



Review

Meat quality assessment using biophysical methods related to meat structure

Jean-Louis Damez*, Sylvie Clerjon

INRA, UR370 QuaPA, F-63122 Saint Genès Champanelle, France

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ABSTRACT

This paper overviews the biophysical methods developed to gain access to meat structure information. The meat industry needs reliable meat quality information throughout the production process in order to guarantee high-quality meat products for consumers. Fast and non-invasive sensors will shortly be deployed, based on the development of biophysical methods for assessing meat structure. Reliable meat quality information (tenderness, flavour, juiciness, colour) can be provided by a number of different meat structure assessment either by means of mechanical (i.e., Warner–Bratzler shear force), optical (colour measurements, fluorescence) electrical probing or using ultrasonic measurements, electromagnetic waves, NMR, NIR, and so on. These measurements are often used to construct meat structure images that are fused and then processed via multi-image analysis, which needs appropriate processing methods. Quality traits related to mechanical properties are often better assessed by methods that take into account the natural anisotropy of meat due to its relatively linear myofibrillar structure. Biophysical methods of assessment can either measure meat component properties directly, or calculate them indirectly by using obvious correlations between one or several biophysical measurements and meat component properties. Taking these calculations and modelling the main relevant biophysical properties involved can help to improve our understanding of meat properties and thus of eating quality.

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* Corresponding author. Tel.: +33 4 73 62 41 87; fax: +33 4 73 62 40 89.
 E-mail address: damez@clermont.inra.fr (J.-L. Damez).

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1. Introduction

One challenge facing the meat industry is to obtain reliable information on meat quality throughout the production process, which would ultimately provide a guaranteed quality of meat products for consumers. To meet this challenge requires fast, accurate and non-invasive techniques for predicting technological and sensory qualities. Over the last few years, a number of methods have been developed to objectively measure meat quality traits. The majority of these methods are invasive, meaning that a sample has to be taken or that they are difficult to implement on-line. In muscle food, the pivotal qualitative characteristics that need to be determined are texture, nutritional value, and appearance. Several very promising measurement techniques are currently being studied and used in laboratories, some of which will shortly be ready for industrial deployment.

The great variability in raw meat leads to highly variable products being marketed without a controlled level of quality. This problem is aggravated when the industry is unable to satisfactorily characterize this level of quality and cannot therefore market products with a certified quality level, which is an otherwise essential condition for the survival and development of any modern industry. Meat quality depends on the same criteria generally attached to other food. The basic traits relate to nutritional content such as proteins, fat, fibers, vitamins and minerals, mainly iron. Another key criterion is safety. The food must be clean in terms of agro-chemical residue, heavy metals, pathogenic micro-organisms, and any other substance representing a potential health hazard. The other aspect of quality deals with “functional” characteristics, i.e., related to the sensory properties of taste and appearance (Grunert, 1997). The variability in functional traits is in part related to the biological diversity of the animals from which meat is obtained. Numerous studies have shown the important influence of zootechnical characteristics on meat tenderness, other studies have focused on collagen and myofibrillar structure. Factors influencing meat properties are partly related to breed, age and sex (Judge & Aberle, 1982; Huff-Lonergan, Parrish, & Robson, 1995; Horcada, Beriain, Purroy, Lizaso, & Chasco, 1998). These factors are either known or can be contained and controlled. However, the variability in myofibrillar and conjunctive components remains uncontrolled. In fact, meat toughness depends mainly on these two structures: the myofibrillar structure, and conjunctive tissue. Myofibrillar structure is strongly influenced by the animal rearing conditions. For instance, (Greenwood, Harden, & Hopkins, 2007), found that single- or multiple-reared lambs present significant differences in

myofiber types and so in myofibrils, whereas (Gondret, Combes, Lefaucheur, & Leuret, 2005), found changes in myofiber types according to indoor or outdoor rearing systems. On the other hand, conjunctive tissue is directly related to the zootechnical characteristics of the animal at slaughter. These components need to be assessed not only in terms of quantity but also in terms of intramuscular distribution. The spatial organization of the conjunctive network of fat and meat fibers bundles, which defines the “meat grain” and marbling, is one of the meat structure traits strongly connected to meat tenderness. The assessment of this trait is of prime interest, not only for the development of a diagnostic system making it possible to determine the muscular origin of a meat sample and therefore optimize production processes, but also as a non-invasive method of sorting muscle meat in terms of potential tenderness, since consumer demand is for consistency in meat tenderness.

Beyond tenderness, meat structure, as considered in this paper, groups together sensory properties associated with meat eating like texture, pastiness, crusting, palatability, chewiness, juiciness and of course tenderness. These sensory properties are associated with several objective physical properties of the product: fat content, fat spatial organization, collagen content, collagen spatial organization, myofibers spatial organization, myofibers type, size, shape and density, sarcomere length, Z lines and I bands integrity, membranes integrity and sarcolemma attachment to myofibrils. Water content also takes part in these physical properties because it is connected with juiciness and with pale, soft and exsudative (PSE) and dark firm dry (DFD) defects. These defects are more precisely related to water holding capacity (WHC), a water property correlated with water activity (Kuo & Chu, 2003). PSE and DFD defects can also be accessed thanks to the measurement of myofibers metabolism. Another indirect marker of structure is salt diffusion which depends on structure integrity. This review gives a non-exhaustive overview of biophysical methods which can measure one or another of all these sensory and direct or indirect physical parameters. These methods are summarized in Table 1 with the type of information they give and with their main advantages and drawbacks.

Biophysical methods of assessment can either measure meat component properties directly or calculate them indirectly (Monin, 1998) by using obvious correlations between one or several biophysical measurements and meat component properties (Brunton, Lyng, Zhang, & Jacquier, 2006; Swatland, 1997b). This paper pinpoints five groups of biophysical assessment: mechanical methods, optical methods, dielectrical methods, X-ray measurements, and

Table 1
Summary of biophysical methods with the type of information they give in meat science and their main advantages and drawbacks

Methods	Type of information	Advantages	Drawbacks
<i>Instrumental mechanical methods</i>			
Warner–Bratzler shear force test	Tenderness		Destructive, anisotropic
Slice shear force test	Tenderness	More strongly correlated with tenderness than wbsf	Destructive, anisotropic
20% and 80% of meat sample deformation	Tenderness	Assess myofibrillar proteins (20%) and intramuscular connective tissues (80%) separately	Destructive, anisotropic
Armor tenderometer	Tenderness	Portable, non-destructive	Invasive
Torque tenderometer	Tenderness	Portable, non-destructive	Invasive
Tendertec penetrometer	Tenderness	Portable, non-destructive	Invasive
<i>Ultra sound</i>			
Ultrasonic spectral analyses	Texture, fat content, collagen content	3D, on live animals, on whole carcasses	
Ultrasonic elastography (or transient elastography)	Local viscoelastic properties	3D, non-invasive	
<i>Optical spectroscopy</i>			
Infrared spectroscopy	Structure of molecules	Non-contacting, rapid	Need complex data analysis
Near infrared spectroscopy	Structure of molecules, instrumental texture, sensory tenderness, pastiness, crusting, juiciness, discrimination between fresh and frozen-thawed products, water holding capacity ^a	Non-contacting, rapid	Need complex data analysis
Raman spectroscopy	Structure of molecules, interaction of molecules, structure of proteins, water activity, fat organization, water holding capacity, instrumental texture, tenderness	Non-contacting, rapid, can be performed in vivo using optical fiber, small sample portion	Need complex data analysis
Visible spectroscopy, colorimetry	Colour, sarcomere length, myofilaments organization, PSE, tenderness, water holding capacity, drip loss, collagen content, fish freshness	Often performed with polarized light	
Fluorescence spectroscopy	Tryptophan microenvironment, connective tissue content, palatability, chewiness, fish freshness, tenderness, myofiber organization, collagen destructure with heating, aging, sarcomere length	Non-contacting, rapid, can be performed in vivo using optical fiber, often performed with polarized light	Often need extrinsic fluorophore probe, high temperature sensitivity
<i>Microscopy</i>			
Optical microscopy	Fat organization, collagen organization, myofiber typing, discrimination between fresh and frozen-thawed products, myofiber spacing, Z line degradation, sarcomere length, endomysium structure, myofiber diameter, myofiber density, myofiber organization, PSE, specific proteins detection, collagen typing, myofilaments organization	Selective analyse, 3D reconstruction with confocal microscopy	Sample preparation: thin cuts (except for confocal microscopy) and often staining
Electron microscopy	Myofilaments structure changes, structure of proteins, connective tissue organization, endomysium and perimysium structure, Z lines degradation, I band breaks, sarcolemma attachment to myofibrils	Greater resolution and magnification, 3D reconstruction for the SEM, observation of samples without dehydration or freezing with environment scanning electron microscopy	Sample preparation: cryofixation, dehydration, embedding, or staining with heavy metal
Macroscopic imaging	Collagen organization, lipids organization, tenderness, fat content, collagen content, juiciness	Non-contacting, rapid, easy to use	
Impedance measurement	Membranes integrity, aging, discrimination between fresh and frozen-thawed products, pH, fat content, tenderness	Non-destructive, inexpensive	Invasive
Microwave measurement	Water activity, aging, fish freshness	Potentially non-contacting	
X-ray measurement	Fat content, myofilaments structure changes		Use of ionising radiation
<i>Magnetic resonance</i>			
NMR spectroscopy and imaging	Water activity, water content, salt content, discrimination of Na ions population, water holding capacity, denaturation of connective tissue, pH, cooking losses, fat content, fat organization, PSE, DFD, collagen content, collagen organization, myofiber typing	Accuracy, 3D reconstruction	Expensive
Magnetic resonance elastography	Local viscoelastic properties	3D reconstruction of mechanical properties	Expensive

^a Debated.

nuclear magnetic resonance (NMR) measurements. Other reviews have dealt with the on-line evaluation of meat (Swatland, 2003)

or food (Scotter, 1997) quality, but we focused here on structure assessment.

