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Muscle characterisation by NMR imaging and spectroscopic techniques

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Abstract

This work illustrates the potential of nuclear magnetic resonance (NMR) for better characterisation and understanding of meat characteristics. Magnetic resonance spectroscopy (MRS) allows energy metabolism in muscle to be followed, and the fatty acid composition of animal fat to be studied. Magnetic resonance imaging (MRI) affords a spatial resolution that characterises body composition and allows at each point of an NMR image quantitative measurement of parameters closely correlated with meat properties such as pH, cooking yield and water holding capacity. NMR is thus a powerful tool for meat research, and a reference for other less expensive techniques.

1. Introduction

High quality/price ratios and consistent quality are expected by both meat manufacturers buying raw material for further processing, and consumers purchasing meat. This demand has led to an increasing need for techniques to evaluate meat quality. Meat research has developed new techniques to study the mechanisms underlying meat quality (Monin, 1998); this paper focuses on nuclear magnetic resonance (NMR). NMR has proved an irreplaceable research tool in both analytical chemistry and biology. However, NMR has not been widely used in meat science, even though a broad range of applications is potentially feasible. The high cost of NMR spectrometers may be an obstacle. Magnetic resonance spectroscopy (MRS) provides non-invasive means of monitoring small molecules in solution. ^{31}P-MRS has been used to study the post-mortem catabolism of high-energy phosphate compounds and the associated pH variation in muscle. Fatty acid chain composition has also been studied using ^{13}C-MRS. The ^{1}H spectrum of a muscle displays two signals: a small peak associated with fat protons, and a very intense water resonance, which has hitherto impeded investigations by ^{1}H-MRS. Magnetic resonance imaging (MRI) gives a spatial resolution (about 300 μm) that allows characterisation of the tissue morphology by ^{1}H-MRI. In animal product research, MRI is useful for the determination of body composition. Few studies have been reported with other nuclei because of their low relative NMR sensitivities. The use of ^{23}Na imaging to follow brine diffusion is possible however, because sodium ion concentration is massively increased in cured meat (Renou, Benderbous, Bielicki, Foucat & Donnat, 1994).

The aim of this paper is to describe the techniques used to characterise muscle by NMR imaging and spectroscopic techniques. It also focuses on new developments which give access to quantitative parameters with greater accuracy.

2. Muscle composition in MRI

Quantity and distribution of fat and connective tissue are of paramount importance for the evaluation of meat quality. One criterion for consumer acceptance is the appearance of the meat, which is related to the visible fat, i.e. intramuscular and intermuscular fat. The amount, type and distribution of connective tissue determine the meat toughness, which is a parameter of eating quality. ^{1}H-MRI is a non-invasive tool that provides insight into the structural component of the meat sample: muscle fibres, fat and connective tissue.

MRI offers a wide range of imaging sequences that provide information at different scales, from lean and adipose tissue discrimination at low resolution level, to water and lipid content quantification at a higher level.

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These MRI sequences include maximising contrast (difference in signal intensities) between the tissues (Foster, Hutchinson, Mallard & Fuller, 1984), constructing a parametric image (Chang, Kuriashkin & Clarkson, 1997; Kamman, Bakker, Van Dijk, Stomp, Heiner & Berendsen, 1987) or specifically eliminating the signal from a particular tissue (Bydder & Young, 1985; Dixon, 1984; Haase, Frahm, Hänicke & Matthaei, 1985).

