscopy

Raman spectroscopy (RAM; Figure 8), in contrast to NIRS and MIRS, is not based on the absorption of light but on the inelastic scattering of light which occurs when laser light interacts with molecules. The incident light excites molecular vibrations in the material leading to a red-shift of the scattered light which is analyzed. Thus, the Raman spectrum is fundamentally vibrational and may be regarded as a fingerprint of the scattering material providing qualitative and quantitative information of both the molecular composition and structure. A good correlation was found for traits such as cooking loss (R^2 = 0.77) and shear force (R^2 = 0.71) in pork meat (Beattie et al., 2008) and for instrumental hardness, cohesiveness, adhesiveness and springiness in pork meat (R^2 = 0.92-0.98) (Herrero et al., 2009). Sensory characteristics such as juiciness (R^2 = 0.62) and tenderness (R^2 = 0.65) in silversides from bulls (Beattie et al., 2004), and SFA, MUFA, PUFA and iodine value (R^2 = 0.97-0.99) are reported to be highly correlated to RAM spectra (Olsen et al., 2007). Pork meat quality assessment on the slaughter line using a portable device has also been recently published (Scheier et al., 2015).

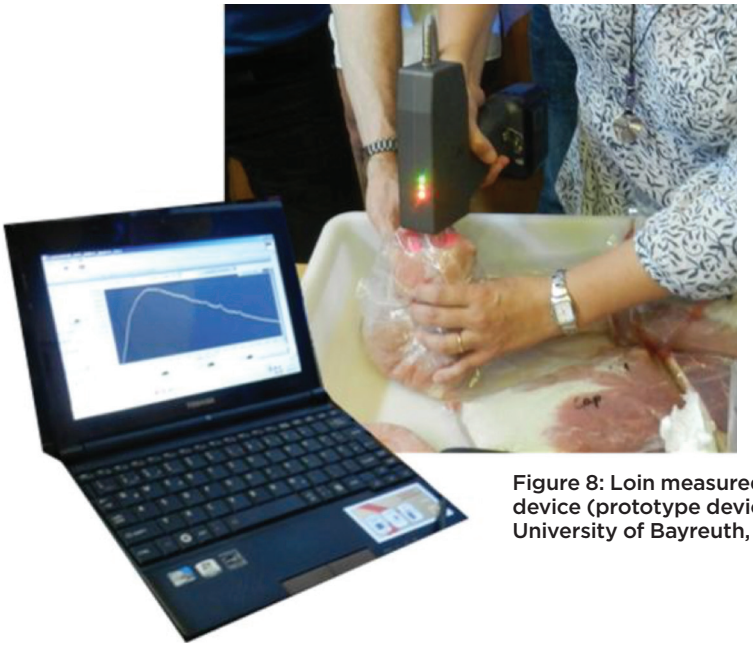


Figure 8: Loin measured with a portable RAMAN device (prototype device of Heinar Schmidt, University of Bayreuth, Germany).

9.2.7 Dielectric microwave spectrometry

Dielectric microwave spectrometry allows the determination of the dielectric properties of a sample. When a microwave frequency radiation interacts with the sample, a change in the rotation of the polar molecules is produced and dielectric parameters, which can be related to composition and quality parameters, can be calculated. There are transmission and other measurement systems such as **Time Domain Reflectometry** (TDR) which show the response of the interaction of an electromagnetic pulse containing a wide range of frequencies simultaneously. Microwave dielectric spectrometry has been used to determine the presence of added water in different pork products (Kent et al., 2002), to control pork salting process (Castro-Giráldez et al., 2010a) and to identify pork quality (Castro-Giráldez et al., 2010b). Dielectic TDR portable devices has also been used to develop models for fast estimation of water (RMSE = 1.67%) and salt contents (RMSE = 0.22%) in dry-cured ham (Fulladosa et al., 2013).

9.2.8 Ultrasound

Real-time ultrasound (US) (Figure 9) technology is based on sound waves with a frequency greater than 20 kHz which is approximately the hearing limit of the human ear. The low energy ultrasound, with frequencies higher than 100 kHz and intensities lower than 1 W·cm^2 is non destructive and very commonly used in live animals, carcass and food evaluation. The sound waves of various frequencies produce images by vibration-reflection of internal organs and tissues. The most commonly used ultrasound techniques are **pulse-echo wave ultrasound** based in ultrasonic pulses that are partially transmitted and partially reflected and **continuous wave ultrasound** based on electrical continuous pulses. There are also **pitch and catch** techniques and **ultrasonography** technologies that are less commonly used. In US imaging two models can be used, A-mode or amplitude modulation, with a single transducer and the B-mode with a linear array of transducers that generates 2D images. US is mainly used in the estimation of intramuscular fat and results are very variable between studies. In live cattle accuracy in terms of R^2 varied between 0.32 and 0.80 (Aass et al., 2006; 2009; Brethour, 2000) and in live pigs between 0.18 and 0.38 (Bahelka et al., 2009). In pork meat R^2 varied between 0.48 and 0.93 (Lakshmanan et al., 2012; Maignel et al., 2010; Mörlein et al., 2005; Newcom et al., 2002) and in beef meat between 0.64 and 0.81 (Hassen et al., 2001; Park et al., 1994). Determination of fat content in raw hams (De Prados et al., 2015) and salt content in dry-cured ham (Fulladosa et al., 2015) has been recently reported.