2. Mechanical methods

Mechanical methods for assessing textural sensory attributes have been widely used since the thirties. They include invasive methods, such as compression, traction and shearing, which require sampling, and non-invasive methods, such as direct or resonance tests that can be performed on intact muscles.

2.1. Instrument measurements

The Warner–Bratzler shear force (WBSF) test uses a Warner–Bratzler apparatus to measure maximum shear force. Classical WBSF measurements are widely used, but the results usually show tenderness discrepancies when estimated by a trained sensory panel (TSP) or other objective measurements (Lepetit & Culioli, 1994; Shackelford, Wheeler, & Koohmaraie, 1995; Timm et al., 2003). These discrepancies stem from the orientation of the probe, with measurements taken in the parallel orientation (along the length of the muscle) being more consistent in predicting tenderness than measurements in the perpendicular orientation. Moreover, WBSF measurements differ between raw and cooked meat (Tornberg, 1996). Despite (i) the precautions that have to be taken orienting the measurement probe due to variations in muscle fiber direction (Stephens et al., 2004) and (ii) the destructive and time-intensive nature of the method, WBSF remains the most widely used instrument technique for assessing meat toughness. Attempts have been made to improve or streamline instrument methods using flat blades (slice shear force, SSF) instead of the V-shaped blade used with WBSF. (Shackelford, Wheeler, & Koohmaraie, 1999), reported that SSF measurements are more strongly correlated ($r = -.82$) with TSP tenderness rating than WBSF ($r = -.77$), and it has been reported that SSF can accurately identify “tender” beef (Wheeler et al., 2002). As highlighted by (Bouton, Ford, Harris, & Ratcliff, 1975; Carroll, Thiessen, Rollins, & Powers, 1978; Sacks, Kronick, & Buechler, 1988), in compression tests on raw meat, as deformation increases, the three structural components – myofibrillar proteins, intramuscular connective tissue, and perimysium – successively play a role in mechanical resistance. This prompted the development of complementary mechanical methods to assess myofibrillar proteins and intramuscular connective tissues by, respectively, measuring strain at 20% and 80% of meat sample deformation (Lepetit & Culioli, 1994). Portable apparatuses have been developed: the modified “Armor Tenderometer” with six sharp needles, as described by Timm et al. (2003), which gives pretty good results in predicting TSP toughness on raw meat (Stephens et al., 2004), the “TorqueTenderometer” from MIRINZ, New Zealand, and the Tendertec mechanical penetrometer from the Australian Meat Research Corporation (Ferguson, 1993) which gives a good correlation with meat toughness (Belk et al., 2001).

2.2. Ultrasound methods

Analyzing the acoustic parameters of waves propagating in a medium makes it possible to assess the characteristics of the propagation medium and to characterize it. Two methods using ultrasound can be used in functional quality assessments of muscle food: ultrasonic spectral analysis (Abouekaram, Berge, & Culioli, 1997) and ultrasonic elastography or “transient elastography” (Ophir, Miller, Ponnekanti, Cespedes, & Whittaker, 1994). The acoustic parameters taken into account include the velocity of the propagating waves, and spectral parameters such as attenuation and backscatter coefficient in the medium. Ultrasonic wave propagation in meat depends not only on the composition (e.g., water and lipid content) but also the structure (e.g., orientation of muscle fibers, organization of connective tissue). Some studies

discriminating muscle samples in terms of fat and collagen contents reported better results than those obtained by the mere analyse of the chemical and mechanical properties (Abouekaram, Laugier, Fink, & Culioli, 1992; Abouekaram et al., 2000; Morlein, Rosner, Brand, Jenderka, & Wicke, 2005). Fat content has been reported to be correlated with ultrasound propagation speed, with fat and lean showing reverse temperature dependencies on sound velocity (Abouekaram et al., 2000; Benedito, Carcel, Rossello, & Mulet, 2001). As reported by Monin (1998), ultrasonic measurements give a good prediction of meat texture on live animals and whole carcass, while at the same time being inexpensive and non-invasive.

Biological tissues behave as viscoelastic materials, i.e., they present both fluid viscosity properties and solid elasticity properties. Since acoustic wave propagation is directly linked to these mechanical properties, following the tissue propagation of acoustic waves could be a solution for measuring local viscoelastic properties. This can be done by means of an echographic system, via a technique called “transient elastography”.

Complementary with ultrasound analysis, transient elastography is a novel and non-invasive technique for evaluating the local mechanical properties of biological tissues. It consists in an ultrasonic transducer, which is applied at the surface of a biological tissue, coupled with an ultrasonic pulse echo system. The basic idea is that the low frequency vibrations generated by the transducer itself induce a low frequency motion of the scatterers inside the media. This motion can be detected and measured with the conventional echographic system, and via an inverse problem resolution this motion can yield the local viscoelastic properties. The same technique is applied in the evaluation of fibrosis in chronic liver disease.

Recent reports have described that transient elastography is able to work in anisotropic media like muscle (Gennisson, Catheline, Chaffai, & Fink, 2003; Gennisson, Cornu, Catheline, Fink, & Portero, 2005; McAleavey, Nightingale, Stutz, Hsu, & Trahey, 2003) thanks to the polarization of the low frequency shear strain waves. Sabra, Conti, Roux, and Kuperman (2007) worked on a low-cost transient elastography technique for monitoring biomechanical muscle properties *in vivo*, an approach that could be useful for industrial prototyping.

In the field of meat science, the same team has transposed this technique to beef meat structure evaluation (Catheline et al., 2004). A *post rigor* biceps femoris beef muscle was submitted to an acoustic wave from 50 to 350 Hz. Local displacements resulting from this mechanical excitation were accessed with a classical medical transducer array. These local displacements (speed and attenuation) are then used to compute viscoelastic properties in accordance with the mechanical Voigt's model. (Berg et al., 1999) assessed pork quality with this same technique.

A similar approach is used in combining NMR and mechanical low frequency shear waves in magnetic resonance elastography (see paragraph 6.3.). Dutt et al. (2000) have published a comparison of ultrasound elastography and magnetic resonance elastography.

3. Optical methods

3.1. Spectroscopic methods

Waves propagate and the study of radiation, absorption and more generally of any interactions between electromagnetic radiation and matter is called spectroscopy. From low frequencies to high frequencies, optical spectroscopy covers near-infrared (NIR), infrared (IR), visible, and ultra-violet (UV) (including fluorescence). Spectroscopic methods are widely used for muscle food quality

assessment and control, in both laboratory and meat industry settings (Hildrum, Wold, Vegard, Renou, & Dufour, 2006). Optical spectroscopy offers a panel of useful techniques for on-line characterization because of its non-contacting possibilities and because of the fibre-optical components which make it easy to design portable devices. It has been widely investigated in the field of meat science as a means of gaining structural information. Polarized light gives additional organizational data and are therefore often used for these applications. Below is an outline of the main methods developed and used in meat science and sometimes in the biomedical field, where tissue organization is also of interest.

3.1.1. Infrared spectroscopy

Infrared (IR) spectroscopy is a spectroscopic method that deals with the infrared region of the electromagnetic spectrum (from about 800 to 2500 nm). It is typically employed in pharmaceutical applications, medical diagnostics, food and agrochemical quality control, and combustion research. Infrared spectroscopy is based on the principle that the chemical bonds in organic molecules absorb or emit infrared light when their vibrational state changes. In the near infrared area spectrum, there are major changes in vibrational state. A major challenge in meat science applications for near infrared spectroscopy is sample presentation. Transmission is the most powerful method well-suited to liquids and gases but is inappropriate for undiluted solids. Reflection spectroscopy offers the alternative that is almost always used in meat and muscle studies, and it has been widely investigated as a means of indirectly measuring meat structure. Indeed, although infrared spectroscopy gives direct molecular-level information, research shows that it can be successfully used to determine macroscopic structural changes associated with meat or muscle structure.

New developments in IR spectroscopy will expand its applications further. These include hand-held fibre-optic-enabled instruments able to make instantaneous measurements in almost any part of a product. IR spectroscopy, with its speed, ease of use and versatility, may well become one of the most powerful analytical techniques available to final meat product evaluation (van Kempen, 2001).

Fourier transform infrared (FT-IR) spectroscopy is a fairly new technique for collecting infrared spectra. Instead of recording the amount of energy absorbed when the infrared light frequency is scanned (monochromator), the IR light is guided through an interferometer. After passing through the sample, the measured signal is the interferogram, a time-domain signal. Performing a mathematical Fourier transform on this signal results in a spectrum identical to that from conventional (dispersive) infrared spectroscopy, and measuring a single spectrum is faster. Due to the advantages offered, virtually all modern infrared spectrometers are FT-IR instruments.