MRI has been widely used for the global measurement of body composition in terms of lean and adipose tissues. Quantitative information is simply obtained by counting the number of pixels belonging to each tissue using basic segmentation schemes (Colin, Erbland, Datin, Boire, Veyre & Zanca, 1995). Comparison with commonly used techniques for assessing total body adipose tissue argue for MRI (Fowler, Fuller, Glasbey, Foster, Cameron, McNeill & Maughan, 1991) as also does the comparison between different imaging techniques (ultrasound imaging, computer assisted tomography) (Fuller, Fowler, McNeill & Foster, 1994). Total body fat can be determined with whole body MR images, but Fowler, Fuller, Glasbey, Cameron and Foster, (1992) have demonstrated that percentage of adipose tissue determined on four MRI images is sufficient to deduce total body fat. A close correlation between poultry breast weight and volume calculated on MR images has also been demonstrated (Davenel, Collewet, Cambert & Marchal, 1998).

Unlike most techniques for body composition determination, MRI allows the visualisation of adipose tissue distribution through slices in any orientation with an excellent spatial resolution (Foster, 1984; Mitchell, Wang & Elsasser, 1991; Tingle, Pope, Baumgartner & Sarafis, 1995). This allows quantification of different adipose tissue compartments (intermuscular, subcutaneous, and intra-abdominal). Measurements of adipose tissue calculated by MRI have been validated for reference measurements (Abate, Burns, Peshock, Garg & Grundy, 1994; Seidell, Bakker & Van Der Kooy, 1990; Fowler et al., 1992).

The two main MRI sequences (inversion-recovery and spin-echo) used for separating lean and adipose tissue both have drawbacks. The first suffers from a low signal-to-noise ratio, and the second is hindered by a chemical shift artefact and partial volume problems, i.e. the signal from a voxel is the sum of signals from more than one tissue. The signal intensity changes smoothly from adipose tissue to muscle, and the dividing line between the two tissues is not clearcut. A new imaging sequence called chemical shift selective inversion recovery (CSS-IR) (Kaldoudi, Williams, Barker & Tofts, 1993), which overcomes these drawbacks, has been tested on bovine muscle samples. The sequence produces two images, one in which the signal from muscle is suppressed [water-suppressed (WS) image] and the other in which the signal from adipose tissue is suppressed [fat-suppressed (FS) image]. An example of a CSS-IR sequence on a *triceps brachii* bovine muscle is shown in Fig. 1. Intramuscular fat (resp. muscle) shows a hyposignal (resp. hypersignal) on the FS image and an hypersignal (resp. hyposignal) on the WS image.

In body composition studies, a pixel is classified as lean or adipose tissue whether or not its intensity