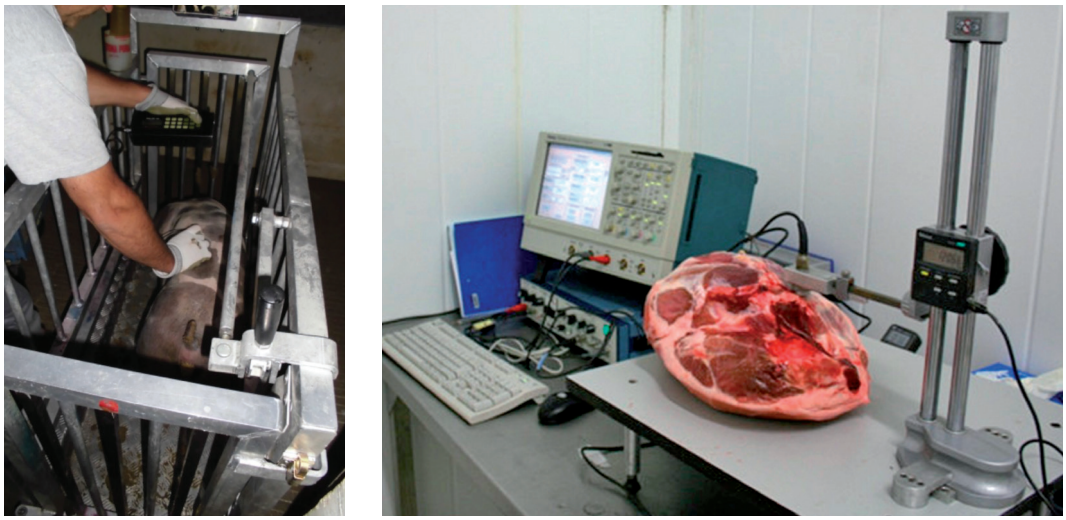


Figure 9: Ultrasound measurement of fat in live pigs (left) and ham (right).

9.2.9 Sensors

Recently **voltametric** and **impedimetric sensors** have been developed to determine NaCl, nitrate and nitrite in pork minced meat (Labrador et al., 2010) as well as an **electronic nose** to determine some microbial quality of beef fillets (Papadopoulou et al., 2013). **Gas-sensor array technology** (electronic nose) was also studied for detection of boar taint which presently receives a lot of attention due to the possible ban of surgical castration of male piglets. The electronic nose *per se* is a precise technique based on sensitive sensors. Results show that it can predict androstenone with high accuracy but for skatole results are less good. Its feasibility is also limited due to the factors that demand more harmonisation like sample preparation, detection thresholds, cut-off limits, etc. (Haugen et al., 2012). In general, the ability of sensors could be improved through the use of combined technologies (Damez and Clerjon, 2008).

9.3 Some points of interest

According to the studies published using new technologies for the determination of meat quality parameters some points should be noted:

- There are increasing numbers of scientific papers evaluating the reliability of different spectroscopic and imaging technologies to determine meat quality in which the industrial implementation is also evaluated.
- Performance of different technologies in the determination of meat quality properties depends on the technology used and the trait studied. It is important to find, for each quality trait of interest, the most appropriate technology in terms of the accuracy, cost, efficiency and other requirements. The combination of technologies could allow a better determination of meat properties.
- Some technologies are suitable to be used on line although further works are needed to prepare them for this purpose in order to 1) avoid or minimize pre-treatment of the samples, 2) allow a continuous measurement in the carcass or the meat and 3) get an appropriate production speed as well as other necessities that could difficult the implementation in the industry.
- The value of the technologies is not only in the industrial use. These methods can serve for screening purposes or for breeding programs where lower accuracy of rapid methods (as compared to analytical values) is compensated by high number of results.
- The chemometric treatment used to analyse images and other comprehensive data from modern technical devices is complex and considerably influences the performance of the method. Thus, it is also important to improve and optimise the automation of data/image analysis to allow a good implementation of the technology on line and to provide quick results.

9.4 References

9.4.1 Review papers

As already explained, a lot of different papers have been published on the application of modern technologies for different meat analyses, indicating the increase of the use of these technologies. Some of them have been included/mentioned in the information above, but it is not possible to discuss all of them. More information can be found in the reviews (see below) of the different technologies applied to livestock and food science:

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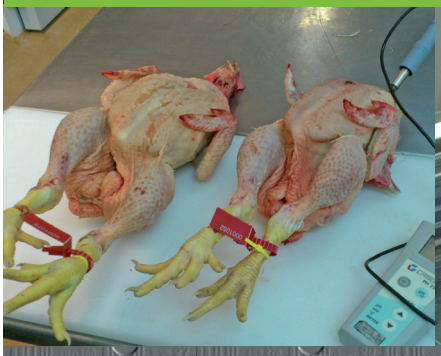

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