Near infrared spectroscopy, often extended to the visible region, is under investigation in several laboratories with the aim of evaluating its potential use for meat structure control. Most of these laboratories are working on eating meat quality, focusing on instrumental texture, sensory tenderness, pastiness, crusting and juiciness. For instance, Andres et al. (2007) are working sorting extreme samples of lamb meat into a high-quality class in term of tenderness and juiciness. This result may have practical implications for sorting meat into a high quality class, which could be branded and sold at a higher price. Ellekjaer, Isaksson, and Solheim (1994) have published results on sausage quality. Fiber-optic probes have been used to predict pastiness or crusting of dry-cured ham (Garcia-Rey, Garcia-Olmo, De Pedro, Quiles-Zafra, & de Castro, 2005; Ortiz, Sarabia, Garcia-Rey, & de Castro, 2006), and beef tenderness has also been investigated: Liu et al. (2003) classified tender and tough samples correctly to 83%, Shackelford, Wheeler, and

Koohmaraie (2005) performed on-line tenderness classifications on US beef carcasses, while Byrne, Downey, Troy, and Buckley (1998) Park, Chen, Hruschka, Shackelford, and Koohmaraie (1998) worked on the prediction of WBSF and sensory tenderness. Meullenet, Jonville, Grezes, and Owens (2004) showed that NIR spectroscopy could be used to predict the instrumental texture of cooked poultry meat and to classify muscles according to tenderness levels.

IR spectroscopy has also made it possible to discriminate between fresh and frozen-thawed products from broiler breast meat (Lyon, Windham, Lyon, & Barton, 2001) or fish (Uddin et al., 2005) which is of high interest for the control of fraudulent freezing-thawing cycle.

The evaluation of the pork water holding capacity (WHC) is a more controversial topic. While Pedersen and Engelsen (2001) present encouraging results, Hoving-Bolink et al. (2005) are more prudent about the ability of IR spectroscopy to predict this default. It has been demonstrated that microstructure changes in salted pork can be detected by FT-IR microspectroscopy (Bocker, Ofstad, Bertram, Egeland, & Kohler, 2006). Uddin, Okazaki, Ahmad, Fukuda, and Tanaka (2006) also investigated protein denaturation and changes in water state in fish–meat gels while heating with IR spectroscopy. To our knowledge, Swatland is the only author who used polarized IR light to access structural information on meat. The polarization gives additional information on sample organization that can be exploited to detect cold shortening in pork (Swatland, 1995) and beef (Swatland, 1996). Swatland and Barbut (1995) also showed that myofibrillar near-IR birefringence in turkey meat is correlated with WHC of raw meat and with fluid loss during cooking.

3.1.2. Raman spectroscopy

Raman spectroscopy is also a vibrational spectroscopic technique used in condensed matter physics, biomedical applications and chemistry to study vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering of monochromatic light, usually from a laser in the visible, IR, or near-UV spectra. It gives similar but complementary information to IR spectroscopy.

Raman spectroscopy has great potential for biochemical tissue analysis at both the macroscopic and microscopic scale. One of the great advantages of this technique is its ability to provide information on the concentration, structure and interaction of biochemical molecules in their microenvironments within intact cells and tissues (i.e., *in situ*), non-destructively, and without homogenization, extraction, or the use of dyes, labels, or other contrast-enhancing agents. Furthermore, Raman spectroscopy can be performed *in vivo* using optical fiber technology.

In a recent review, Herrero (2008) compared Raman spectroscopy to various conventional methodologies such as protein solubility, apparent viscosity, WHC, instrumental texture methods, dimethylamine content, peroxide values, and fatty acid composition, all commonly used to determine quality in fish and meat muscle treated under different handling, processing and storage conditions through the changes in the proteins, water and lipids of muscle food. It has been shown that Raman spectroscopy data are related to the results obtained with these conventional quality methods and could be used to evaluate muscle food quality. In addition, it has been shown that Raman spectroscopy provides structural information on the changes of proteins (Li-Chan, Nakai, & Hirotsuka, 1994), water (Herrero, Carmona, Garcia, Solas, & Carceche, 2005) and lipids (Beattie, Bell, Borgaard, Fearon, & Moss, 2006) of muscle food that occur during the deterioration. Furthermore, this spectroscopy technique has several advantages compared to traditional methods, since it is a direct and non-invasive technique that requires only small sample portions.

Before being investigated in food science, Raman spectroscopy had been widely used in biomedical applications where it is well known for its ability to determine the degree of saturation in fatty acids, a very significant nutritional aspect. A review in this field (Manoharan, Wang, & Feld, 1996) outlined the main applications for analysis of biological tissue, describing the advantages and disadvantages of visible, N-IR and UV excitations and addressing the problems and prospects of using these methodologies for disease diagnosis. Still in the biomedical field, Buschman et al. (2001) explored cellular and extracellular morphologic structures, and Brennan, Wang, Dasari, and Feld (1997) developed a N-IR Raman spectrometer for *in situ* clinical investigation. This portable device was equipped with an optical fibre for *in vivo* measurements.

Marquardt (2001) has also reported on the development of an on-line probe designed and optimized for performing Raman measurements in both laboratory and industrial environments. Developing this kind of Raman spectrometer in the biomedical field has paved the way to development in food science. Moreover, the components contributing to Raman scattering in muscle include collagen and elastin, both well known for their role in meat structure. At the protein level, Beattie, Bell, Farmer, Moss, and Desmond (2004) demonstrate the ability of Raman spectroscopy to measure changes in secondary structure and then to be useful for determining textural properties such as beef meat tenderness. N-IR FT-Raman spectroscopy was also used to investigate protein structure changes in food during heating (Beattie et al., 2004; Ozaki, Cho, Ikegaya, Muraishi, & Kawauchi, 1992).

In a recent study, porcine muscle tissue was subjected to different processing factors, including ageing, salting and heat treatment, in order to induce structural changes. These changes were then investigated with both FT-IR and Raman microspectroscopy in order to compare the techniques. Bocker et al. (2007) concluded that Raman is as strong as IR spectroscopy in detecting muscle structure changes. Still in the field of pork processing, it has been shown that Raman scattering is able to predict WHC early in fresh meat (Pedersen, Morel, Andersen, & Engelsens, 2003). Ellis, Broadhurst, Clarke, and Goodacre (2005) proposed an approach that would aid food regulatory bodies to rapidly identify meat and poultry products, highlighting the potential application of Raman spectroscopy for rapid assessment of food adulteration and discrimination between both species and distinct muscle groups within these species.

Again, polarizing the exciting light can improve the performance of Raman spectroscopy in investigating structured materials. Smith and Berger (2005) performed polarized Raman spectroscopy on a two-layer diffusing biological tissue. Polarized light directly backscattering off from the superficial layer partially retains its sense of polarization, whereas deeper-probing light will be increasingly depolarized by diffusion. This technique leads to depth-dependent selective information, which can be useful in complex foods studies.

3.1.3. Visible spectroscopy and colorimetry

This field of biophysical methods covers the visible spectra (often extended to near-UV and/or N-IR regions) and the CIE $L^*a^*b^*$ colour space as objective and non-destructive tools for tissue characterization.

There has been a great deal of biomedical research into tissue characterizing using these techniques. As this research is often focussed on gaining structural information, it merits coverage here due to the potential for use in muscle – and therefore meat – structure evaluation.

The use of visible light to access muscle structural information is not a new concept. Rome (1967) utilized light scattering on isolated rabbit muscle to measure sarcomere length. Haskell, Carlson, and Blank (1989) worked on muscle birefringence to measure sar-

comere length and characterize orientation disorders in the myofibril array. Birefringence measurements were performed using polarized light. The birefringence of rabbit muscle was also used to study and better understand the muscle cell biology of the rigor to relaxation phase (Taylor, 1976). More recently, Xia, Weaver, Gerrard, and Yao (2006) reported using light scattering to evaluate sarcomere structure changes in whole muscle. Visible spectroscopy is often performed with polarized light to provide quantitative morphological data on structural changes (Sokolov, Drezek, Gossage, & Richards-Kortum, 1999). For instance, malignant tissues are less organized than others, and anisotropic scattering and absorption parameters can be exploited to detect them (Ghosh, Mohanty, Majumder, & Gupta, 2001; Kim et al., 2003). Refocusing on muscle studies, Binzoni et al. (2006) worked on anisotropic photon migration in human skeletal muscle as a tool to access information on fibre organization.

In the field of meat science, early detection of pale, soft and exsudative (PSE) meat is a major potential application of visible spectroscopy and colorimetry for both pork (Chizzolini, Novelli, Badiani, Rosa, & Delbono, 1993; Swatland, 1997a; Swatland & Irie, 1992; Xing, Ngadi, Gunenc, Prasher, & Garipey, 2007) and poultry meat (Barbut, 1993; Marquez, Wang, Lin, Schwartz, & Thomsen, 1998; Sante, Lebert, Le Pottier, & Ouali, 1996).

Valkova, Salakova, Buchtova, and Tremlova (2007) showed the ability of the CIE $L^*a^*b^*$ system to predict sensory tenderness in cooked pork ham. Liu and Chen (2001) studied changes in visible spectra versus *post mortem* chicken meat degradation. Visible spectroscopy with polarized light can be used to predict toughness, WHC and drip loss in salted comminuted chicken breast meat (Swatland & Barbut, 1999).