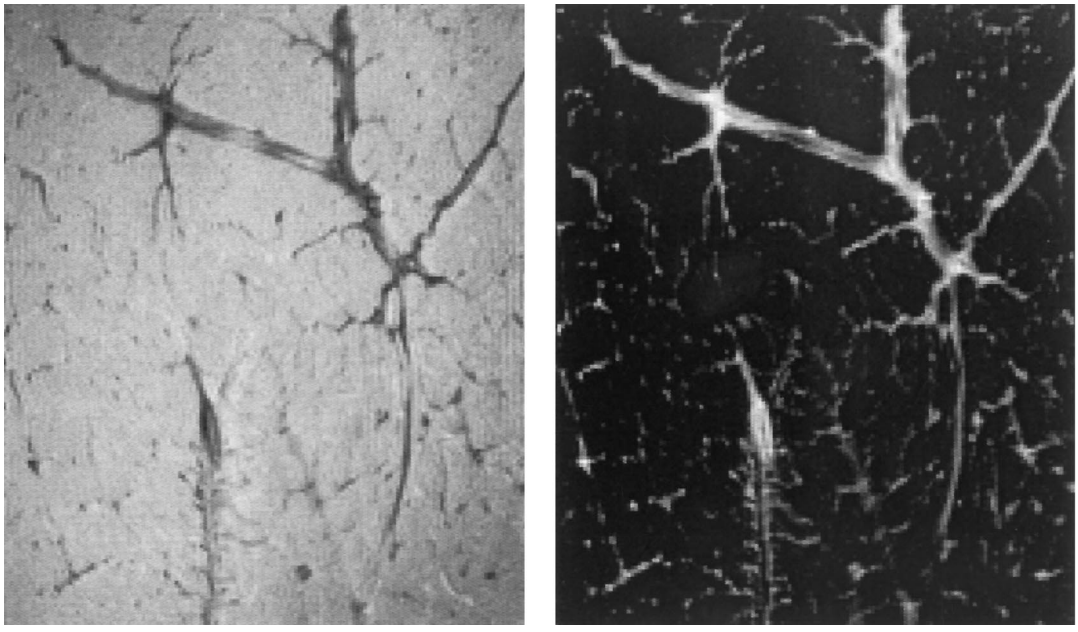

Fig. 1. Fat-suppressed (left) and water-suppressed (right) MR images of a *triceps brachii* bovine muscle. Field of view is 45×50 mm, slice thickness 2 mm and in-plane resolution 300×300 μm.

exceeds a threshold. However, there may be moderate differences in water and lipid content between tissues of the same kind, or even inside the same tissue. The information on water and lipid content is nonetheless accessible owing to the quantitative aspect of MRI (the signal intensity of a pixel can be related to its content). Several authors have shown correlations between the water content in a homogeneous sample determined by MRI, and the water content determined by oven drying (Duce, Ablett, Guiheneuf, Horsfield & Hall, 1994; Rajanayagam, Fabry & Gore, 1991). Other authors have found a close agreement between the percentage of lipid determined by MRI and by lipid extraction (Poon, Szumowski, Plewes, Ashby & Henkelman, 1989; Wong, Northrup, Herrick, Glombicki, Wood & Morrisett, 1994). MRI has also been used to discriminate between different muscles or between different physiological conditions according to its intramuscular lipid content (Datin, et al., 1996).

The ability of the CSS-IR (Kaldoudi et al., 1993) to discriminate between different lipid contents has been tested in our laboratory. A high correlation between the lipid content determined by MRI and by Soxhlet extraction was found on samples with lipid contents ranging between 0 and 15% in 5% steps ($R^2 = 0.99$) and on samples with lipid content ranging between only 1 and 6% in 1% steps ($R^2 = 0.97$) (Laurent, Bonny & Renou, 1998). These correlation coefficients depend on the range of the lipid contents studied, and on the characteristics of the MR spectrometer.

No data are available to our knowledge concerning the determination and quantification of the collagen network in meat by MRI, but links can be made with the MRI of tendons (Gold, Pauly, Macovski & Herfkens, 1995; Koblik & Freeman, 1993; Schick, Dammann, Lutz & Claussen, 1995). On conventional MR images, the tendon is characterised by a low signal-to-noise ratio and is quite difficult to identify with no a priori knowledge. Recently, an approach exploiting the order in biological tissues was proposed for specific imaging of tendons, cartilage and ligaments (Tsoref, Shinar, Seo, Eliav & Navon, 1998). However, all these studies were directed towards assessing morphological changes in tendons (injury, recovery) and not to measurement of the tendon volume.

Fat- and water-suppressed images of bovine muscle are shown in Fig. 2. The long thin structure in Fig. 2 that cuts the sample into two pieces shows a hyposignal in the fat-suppressed image and no signal at all in the water-suppressed image. This structure is identified as a micro-tendon insertion. On the FS image of Fig. 2, lipid aggregates that follow the borders of the micro-tendon insertion are remarkably well delineated.

Muscle fibres in the s*emitendinosus* (ST) bovine muscle are regularly orientated. In Fig. 3, an MR image of an ST muscle in which the orientation of the imaging plane is orthogonal to the fiber orientation is shown. This particular orientation underlines the network organisation of the perimysium.

3. Characterisation of muscle fibres

Metabolic type characterisation of muscle fibres is of importance in animal physiology (exercise ability criteria) and meat production. In the latter case, metabolic type influences, and very often determines, many characteristics of meat products (colour, composition, texture, and flavour). A non-invasive method would be particularly useful in each of these cases.