There have also been studies on beef meat, such as to measure sarcomere length and collagen content (Xia, Berg, Lee, & Yao, 2007) or to select animals with low-temperature gelatinization of connective tissue (Swatland, 2006), based on the fact that connective tissue is closely related to beef meat tenderness. Lastly, the European FAIR project (MUSTEC) investigated these methods for on-line predicting of fish freshness (Olafsdottir et al., 2004).

3.1.4. Fluorescence spectroscopy

Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually UV light, that excites the electrons in molecules of certain compounds and causes them to emit a lower-energy light. In fluorescence spectroscopy, the species is first excited, by absorbing a photon of light, from its ground electronic state to one of the various vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state. The molecule then drops back down to one of the various vibrational levels of its ground electronic state, emitting a photon in the process. As molecules can drop down into any of several vibrational levels in the ground state, the emitted photons will have different energies, and thus frequencies. Therefore, analyzing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, makes it possible to determine the structure of the different vibrational levels.

Tryptophan is an important intrinsic fluorescent probe that can be used to assess the nature of the tryptophan microenvironment. Proteins that lack tryptophan can be attached to an extrinsic fluorophore probe.

For opaque samples such as meat and meat products, front face fluorescence is used, and because these products contain tryptophan, this technique has been used in meat and muscle science to investigate sample structure without using extrinsic fluorophore probes.

It is well known that the amount of connective tissue in meat is directly linked to meat tenderness (Light, Champion, Voyle, & Bailey, 1985). Since connective tissue is the most intrinsic fluorophore in meat, its fluorescence intensity should be a good marker of meat tenderness.

Since as far back as 1987, Swatland has been published research on the potentiality of autofluorescence for gauging meat quality. He has correlated connective tissue fluorescence with meat quality parameters like palatability (Swatland, Gullett, Hore, & Buttenham, 1995) or chewiness (Swatland, Nielsen, & Andersen, 1995) and has also developed on-line probes combining fluorescence measurements with other optical measurements like reflectance (Swatland, 2000). In 1993, Swatland (1993) highlighted the necessity of controlling temperature when connective tissue fluorescence is used to detect tough meat. Front face fluorescence spectroscopy was also investigated to measure texture of meat emulsions (Allais, Viaud, Pierre, & Dufour, 2004), fish freshness (Andersen & Wold, 2003; Dufour, Francia, & Kane, 2003) and meat tenderness (Egelandsdal, Wold, Spornich, Neegard, & Hildrum, 2002), while (Christensen, Norgaard, Bro, & Engelsen, 2006) reviewed autofluorescence in food and particularly in meat and fish products.

All previous articles treat non polarized light approaches. As structure is often connected with preferential alignments, the use of polarized light in fluorescence spectroscopy is an improvement for structure study. Polarization allows preferential excitation of fluorophore with transmission moments parallel to the direction of polarization. Several authors have used this property. The studies on polarization of tryptophan fluorescence began in the 60s with (Aronson & Morales, 1969) and was used in biological tissues studies in the biomedical field (Borejdo et al., 2004), in particular to detect malignant tumours (Ghosh et al., 2001; Mohanty, Ghosh, Majumder, & Gupta, 2001). Moreover, proteins can be labeled with fluorescent dyes for muscle study in polarized light (Dale et al., 1999; Van Der Heide, Orbons, Gerritsen, & Levine, 1992).

In the field of food science, Marangoni (1992) used polarization fluorescence spectroscopy to determine microviscosity and structural order in complex lipid systems. More recently, Yao, Liu, and Hsieh (2004) investigated fluorescence polarization spectroscopy for characterizing fiber formation in meat analogues.

The INRA has investigated the fluorescence of tryptophan and connective tissues in meat with polarized excitation and emission beams to evaluate the potential use of this tool for meat structure control (Luc, 2007; Luc, Clerjon, Peyrin, & Lepetit, 2008). For a well-ordered biological tissue, fluorescence is anisotropic and this anisotropy tends to disappear with structural degradation. Fig. 1 presents the decrease of fluorescence anisotropy with collagen heating. Destructuring processes like ageing, grounding or heating have been successfully investigated. The technique also appears useful for detecting cold shortening bovine muscle (Luc, Clerjon, Peyrin, Lepetit, & Culioli, 2008).

3.2. Imaging

3.2.1. Microscopic imaging

Microscopy has been widely used to control meat and meat product structure. It can be split into two fields: “optical microscopy” and “electron microscopy”.

3.2.2. Optical microscopy

Optical microscopy offers the simplest way to obtain magnified images of biological tissues. This field covers a large range of techniques that have been used for years to characterize meat and meat product structures. Techniques can be classed simply depending on whether samples must be prepared in thin cuts or not. Non-thin-cuts samples were used for the very early phase contrast measurement (Ranvier, 1889) allowing the detection of A and

I bands in muscle and for the new confocal laser scanning microscopy, which will be discussed later.

3.2.3. Histology

Histology is a widely used method for observing biological tissues at the microscopic level, particularly as a tool for controlling meat texture in food science. The technique may or may not require tissue staining with specific dyes. However, histology always needs very thin sample cuts. In biological microscopy, it is almost always necessary to enhance contrast by using specific dyes to make certain biological components more visible during histological observation.

This technique can be used to enhance lipids: for instance, Thakur, Morioka, Itoh, and Obatake (2003) worked on the effect of composition and deposition of lipids stained with Sudan dye on fish meat texture. Collagen architecture can also be accessed, either for fiber typing in pork with myosin ATPase or NADH staining (Oshima et al., 2007), or for detecting changes in structure with frozen process in carp myofibrillar proteins stained with SDH enzyme or myofibrillar ATPase (Jasra, Jasra, & Talesara, 2001).

Several studies have conducted to correlate *post mortem* processes with histological muscle fiber traits in meat. For instance, Ichinoseki, Nishiumi, and Suzuki (2006) studied the effect of high pressure on intramuscular collagen fibrils in bovine connective tissue, while Rusman, Gerelt, Yamamoto, Nishiumi, and Suzuki (2007) reported on the effects of high pressure and heat on histological characteristics of bovine muscle, such as inter-myofiber space, Z line degradation, sarcomere length decrease and endomysium structure. The cold shortening and structural characteristics of single pork muscle fibers (Willems & Purslow, 1996) and structural characterization of mechanically recovered meat (Tremlova, Sarha, Pospiech, Buchtova, & Randulova, 2006) have also been investigated. Vonlengerken, Maak, Wicke, Fiedler, and

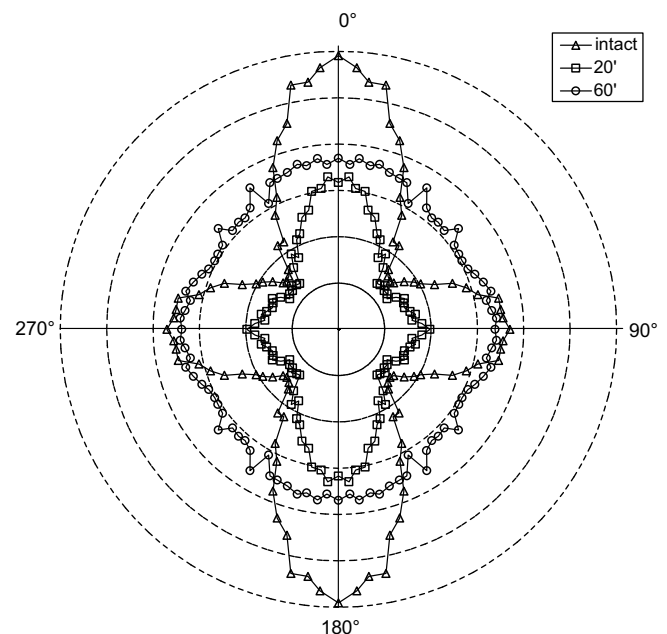


Fig. 1. Angular plot of bovine collagen intrinsic fluorescence anisotropy (arbitrary units). Angular coordinates are relative to the angle between collagen fibres main direction and exciting light polarisation direction. Collagen is heated at 60 °C during 0 min (intact sample), 20 and 60 min. Destructuration with heating is pointed out by the disappearance of angular dependence of anisotropy: after 60' of heating, anisotropy tends to a constant value whatever the exiting light polarisation direction (Luc, 2007).

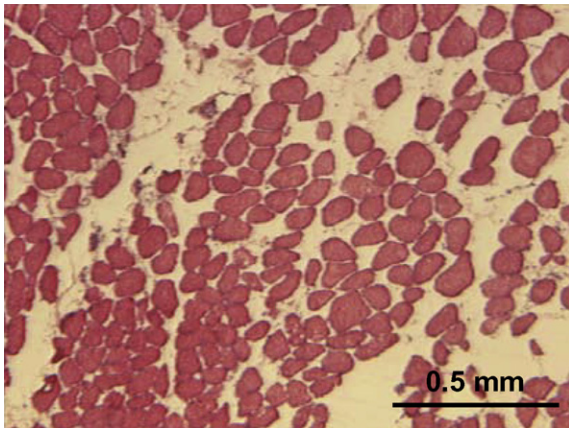


Fig. 2. Observation of increase in fiber spacing to characterize PSE zones in pig with hematoxyline eosine saffran staining. (Laville et al., 2005).