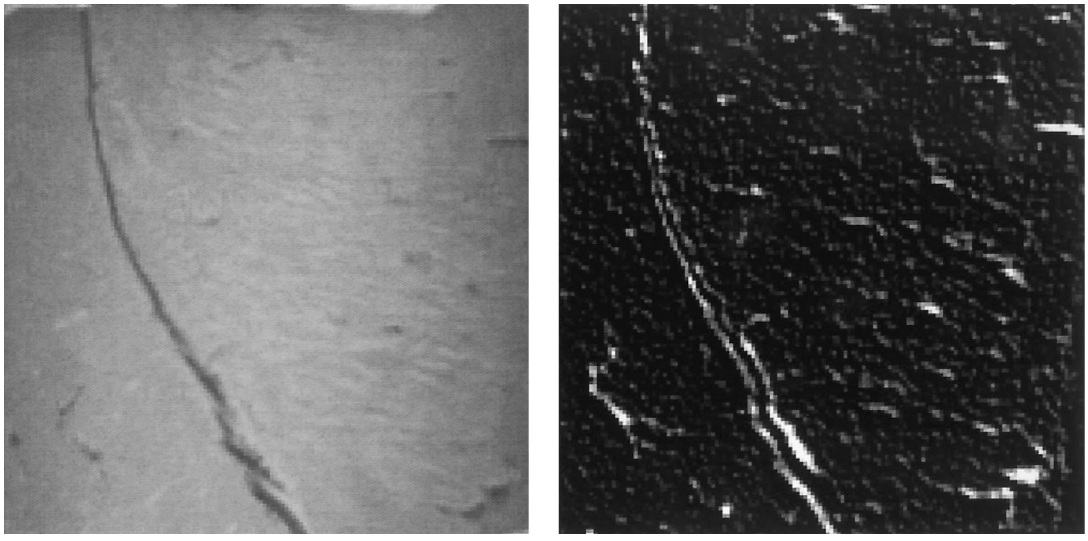

Fig. 2. Fat-suppressed and water-suppressed MR images of a *triceps brachii* bovine muscle showing a micro-tendon insertion. Field of view is 45×45 mm, slice thickness 2 mm and in-plane resolution 300×300 μm.

3.1. MRS

Some ^{31}P-NMR studies (Kushmerick, Moerland & Wiseman, 1992; Meyer, Brown & Kushmerick, 1985; Renou, Canioni, Gatelier, Valin & Cozzone, 1986) have examined the relationships between relative levels of phosphorylated metabolites and fibre composition. ^{31}P-NMR spectroscopy detects the soluble metabolites such as adenosine triphosphate (ATP), creatine phosphate (CP) and inorganic phosphate. The total content of phosphorylated metabolites was found constant (50 μmol/g of fresh tissue). However, glycerophosphoryl choline was detected at a very high level (14 μmol/g of fresh tissue) in pure slow-twitch oxidative fibres of rabbit muscle and seems associated with the slow contractile type fibres (Renou, et al., 1986). Also, lower levels of ATP and CP were measured in pure slow-twitch oxidative fibres than in fast twitch muscles (Kushmerick et al., 1992).

3.2. MRI

With conventional MRI, no contrast has been detected related to fibre composition of muscle. Alternative studies have been conducted on post-mortem muscles to detect more subtle changes based on measurement of NMR relaxation times. They report a higher proportion of type I fibres associated with longer T_2 relaxation times (Adzamli, Jolesz, Bleier, Mulkern & Sandor, 1989; Polak, Jolesz & Adams, 1988; English, Joy & Henkelman, 1991), or with shorter T_2 times (Kuno, Katsuta, Inouye, Anno, Matsumoto & Akisada, 1988) or no correlation at all (Houmard, Smith & Jendrasiak, 1995; Le Rumeur, De Certaines, Toulouse & Rochcongar, 1987; Mardini, McCarter & Fullerton, 1986). These rather inconsistent results can be explained by variations in many biological factors (genetic type, temperature, pH, time post-mortem) affecting the onset of rigor mortis and thus the measured relaxation times. To circumvent this difficulty, another MRI study was performed in vivo on pure slow-twitch and fast-twitch rabbit muscles (Bonny, Zanca, Boespflug-Tanguy, Dedieu, Joandel & Renou, 1998). It was shown that the muscles with high proportions of oxidative slow-twitch fibres had higher T_2 relaxation times than the other muscles. Thus, on a T_2 parametric image where the pixel value is directly proportional to the T_2 relaxation time, the pure type I *Semimembranosus proprius* muscle is brighter than the other muscles, and can be clearly delineated (cf. Fig. 4). The water–myosin interactions, fat content and myoglobin state are the biochemical and physiological characteristics that might explain the observed T_2 differences in muscle characterised by slow-twitch oxidative fibre types.

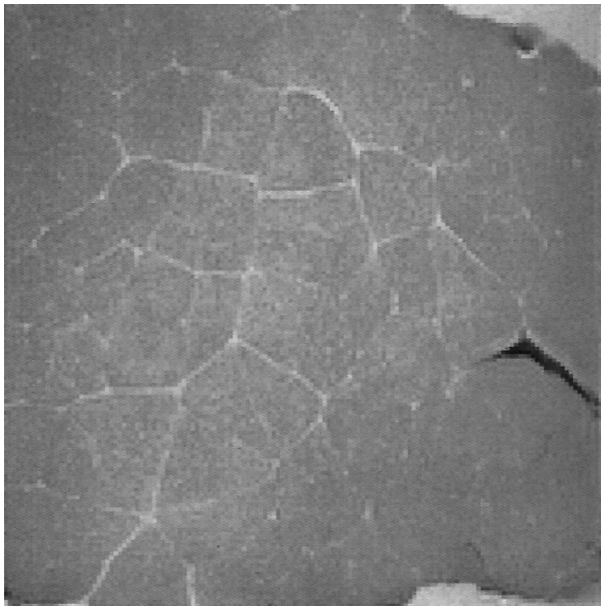

Fig. 3. Fat-suppressed image of a *semitendinosus* bovine muscle highlighting the perimysial network.

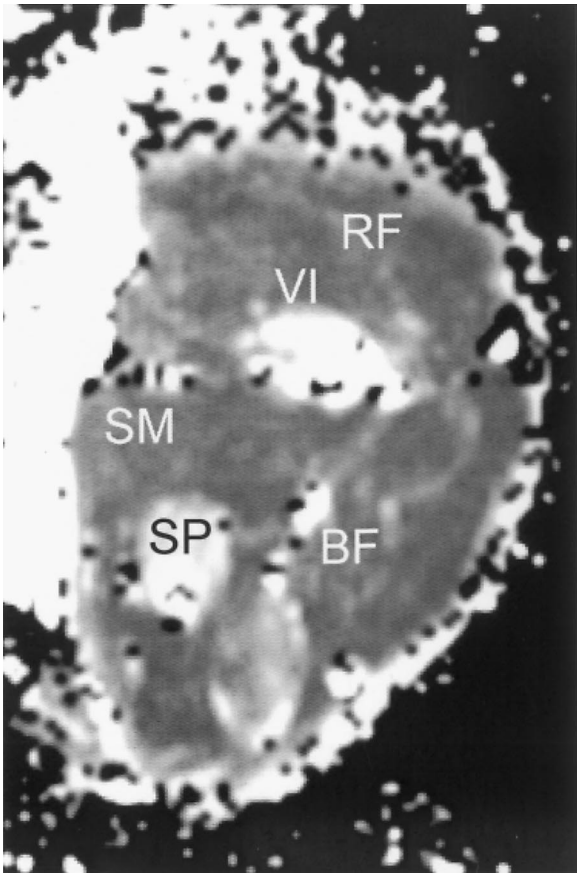

Fig. 4. Characteristic T_2 parametric image of a rabbit thigh transverse slice. BF, *Biceps femoris*; RF, *Rectus femoris*; SM, *Semimembranosus*, SP, *Semimembranosus proprius* VI, *Vastus internalis* [reprinted from Bonny et al. (1998) with permission from Elsevier Science].