Ender (1994) have worked on myofiber's structural and functional traits (type, diameter and number of fibers) in muscle for genetic improvement of pork. Fiber disorganization, fiber misalignment, and increase in fibers spacing (Fig. 2) have been analyzed to characterize PSE zones in pigs with hematoxylin–eosin–safran staining (Laville et al., 2005).

Karlsson, Klont, and Fernandez (1999) reviewed skeletal muscle fibres as factors for pork quality. The histochemical properties of a muscle, such as fibre type composition, fibre area, oxidative and glycolytic capacities, and glycogen and lipid contents, are factors that have been found to influence meat quality. Similarly, histochemistry, combined with several other staining protocols such as Sudan black-B, myosin ATPase or NADH-tetrazolium reductase, has been used to classify fibres.

Immunohistochemical labelling is more selective because antibodies are used to visualize specific proteins and lipids: the secondary antibody is labelled with an enzyme or a fluorescent component. Several applications of immunoenzymology have focused on collagen architecture and collagen typing in pork (Nakamura et al., 2003) and poultry (Oshima et al., 2007; Roy et al., 2006). Astruc, Marinova, Labas, Gatellier, and Sante-Lhoutellier (2007) localized oxidized proteins in muscle to pinpoint the role of membrane proteins in oxidation. The occurrence of fast and slow myosin isoforms in fibre types was also detected by immunohistochemistry in pigs (Fiedler, Dietl, Rehfeldt, Wegner, & Ender, 2004; Fiedler et al., 1999).

When antibodies are labelled with fluorescent dyes, we are working with immunofluorescence, a common technique for visualizing sub-cellular distribution of biomolecules of interest. Immunofluorescent-labelled tissue sections are observed using a fluorescence microscope or by confocal microscopy, as explained further down. Immunofluorescence has been used to study PSE in turkey breast muscle (Pietrzak, Greaser, & Sosnicki, 1997) and measure thin muscle filament lengths of beef, rabbit, and chicken myofibrils (Ringkob, Swartz, & Greaser, 2004). However, histological techniques are often used in combination to obtain a maximum of information from a given tissue.

3.2.4. Confocal laser scanning microscopy

Confocal laser scanning microscopy is a fluorescence technique for obtaining high-longitudinal resolution optical images. It is an evolution of the more traditional fluorescence microscopy, its key feature being the ability to produce point-by-point in-focus images of thick specimens, allowing 3D reconstructions of complex tissues. Because this technique depends on fluorescence, samples

usually need to be treated with fluorescent dyes to make objects visible, but contrary to the histological techniques, there is no need for thin cuts.

In meat science, Straadt, Rasmussen, Andersen, and Bertram (2007) recently applied confocal laser scanning microscopy to monitor changes in fresh and cooked pork muscle during ageing. Two different magnifications, i.e., at $\times 10$ and $\times 200$, give spectacular views of myofibers and myofilaments, respectively.

Nakamura et al. (2007) successfully studied changes in bovine connective tissue according to animals feeding (concentrate- and roughage-fed groups) using confocal laser scanning microscopy coupled with immunohistochemical typing of collagen and protein structure evaluation in perimysium and endomysium. Immunohistochemical/confocal laser-scanning microscopy is a useful tool for studying structural relationships among connective tissue components in skeletal muscle.

3.2.5. Electron microscopy

The use of electrons beam to illuminate a specimen and create an enlarged image leads to image observation that has a much greater resolving power than with optical microscopes. The greater resolution and magnification of electron microscopy stems from the electron wavelength which is much smaller than light photon wavelength.

Biological investigations use scanning (reflection method) or transmission electron microscopy according to the application. We can also cite here the coupling of an X-ray probe to the electronic microscope to perform X-microanalysis, i.e., the local measurement, at microscopic level, of the X-ray spectrum.

3.2.6. Scanning electron microscopy

Scanning electron microscopy (SEM) gives images with great depth-of-field yielding a characteristic 3D display that provides greater insight into the surface structure of a biological sample. For SEM, samples require preparation, such as cryofixation, dehydration, embedding (in resin...), or staining (with heavy metal).

SEM is a high-performance tool for investigating process-related changes in meat ultrastructure. In combination with histological analyses, Tornberg (2005) reviews effects of heating on changes in secondary, tertiary and quaternary structure of proteins and then on cooked meat quality. This paper shows how SEM is a powerful tool to better understand relations between proteins structure and meat quality. Palka and Daun (1999) have also worked on structural changes during heating in bovine muscle. Structural changes in intramuscular connective tissue during tenderization of bovine meat by marinating in a solution containing proteolytic enzyme (Chen, He, Jiao, & Ni, 2006) is also of interest in meat process control and can be accessed with SEM.

Laarre et al. (2007) outlined the comparisons and complementarities of SEM, cryo-SEM and transmission electron microscopy for the measurement of process-related ultrastructural changes in ham. Lastly, Yang and Froning (1992) reported structural differences in washed or unwashed mechanically deboned chicken meat, while Nishimura, Hattori, and Takahashi (1999) focusing on the *ante-mortem* stage, studied structural changes in intramuscular connective tissue during fattening.

Cryo-scanning electron microscopy consists in SEM observation after cryofixation, and is well adapted to biological tissues that are relatively unaffected by this specific sample preparation. More specifically, cryofixation is a solution for visualizing the electrolytes that are suppressed in a classical dehydration preparation. Garcia-Segovia, Andres-Bello, and Martinez-Monzo (2007) studied the effects of cooking temperature and cooking time on losses, colour and texture of beef steaks using cryo-SEM to assess the endomysium and perimysium microstructure. Pig muscle cell ultrastructure *versus* freezing rate and storage time has also been

investigated with this technique (Ngapo, Babare, Reynolds, & Mawson, 1999). Cryofixation is also used in transmission electronic microscopy.

Environmental scanning electron microscopy (ESEM) is a new development in the field of electron microscopy. It opens up the possibility of observing samples at almost normal atmospheric pressures (unlike classical SEM) without having to dehydrate or freeze them. Yarmand and Baumgartner (2000) used ESEM to study the structure of semimembranosus veal muscle. Even though ESEM offer promising possibilities for observing intact samples, contrast is often less good than in traditional SEM when the sample is stained with a heavy metal.

3.2.7. Transmission electron microscopy

In transmission electron microscopy (TEM), electrons are passed through the sample. Resolution is higher than in SEM and the sample can be stained with heavy metals to improve image quality. Unfortunately, samples have to be prepared in very thin slices and put on a grid for the observation, making the technique difficult to implement.

This technique has been used to observe Z line removal during culled cow meat tenderization by proteolytic enzymes (Gerelt, Ikeuchi, & Suzuki, 2000) and by calcium chloride (Gerelt, Ikeuchi, Nishiumi, & Suzuki, 2002) following osmotic dehydration.

Ho, Stromer, and Robson (1996) used TEM to study the effects of electrical stimulation of bovine carcasses on *post mortem* change in skeletal muscles. Nakamura, Ando, Seoka, Kawasaki, and Tsukamasa (2006) used the same technique to study changes in the ultrastructural properties of tuna muscle during chilled storage. Sen and Sharma (2004) also used TEM to show that freezing/thawing cycles do not significantly change the ultrastructural properties of buffalo muscle if freezing is applied in good conditions.

To round off, TEM has also been used to quantify ageing-related ultrastructural changes, I band breaks and sarcolemma attachment to myofibrils, in game meat (Taylor, Fjaera, & Skjervold, 2002). Even when aged for a long time (14 days), game meat presents very few I band breaks. These results are not in agreement with game meat high tenderness which seems to be attributed to myofiber small size better than to ultrastructural *post-mortem* changes. Myofiber attachment in salmon fillets (Taylor, Labas, Smulders, & Wiklund, 2002) was also investigated with TEM. Authors have demonstrated that the structural change associated with loss of muscle hardness is breaks in myofiber-to-myofiber attachments, and that loss of rigor stiffness is associated with breaks in myofiber-to-myocommata attachments. These results imply that ultimate fillet texture occurs by a combination of these distinct structural changes.

As for histological methods, immunoelectron microscopy allows the detection of specific proteins in ultra-thin tissue sections. This technique is actually a TEM technique conjugated with specific antibodies labelled with heavy metal particles (often gold). An original application studying ageing and structural weakening of myofibrils and collagen is given in Takahashi (1996).

3.2.8. Macroscopic imaging

Visual inspection is used extensively for quality assessments on meat products applied to processes running from initial grading through to consumer purchases. Numerous laboratories have investigated the possibility of using image-based meat quality evaluation, and a number of reviews have listed the main applications for food and more specifically meat science (see Brosnan & Sun, 2004; Du & Sun, 2004). In meat science, image analysis consists in analyzing the texture of images produced from muscle and meat sections at one or more wavelengths. The technique makes it possible to clearly highlight the collagen and lipid structures of muscular tissue.

Kirschner, Ofstad, Skarpeid, Host, and Kohler (2004) used FT-IR imaging (associated with FT-IR microscopy) to monitor thermal denaturation processes in aged beef loin. Recently, the same team developed an algorithm for analyzing sets of FT-IR microscopy images of tissue sections, which was applied to FT-IR microscopy images of beef loin muscles containing myofiber and connective tissue regions. The images were investigated for variations due to ageing duration and due to homogeneity in the connective tissue regions. Keeping with beef quality, it has been shown (Shackelford, Wheeler, & Koohmaraie, 1998) that early image analysis of a steak from the 12th rib region can predict tenderness classification. Shiranita, Miyajima, and Takiyama (1998) described a method for determining meat quality using the concepts of “marbling score” and texture analysis. Marbling score, which was a measurement of fat distribution density in the rib-eye region, was considered as a texture pattern. Texture analysis has also been used to classify photographic images of beef meat slices (Basset, Buquet, Abouelkaram, Delachartre, & Culioli, 2000). Among the multiple muscular tissue characteristics that influence meat quality, connective tissue content and spatial distribution, which define meat grain, are particularly important since they are directly related to tenderness. Connective tissue contains two key components, namely fat and collagen, which vary according to muscle, breed, and age. These components are clearly visible on photographic images. Finally, image processing techniques have been developed to predict cooked-beef tenderness from fresh-beef image characteristics (Li, Tan, Martz, & Heymann, 1999). Another recent paper (Chandraratne, Samarasinghe, Kulasiri, & Bickerstaffe, 2006) investigated how the surface characteristics (geometric and texture) or raw meat could be used to predict the tenderness of the cooked lamb meat. The prediction showed encouraging results indicating that there is significant relation between the raw meat surface and the cooked meat tenderness using non-linear and artificial neural networks analyses ($R^2 = 0.602$ and 0.746 , respectively). It has also been possible to analyze pork quality and marbling level using a hyperspectral (400–1000 nm) imaging system (Qiao, Ngadi, Wang, Gariepy, & Prasher, 2007). Hyperspectral analyses provide supplementary information than classical visible imagery, and a neural network model based on only 10 principal components extracted from the hyperspectrum of raw meat can classify meat according to its firmness and exudation. In spite of these promising results, authors do not succeed in proving that spectrum widening improves classification results.

Meat analogues produced from vegetable proteins have generated the need for a new imaging system and reliable non-destructive techniques for determining the textural properties of these new products (Ranasinghesagara, Hsieh, & Yao, 2005). Image analysis could also be used to determine fibre orientation in meat products.

The fact that meat structure is closely linked with muscle fibre parameters prompted the INRA to develop dedicated software (Buche & Mauron, 1997) to determine fibre structural parameters (area, shape factors, etc.) in pork, chicken, trout, beef, rabbit, mutton and turkey muscles. Imaging analysis of magnetic resonance and histological images can generate precise and objective measurements of the two main structural component of muscle tissue (myofibers and intramuscular connective tissue) (Sifre-Maunier, Taylor, Berge, Culioli, & Bonny, 2006).

Multispectral image analysis (MIA) on images acquired at different wavelengths in the UV-Visible range was used to correlate meat components with sensorial and physical properties, giving good results in terms of predicting composition (collagen ($R^2 = .88$), lipids ($R^2 = .87$)) and sensory attributes (tenderness ($R^2 = .75$), juiciness ($R^2 = .84$)) (Abouelkaram, Chauvet, Strydom, Bertrand, & Damez, 2006).

4. Dielectric methods

4.1. Impedance measurement

The first research involving impedance measurements on meat was published back in the 1930s (Callow, 1936). Electric impedance is the property of a material to oppose the flow of electric current. When this property is not dependent on the frequency of the current, it is qualified as resistance; otherwise, as it is the case with biological tissues, the impedance has a resistive component and a capacitive component. In schematic terms, biological tissues are composed of cells that are surrounded by an extracellular liquid. The cell membrane acts as an insulator at low frequencies, behaving like a capacitor. Biological tissue, particularly meat, has anisotropic impedance, i.e., impedance varies according to whether the current runs parallel or perpendicular to muscle fiber (Damez, Clerjon, Abouelkaram, & Lepetit, 2007; Swatland, 1980). The impedance of the meat decreases quickly with *rigor* and continues to decrease, albeit much more slowly, during storage (Pliquett, Pliquett, Schoberlein, & Freywald, 1995).

Electric impedance is used for a broad range of purposes in meat technology:

4.1.1. Detection of frozen meats

In the 1970s, it was shown that frozen meat samples have very weak impedance (Sale, 1974). However, we now know that this measurement cannot certify that a meat has been frozen, because meat aged for very long can present similarly low impedance (Damez, Clerjon, Abouelkaram, & Lepetit, 2008).

4.1.2. pH

Since the 1980s, the vast majority of research on impedance has involved using this measurement for controlling the drop in pH or for evaluating ultimate pH. It mainly concerns pig meat (Swatland, 1985) although beef has also been studied (Byrne, Troy, & Buckely, 2000). In the case of pork, one of the major problems is the evaluation of water holding capacity (Schafer, Rosenvold, Purslow, Andersen, & Henckel, 2002) and the detection of pale soft exudative (PSE) meat, which has a low pH and is highly exudative, and thus unsuitable for processing. In the case of beef, the problem is the dark firm dry (DFD) meat with high pH. These two defects are associated with modifications of membranes and extracellular fluids, which therefore affect the meat's electrical properties. Electrical measurements have been used to compensate the inaccuracy of pH measurements. The majority of the studies in this field have focused on detecting the defects early, i.e., within 45 min to 1 h post-slaughter. However, recent results show that electrical measurements do not permit the early detection of DFD (Forrest et al., 2000; Guerrero et al., 2004). The difficulty in detecting PSE meats during *rigor* set in is due to the fact that meat evolves rapidly (pH, temperature) during this period whereas the associated metabolic changes will only affect its structure and thus its electric properties later on. However, impedance (conductivity) is more capable of detecting PSE meats once the ultimate pH is reached (Guerrero et al., 2004).

4.1.3. Fat content

Many studies conducted since the 1980s, have attempted to use electrical properties to estimate fat content in animal carcasses or meat. Fat is an electrical insulator and therefore influences the impedance of tissues. Electric impedance methods can obtain remarkable results. A simple electric conductivity measurement on a carcass immediately after slaughter can be associated with anatomical data to give fat content with remarkable accuracy ($R^2 = .95$). This could be explained by the fact that there are no

membrane or extracellular compartment modifications occurring immediately after slaughter, and the measurements are made at a stable temperature. A patented system has been developed (Madsen, Rasmussen, Boggaard, & Nielsen, 1999) for measuring fat content in muscle. This portable apparatus uses electrodes inserted in the muscle, and fat content is estimated via measurements made at several frequencies. Measurements of fat content after *rigor* are not consistent, because impedance in this case is also influenced by membrane state.

4.1.4. Tenderness

A study (Byrne et al., 2000) related the electrical properties of muscle after cooking to tenderness as assessed by WBSF and attempted to establish a link between the electrical properties and the mechanical resistance of meat. The results showed there was no direct relationship between meat tenderness and straightforward electrical measurements. This is due to the fact that connective tissue, which plays a crucial role in tenderness, has similar impedance to muscle fiber and thus cannot be detected by electrical measurements.

4.1.5. Ageing

Ageing involves meat-tenderizing biochemical and physico-chemical processes. These processes include the action of endogenous proteases on muscle fibre structure, a progressive increase in membrane water permeability, and the weakening of connective tissues. Faure et al. (1972) set out to evaluate state of maturation by quantifying these effects. They proposed an approach based on the ratio of low-frequency impedance to high-frequency impedance, which decreases during refrigerated storage. However, Lepetit, Sale, Favier, and Dalle (2002) showed that between-animal variations of this ratio stemmed from variations in ion or fat contents. Furthermore, this impedance ratio cannot reliably indicate the state of meat maturation or destructuring. A similar study reported the ratio of capacity (the dielectric parameter reflecting the insulating state of the membranes) to electrical resistance (Kleibel, Pfützner, & Krause, 1983), but the parameters measured were also affected by tissue adiposity.

Muscle is electrically anisotropic, meaning that muscle and thus meat exhibit changes in electrical properties according to the direction of the electrical fields in the sample. After *rigor mortis*, the electrical impedance of meat decreases linearly with the mechanical resistance of muscle fibres, and electrical anisotropy is a better predictor of muscle fibre strength than impedance alone (Lepetit et al., 2002). The rate of ageing in beef varies tremendously from one animal to another. The strength of muscle fibres can reach its minimum value within a few days, whereas for the same muscle in another animal it can take more than two weeks. It has been shown (Lepetit & Hamel, 1998) that it is possible to select meats which age rapidly if the state of ageing is known at 48-h *post mortem*. This will avoid storing, already-aged meats during long periods. The expected benefits include a 50% cut in storage costs. The study measured ageing state using a destructive mechanical method, but this same information can be obtained from non-destructive sensors. One such sensor made by Damez et al. (2008) uses electrical impedance anisotropy, and has been patented (Lepetit et al., 2007).

4.2. Microwave characterization

The interaction of microwaves and food products has been exploited for heating in many applications for thawing, cooking and disinfection purposes. Recently, however, there has been an emergence of sensor systems based on the interaction of low-power (microwave sources no more powerful than in mobile phone devices) electromagnetic microwaves with biological mat-

ter. In the microwave frequency range (almost 0.3–300 GHz), the dielectric properties of biological tissues are closely correlated with water content and state (Kent & Jason, 1974). In particular, dielectric relaxation spectroscopy determines the molecular motion response of sample polar molecules (mainly water) to a weak external alternative electric field. As electric field frequency increases, it reaches a frequency called 'relaxation frequency' where the polar molecule can no longer rotate with the electric field. Dielectric properties change markedly around this relaxation frequency. The technique has been explored for measuring water activity in proteic gels (Clerjon, Daudin, & Damez, 2003). Water activity is a parameter closely connected to water binding which is related to water holding capacity in meat and so to PSE and DFD phenomena. Moreover, fraudulently added water in meat products can be detected thanks to its higher value of water activity (Kent & Anderson, 1996; Kent, Knochel, Daschner, & Berger, 2001).