4. Characterisation of lipids

4.1. MRS

Compared with low resolution, high-resolution NMR spectroscopy has found very little use in food science. However, ^{13}C-NMR spectroscopy is very useful for the study of fatty acid composition in animal fats. Their strong chemical shifts (200 ppm) allows a high separation of resonances. Resonances around 130 ppm are characteristic of unsaturated carbons. Their intensities compared with selected resonances at low field allow a quantitative determination of the composition of animal fat in terms of saturated, mono- and polyunsaturated fatty acids in triglycerides. The ester carbonyl carbons appeared at high field. Resonances in the region 173.2–173.25 ppm are assigned to ester carbons in the glycerol external $C_{1'3'}$ position, while the peaks in the 172.8–172.85 ppm are assigned to ester carbons in the glycerol internal $C_{2'}$ position. The triglyceride composition and the position of fatty acids on glycerol can be followed according to the animal's diet (Fig. 5) and/or the location of the fatty tissue (Bonnet, Denoyer & Renou, 1990). The NMR results concerning the variation of triglyceride composition agree with those obtained by gas chromatography and provide information on glycerol substitution.

4.2. MRI

MRI gives far less detailed information about the chemical composition of fat tissues. MR parametric images of solid/liquid lipid ratio of adipose tissues were obtained by Davenel and Marchal (1998) in backfat and intermuscular adipose tissues. Since the solid/liquid lipid ratio is correlated to the proportion of triacylglycerol, the MR images provide qualitative (distribution) and quantitative information on the consistency of adipose tissues.

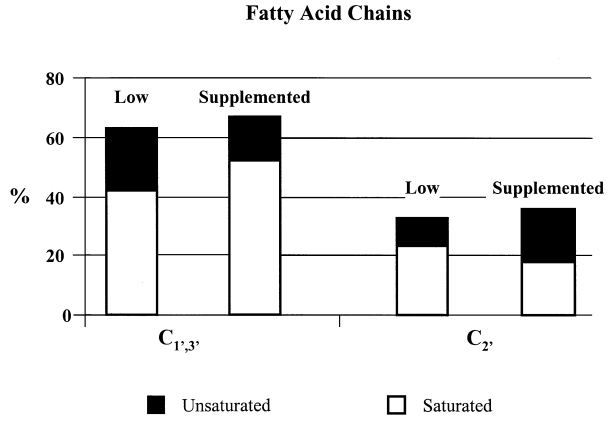

Fig. 5. Percentage of fatty acids at different positions on glycerol for subcutaneous fatty tissue for two diets. $C_{1',3'}$, $C_{2'}$ carbons, respectively, at external and internal position in the glycerol.

5. Process

5.1. Study of rigor mortis onset by MRI

The conditions of rigor mortis onset have great influence on meat quality specially toughness and water holding capacity (WHC). The NMR relaxation parameters reflect the dynamics of water, which are closely correlated with meat properties such as pH, cooking yield and WHC (Renou, Monin & Sellier, 1985).

Quantitative (or parametric) maps can be inferred from a set of MR images using post-processing techniques. Each point of this synthetic image gives the value of an a priori chosen parameter such as NMR relaxation time (cf. Section 3.2) diffusion coefficient, water content or temperature. This approach allows a combination of spatial and quantitative information. We present here an example of simultaneous T_2 relaxation time and temperature mapping during rigor onset inside a muscle sample.