Dielectric properties not only depend on water binding in food material but also on food composition. For any given molecular composition, the dielectric spectrum will change with molecular binding. In real material, the complex interplay between molecular composition, presence of ions, electrical charges on proteins, and pH variations leads to a complex dielectric spectrum regulated by several phenomena.

Microwave approaches applied for macroscopic structural measurements are based on the dielectric anisotropy of muscle. The structural organization and composition of muscle makes it a highly anisotropic dielectric material. This dielectric anisotropy was modelled by Felbacq, Clerjon, Damez, and Zolla (2002) to provide insight into microwave-muscle interactions. It tends to decrease during ageing- or process-related cellular degradation. Polarimetric measurements, i.e., with a linearly polarized electric field, make it possible to evaluate anisotropy. This method has been applied to assess meat ageing and fish freshness (Clerjon & Damez, 2005; Clerjon & Damez, 2007), and Brunton et al. (2006) linked dielectric beef muscle anisotropy to proteic changes during cooking.

Tejada, De las Heras, and Kent (2007) reported the results of the Torrymeter Distell Freshness Meter, a commercial microwave sensor dedicated to fish freshness evaluation. At a more macroscopic level, the premature field of microwave tomography (Christensen, 2004; Semenov et al., 2007) shows promise for giving information on tissues organization within a piece of meat.

5. X-ray measurements

X-rays have long been used in medicine and others areas. The principle is to obtain a measurement of the attenuation of the penetrating energy. Different materials have different attenuation properties, and so depending on the level of penetrating energy, it should be possible to obtain quantitative measurements, in particular for bone, lean meat and fat. Multiple technology tools using X-ray beams at different energy levels have been developed, making it possible to discriminate fat, bone and lean meat according to the energy attenuation measured. Over the last 30 years, the meat industry has been using low-energy X-ray systems like the Anyl-Ray system (The Kartridg Pak Co., Iowa) (Gordon, 1973).

Dual-energy X-ray absorption (DXA) is a useful technology for meat fat assessment. Absorption at low X-ray energies (e.g., 62 keV) is dependent on both fat content and sample density, while absorption at higher energies (e.g., 120 keV) mainly depends on the density. Coupling the two measurement and subtracting one from another gives the fat content (Brienne, Denoyelle, Baussart, & Daudin, 2001; Hansen et al., 2003) with very good accuracy compared to chemical analysis (R^2 values from .7 to .97). Other

researchers have attempted to use DXA to predict the tenderness of raw beef and cooked lamb meat, but the method gave moderate and poor results in comparison with WBSF ($R^2 = .69$ and $R^2 = .12$, respectively) (Kroger, Bartle, West, Purchas, & Devine, 2006). As reported by (Mercier et al., 2006) although DXA is too slow for commercial use, it may be used as reference method in carcass composition studies. It should also be noted that Earlier, Diesbourg, Swatland, and Millman (1988) posted encouraging results for X-ray diffraction measurements of *post mortem* changes in the pork myofibrillar lattice.

6. Nuclear magnetic resonance

NMR contributes to the characterization of many products, including muscle food. The high costs involved do make it currently difficult to consider installing NMR systems on production lines. The tool nevertheless has a wide range of research applications, particularly for product assessment, and can be seen as a reference method given the richness of measurements obtained: the diffusion coefficient and the relaxation time being the most useful. NMR is based on the absorption and emission of energy in the radiofrequency range of the electromagnetic spectrum. All nuclei that contain odd numbers of protons or neutrons have an intrinsic magnetic moment and angular momentum. The most commonly measured nuclei are hydrogen-1 (the most sensitive isotope at natural abundance) and carbon-13, although nuclei from isotopes of many other elements can also be observed (^{23}Na , ^{31}P ...). NMR studies magnetic moments by aligning them with an applied constant magnetic field and perturbing this alignment using an orthogonal alternating radiofrequency magnetic field. This perturbation induces a resonant phenomenon which is exploited in NMR spectroscopy and magnetic resonance imaging (MRI).

6.1. NMR spectroscopy

A review (Bertram & Andersen, 2004) and several papers (Bertram, Purslow, & Andersen, 2002; Bertram et al., 2001) describe the status of NMR applications in meat science and explain the potential and relevance of spectroscopic and relaxation-based methodologies to different topics of importance for meat science. The most widely explored area of NMR in meat science is proton relaxometry. The use of relaxometry has been highly successful due to its ability to characterize water and structural features in heterogeneous systems like meat. Venturi et al. (2007) showed how NMR spectroscopy can measure water activity in freeze-dried chicken breast meat by studying of the shape of the T_2 relaxogram. Low-field water-proton NMR T_2 relaxometry has been widely used to determine WHC, which is closely linked to myofibrillar structure, for beef (Tornberg & Larsson, 1986) and processed pork and meat quality by the INRA (Renou, Kopp, Gatellier, Monin, & Kozakreiss, 1989) and the Danish Institute for Agricultural Science (Bertram & Aaslyng, 2007; Straadt et al., 2007; Bertram, Kristensen, & Andersen, 2004; Mortensen, Andersen, Engelsen, & Bertram, 2006). Micklander, Peshlov, Purslow, and Engelsen (2002) underlined the ability of NMR to track structural changes in pork during cooking. A recent study (Wu, Bertram, Bocker, Ofstad, & Kohler, 2007) demonstrated that the changes in water proton T_2 relaxation times affected by heating rate and raw pork quality (DFD, PSE or normal) are closely related to protein secondary structure changes. The same method was exploited by Ahmad, Tashiro, Matsukawa, and Ogawa (2005) to study the gelation characteristics of fish surimi gel by observing its molecular dynamics. Thermal denaturation of bovine connective tissue has also been studied using water proton NMR T_2 relaxometry (Rochdi, Foucat, & Renou, 1999; Rochdi, Foucat, & Renou, 2000).

Relaxation parameters are also a good indicator of water holding capacity (WHC) in meat. WHC is an important meat quality trait for consumer acceptance. Meat WHC depends primarily on the lateral tensing of the myofibrils during the rise in *rigor mortis* changes associated with fluid flow between water compartments in muscular tissue (Offer & Knight, 1988). NMR measurements of water proton relaxation times give information on the dynamics of water. Significant correlations have been highlighted between measured value and indicator relaxation times for meat quality parameters such as pH, WHC or losses to cooking (Fjellkner-Modig, Persson, & Tornberg, 1986; Renou, Kopp, & Valin, 1985).

Since fat content contributes to meat textural properties, we can also cite studies by Renou, Monin, and Sellier (1985) Foucat, Donnat, Martin, Humbert, and Renou (1997) on meat fat content measurement using NMR spectroscopy. Furthermore, *post mortem* metabolism has been studied in pig muscle using ^{31}P NMR to predict PSE and DFD defaults early, either *post mortem* (Miri, Talmant, Renou, & Monin, 1992) or *ante mortem* (Lahucky et al., 1993).

Very recently, a new technique was described for the absolute quantification of double-quantum filtered spin-3/2 nuclei ^{23}Na spectra (Mouaddab, Foucat, Donnat, Renou, & Bonny, 2007). This method paves the way for absolute quantification of both bound and free fractions of Na^+ , which are determining factors in the characterization of salted/brined/dried meat products. Still in the field of salted meat, Foucat, Donnat, and Renou (2003) studied the interactions of sodium and chloride ions with meat products by means of ^{23}Na and ^{35}Cl NMR spectroscopy. The same kind of investigation was done on fresh and frozen-thawed cod fillets (Erikson, Veliyulin, Singstad, & Aursand, 2004).

The *post mortem* evolution of energy-rich compounds can be followed using ^{31}P NMR (Renou, Canioni, Gatelier, Valin, & Cozzone, 1986). This technique makes it possible to measure the concentrations of ATP, creatine phosphates, sugar phosphates and inorganic phosphate (Pi). The pH calculation starts from the chemical shift of the peak associated to Pi. NMR has so far proven very useful tool for studying metabolic changes and pH evolution in the muscle in relation to the technological quality of the meats.

6.2. Magnetic resonance imaging

The major interest of this technique is that it can solve many product control problems during production. NMR sequences run on bovine samples can produce images where the water and lipid signals are selected, making it possible to identify the various components of the conjunctive network. This technique is further enhanced by “susceptibility” imaging which makes it possible to locate the conjunctive network fibers whose thickness is much lower than the dimensions of the NMR image voxels. NMR micro-imaging on meat samples can be used to quantitatively characterize lipids. Fat content analysis by NMR requires choosing a sequence of impulses (Inversion-Recovery, Spin Echo, or others) to be applied to the product. The results highlight the versatility and practicability of the technique, since the equipment involved is compact and the method can be equally well deployed for controlling food composition as for checking food quality.

NMR imaging (MRI) generates a morphological image of a sample distinguishing bone, fat and lean meat. Sample elements can be differentiated by differences in water content and in water mobility in various biological elements. Water content and water mobility are variables that can be studied by measuring particular NMR parameters (proton density, relaxation time, T_1, T_2, T_2^* , diffusion coefficient, etc.). Fat was quantified in ground beef by NMR (Foucat et al., 1997). The results showed excellent correlation ($R^2 = .992$) with the Soxhlet method. For the test range of 5–15% fat content, actual fat content was determined with very good accuracy. It is thus possible to characterize the connective tissue structure of

the perimysium (Fig. 3) (Bonny et al., 2001; Laurent, Bonny, & Renou, 2000).

^{23}Na NMR spectroscopy presented in 6.1. is also used in MRI studies. Salt ingress in muscle products is connected with structural integrity as membranes act as barriers for ions diffusion. Guheneuf, Gibbs, and Hall (1997) showed with ^{23}Na MRI that sodium ions ingress into *post rigor* porcine muscle during brining follow a Fick's second law. Bertram, Holdsworth, Whittaker, and Andersen (2005) introduced the use of combined ^{23}Na MRI and ^{23}Na NMR spectroscopy for the study of the diffusion of sodium ions into the meat during curing. Results revealed a decrease in diffusion coefficient, suggesting that changes occur in the microscopic structure of the meat during curing. ^{23}Na NMR spectroscopy gives here complementary information to MRI by the identification of two sodium populations. ^{23}Na MRI quantification of sodium mobility in pork during brine was also investigated at different pH and *post mortem* ages (Vestergaard, Risum, & Adler-Nissen, 2005) suggesting the diffusion coefficient to be affected by changes in NaCl concentration, swelling and degree of dehydration.

In the past, *post mortem* muscle studies have characterized fiber types (type I: slow-twitch oxidative; type IIa: fast-twitch oxidative glycolytic; type IIb: fast-twitch glycolytic) with T_1 and T_2 values (Adzamlı, Jolesz, Bleier, Mulkern, & Sandor, 1989; Lerumeur, Decertaines, Toulouse, & Rochcongar, 1987), where T_2 can differentiate type I fibers from others with slightly higher values. *In vivo* studies on rabbits highlighted a higher T_2 for muscles characterized by type I fibers (Bonny et al., 1998), which the authors suggested was due to water structuralization in muscle fibers, fat content, and myoglobin state. Fig. 4 illustrates these researches.

6.3. Magnetic resonance elastography

Like transient elastography, magnetic resonance elastography (MRE) measures local viscoelastic properties by following an acoustic wave in a biological tissue. The technique takes advantage of MRI image quality, and microscopic MRE, which is made possible with high-resolution MRI, (Othman, Xu, Royston, & Magin,

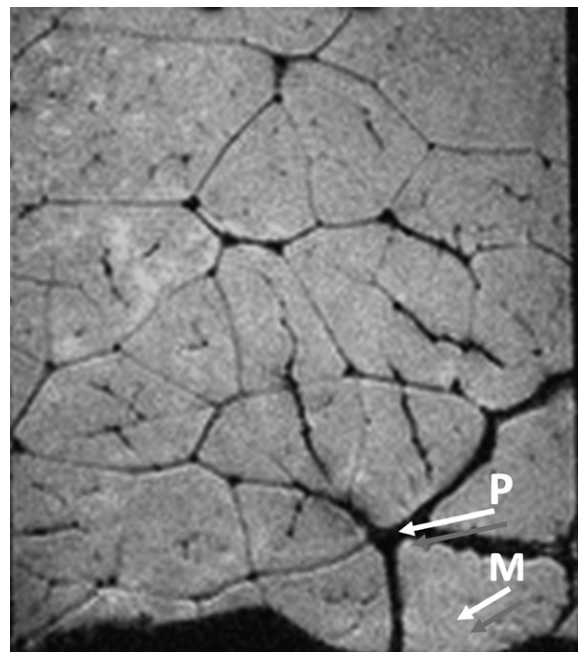


Fig. 3. Magnetic resonance imaging (MRI). Characterization of perimysium (P); myofibers (M). Image of beef *gluteo biceps* 24H *post mortem*, MRI 4.7 Tesla, resolution 300 μm (Bonny et al., 2001).

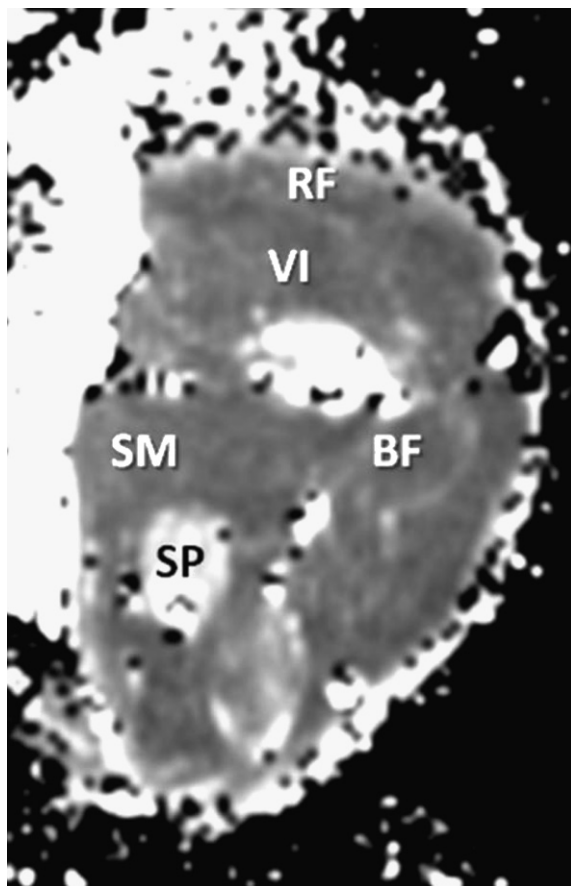


Fig. 4. Characteristic MR T_2 parametric image of rabbit thigh transverse slice distinguishing Type I fiber muscles from Type II fiber muscles. BF, *biceps femoris*; RF, *rectus femoris*; SM, *semimembranosus*; SP, *semimembranosus proprius*; VI, *vastus internalis* (Bonny et al., 1998).

2005), is a promising method for carrying out mechanical investigations at the microscopic scale.

MRE has not been yet used in the field of meat science, but the approach is under development at the INRA in France to measure local mechanical parameters on whole muscle to gain insight into the structural changes occurring in meat products during processing. MRE has already been applied in the biomedical field to study breast and liver diseases (Sinkus et al., 2000; Manduca et al., 2001). Research has been published on muscles, which are an anisotropic material, and the conclusions will drive MRE development in the meat sciences (Basford et al., 2002; Dresner et al., 2001; Papazoglou, Rump, Braun, & Sack, 2006; Uffmann et al., 2004; Sinkus et al., 2005).

7. Discussion

This overview has highlighted how many biophysical methods are able to measure parameters directly or indirectly connected with meat structure. The wide range of physical principles employed means that individual methods give different key information for meat structure assessment. Table 1 gives a summary of these methods with the type of information they give and their main advantages and drawbacks. While comparative analysis is useful (Brondum et al., 2000) the way forward is to combine the technique and thus improve sensor performances. Several laboratories have followed this direction.

Sifre et al. (2005) investigated spatial organization of the perimysium in beef meat using a combined histology and MRI ap-

proach. The technique provided complementary microscopic and macroscopic datasets on intramuscular connective tissue structures, both of which are necessary for predicting sensory tenderness. The same methods are exploited in association with rigor index to predict soft flesh problems in freshwater rainbow trout (Foucat, Taylor, Labas, & Renou, 2004) or to detect frozen-thawed fish (Foucat, Taylor, Labas, & Renou, 2001).

Fourier transform infrared microspectroscopy and low-field proton NMR transverse relaxation measurements were combined to study ageing-related changes in protein secondary structure and water distribution in pork following salting and cooking (Wu et al., 2006).

Swatland's teams have investigated a number of biophysical methods for analyzing meat quality, sometimes in combination. For instance, Swatland (2001) deals with combining spectrophotometry and fluorometry in the same probe to discriminate connective and adipose tissues. They have also coupled optical and mechanical measurements to evaluate meat tenderness (Swatland, Brooks, & Miller, 1998), pairing these two biophysical parameters with electrical properties to develop a three-system probe for meat texture measurement (Swatland, 1999).

Another field under development that is closely linked to biophysical methods in meat science is data processing. Spectral and image analysis or combined biophysical methods are very prolific data generators, and it is a real challenge to find solutions optimizing the data processing steps. Multivariate data analysis (chemometrics) can more efficiently tackle food science problems and, moreover, solve problems that could not be handled before (Bro et al., 2002). For instance Kohler et al. (2007) developed a very useful tool for FT-IR microscopy data processing.

The linear myofibrillar structure of muscle food gives of its biophysical properties strong anisotropy that can be assessed using appropriate techniques. Such techniques, using polarized electromagnetic waves, either visible, IR, UV optical bands, microwaves as well as electrical conductivity and US, have been highlighted.

All the results presented here point out the wealth of potential for using biophysical methods in meat quality investigations. Although a high number of studies have already been conducted, the field of research is vast, and meat scientists still have years of exciting work ahead before offering to meat industry a cheap, robust, reliable, portable, rapid, universal ... magnificent meat quality sensor!

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