T_2 relaxation measurement of water protons affords information about water dynamics and an understanding of how this water interacts with meat material (Renou, 1995). For example, relationships between pH fall and water dynamics have been established in porcine muscle during the onset of rigor mortis using NMR spectroscopy (Renou, Kopp, Gatellier, Monin & Kozak-Reiss, 1989). Considering the spatial information afforded by MRI, our imaging approach provides more information than the previous results. Beside the mono-exponential T_2 determination, temperature was monitored in each pixel. MRI thermometry based on the temperature-dependent proton resonance frequency (PRF) was used. For various ex vivo tissues (including muscle), the temperature dependence of the PRF shift has been reported to be around 0.01 ppm/°C and it exhibits this linear relationship up to 80°C (Peters, Hinks & Henkelman, 1998). The experimental protocol gives the temperature variation as a function of time from the PRF shift measured in each pixel of the image. MR thermometry can then be considered as an accurate, non-invasive and relative thermometric method (Ishihara et al., 1995).

A special MR sequence, called gradient echo sampling of a spin echo (GESSE), was implemented to acquire a series of real and imaginary images. This set is post-processed so that magnitude and phase images were used to calculate respectively T_2 value (Yablonskiy & Haacke, 1997) and temperature.

A bovine muscle sample was placed in the core of a 200 MHz MR spectrometer 2 h after slaughtering. The sample remained at room temperature of about 23°C for 24 h and, during this period, GESSE images were measured every 20 min.

Fig. 6 shows a typical T_2 map of muscle obtained 120 min after slaughtering. An oil reference is placed in the

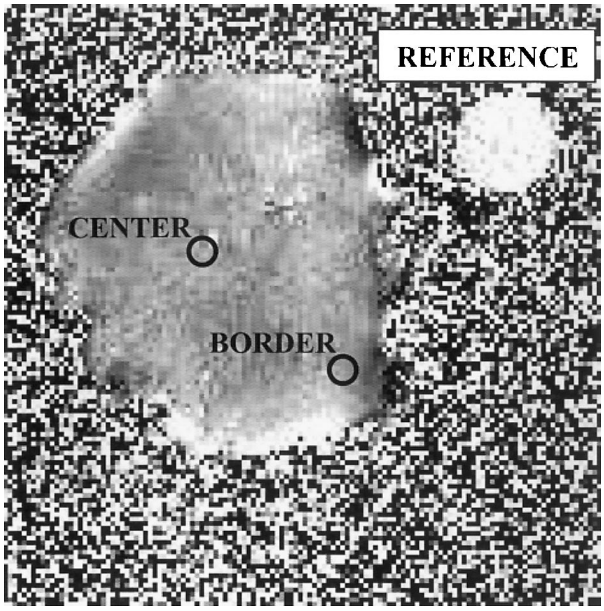

Fig. 6. Typical T_2 map of muscle sample obtained 120 min after slaughtering.

field of view to check the stability of T_2 measurements with time and correct the drift of the main magnetic field. Two regions of interest have been placed on parametric images in the centre and the border of the muscle sample (cf. Fig. 6). T_2 and temperature shift curves are represented as a function of time post-mortem in Figs. 7 and 8. Under our experimental conditions, no spatial temperature variation inside the muscle sample was detected. The observed fluctuations of less than $\pm 1°C$ over 24 h may simply be due to room temperature drift.

During rigor onset, mono-exponential T_2 increases significantly, consistent with the bi-exponential analysis obtained previously by NMR spectroscopy (Renou et al., 1989) indicating that the longest T_2 relaxation component becomes predominant during ageing. NMR imaging protocol limitations prevent a bi-exponential analysis, but this is offset by the spatial information. Two different behaviours were easily detectable by comparing T_2 curves obtained in the centre and in the border of the muscle. TheT_2 plateau is reached at two different post-mortem times.

These results demonstrate the usefulness of MRI for the study of water dynamics in relation to temperature-related process. Non-invasive MR thermometry is available with an accuracy of less than 0.5°C, a temporal resolution of less than a half-hour and a spatial resolution of about $0.6\times0.6\times5$ mm^3.

5.2. Salting

Salting with sodium chloride is one of the oldest methods of food preservation. Sodium chloride is also an essential ingredient in processed meat products since it helps solubilise meat proteins, which affects the binding and texture of final meat products. Increased sodium intake by certain sensitive individuals, however, has been associated with some diseases. For these reasons public health authorities have advocated reducing sodium intake.

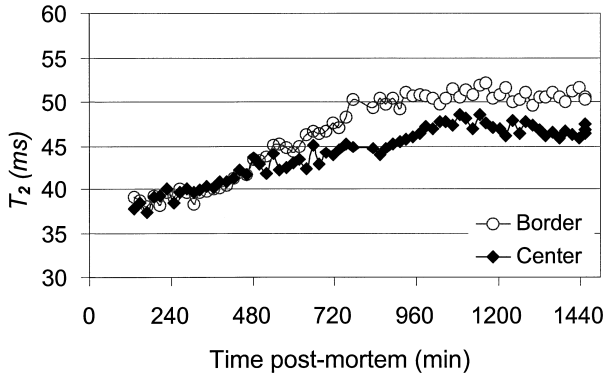

Fig. 7. T_2 curves measured in the regions of interest represented on Fig. 6.

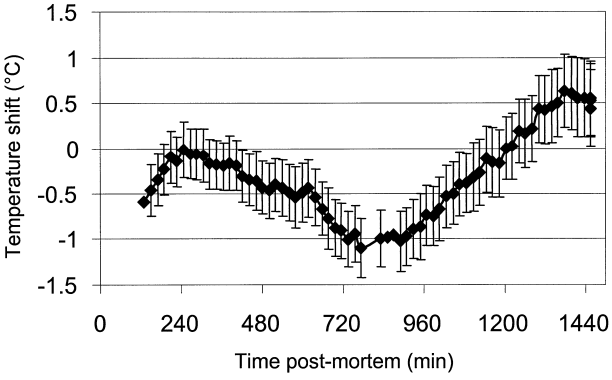

Fig. 8. Temperature shift curve measured in both regions of interest represented on Fig. 6.

Little work has been reported on ^{23}Na imaging in biological systems because of their low sodium concentrations. However, the sodium concentration in cured meat is massively increased and so the Na^+ in brine can serve as a marker. ^{23}Na-MRI, which allows Na^+ concentration mapping in the tissue, is of interest for the analysis of meat treated with NaCl brine. Some results have already been obtained in meat (Renou et al., 1994) for following brine distribution in ham according to the technological process. Tumbling was found to increase the homogeneity of salt distribution, but only cooking afforded full homogenisation of brine in ham.

6. Conclusion

Food science has shown little interest in MRS and MRI, which has remained essentially confined to research activities despite many potential applications: process optimisation of animal production (animal diet,

genetic type), consumer satisfaction, public health, and as a reference tool for the calibration of other techniques.

MRS gives non-invasive access to structural, metabolic and chemical information. Fatty acid chains are studied with ^{13}C-MRS, while ^{31}P-MRS focuses on phosphorylated metabolites. Complementarily, ^{1}H-MRI provides spatial information on water dynamics and fat distribution in tissues, and is used to study body composition, adipose tissue distribution, connective tissue and muscle fibre type non-invasively. The low relative NMR sensitivity of other nuclei limits its domain to ^{1}H-MRI. MRI associates morphological images with parametric images, which allows accurate parameter quantification. It also permits to relate NMR parameters to physical characteristics (temperature, ions). Lastly, NMR opens new ways of research to optimise technological processes. These examples show the potential of NMR spectroscopy and imaging techniques applied to food science.

Acknowledgements

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