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Feasibility of using low field nuclear magnetic resonance (LF-NMR) to measure meat properties on-line

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Executive summary

- There is evidence that NMR can measure changes in water compartments in meat associated with rigor, water binding and cooking. Such changes are important also for NIR measurements of meat tenderness, suggesting the detailed work already undertaken for NIR will underpin NMR studies.

- Tenderisation of meat also involves changes in cytoskeletal proteins and other water compartments.

- Examination of existing literature shows that there is a change in NMR over time in concert with factors potentially related to tenderness.

- Existing bench top units are impracticable.

- The development of new devices by magritek that allow meat to be placed on top of the device may enable changes associated with tenderness and other meat properties to be determined. These units consist of a flat bed design as well as a mouse unit.

- There needs to be a detailed examination of the various NMR parameters in terms of noise, time of acquisition and preparation of the meat in order to obtain known and defined tenderness levels and other meat property relationships.

- The analysis is complex and an NMR study on meat properties cannot be undertaken without an integrated understanding of chemometrics, NMR procedures and meat science.

- Assuming that the NMR study indicates that there is potential for the development of a successful NMR meat measurement system, it will require considerable financial investment and involvement of the meat industry for its acceptance

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1 Possibilities of measurement of meat properties using LF-NMR

There is a strong possibility of using nuclear magnetic resonance (NMR) to measure meat quality attributes on-line in various processing situations based on early published work. However, it is the development of an appropriate device that can be adapted to commercial situations that is the enabling technology.

Sophisticated High-Field NMR units using liquid nitrogen and superconducting magnets may well show the changes in meat but are impractical in a commercial situation (Figure 9), but could be useful in analysing the spectra for interpreting the chemical changes that occur. Bench-top Low-Field NMR (LF-NMR) units (Figure 8) have been used to measure certain aspects of meat quality. LF-NMR has been accepted in Sweden as a standard method for total fat analysis in meat and meat products since 1985 (Croon *et al.*, 1985). Measurement of other meat parameters have been investigated in a purely experimental way and extrapolation suggests there are other properties that can be measured. The greatest contribution to further measurement possibilities is the development of new enabling NMR technology by magritek[1] (see Figures 10, 11, 12).

2 Mechanisms and definitions

Nuclear magnetic resonance (NMR) is a physical phenomenon based upon the magnetic property of an atom's nucleus. All nuclei that contain odd numbers of nucleons and some that contain even numbers of nucleons have an intrinsic magnetic moment. The most often-used nuclei are hydrogen-1 and carbon-13, although certain isotopes of many other elements nuclei can also be observed. NMR studies a magnetic nucleus, like that of a hydrogen atom (protium being the most receptive isotope at natural abundance) by aligning it with a very powerful external magnetic field and perturbing this alignment using an electromagnetic field. The response to the field by perturbing is what is exploited in nuclear magnetic resonance spectroscopy (high-field NMR) and magnetic resonance imaging (MRI) as well as nuclear magnetic relaxometry (low-field NMR).

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about a molecule. It is the only technique that can provide detailed information on the exact three-dimensional structure of biological molecules in solution. Also, NMR is one of the techniques that has been used to build elementary quantum computers.
Low-field NMR (LF-NMR) does not give as detailed information as NMR spectroscopy about molecular structure. Yet following the relaxation processes of the pertubed nuclei gives valuable information about the state of the nuclei and thereby about the physical/chemical state of the surrounding sample matrix. The process called population relaxation refers to nuclei that return to the thermodynamic state in the magnet. This process is also called T1 relaxation, where T1 refers to the mean time for an individual nucleus to return to its equilibrium state. Once the population is relaxed, it can be probed again, since it is in the initial state.

The precessing nuclei can also fall out of alignment with each other (returning the net magnetization vector to a nonprecessing field) and stop producing a signal. This is called T2 relaxation. It is possible to be in this state and not have the population difference required to give a net magnetization vector at its thermodynamic state. Because of this, T1 is always larger (slower) than T2. This happens because some of the spins were flipped by the pulse and will remain so until they have undergone population relaxation. In practice, the T2 time is the life time of the observed NMR signal, the free induction decay. In the NMR spectrum, meaning the

[1] http://www.magritek.com/

Fourier transform of the free induction decay, the T2 time defines the width of the NMR signal. Thus, a nucleus having a large T2 time gives rise to a sharp signal, whereas nuclei with shorter T2 times give rise to more broad signals. The length of T1 and T2 is closely related to molecular motion.

3 Low-field NMR related to meat - to date

The concept of measuring meat ageing using LF-NMR is not new and there has been one paper by Wahlgren and Tornberg (1996) that peripherally involved an author from this work, Carrick Devine. There was a subsequent paper reviewing the changes relating to water pre rigor by Tornberg, Wahlgren, Brøndum and Engelsen (2000) where they looked at meat properties both pre and post rigor with LF-NMR. This and other studies showed promise and have a strong bearing on anything we might do for measurement of meat properties. A PhD thesis on the application of NMR in meat science was published by Hanne Bertram (2002), who has continued to work in this area. LF-NMR has been used for determining water compartments (Bertram *et al*., 2002; Bertram, *et al.* 2004a) and cooking (Bertram, *et al.,* 2004b). A good correlation between LF-NMR and water holding capacity was found (Bertram, *et al.,* 2001). Several other meat properties were studied as well as pre- and post rigor changes. Elisabeth Micklander, an author in this work, studied changes in meat during cooking (Micklander, *et al.,* 2002) as well as changes in pre-rigor meat (Micklander, *et al.,* 2005). There have also been studies in rabbit meat (Capozzi *et al.*, 1999; Bertram, *et al.,* 2003).
There are some issues that need to be clarified. Although a bench top unit (e.g. Figure. 8) has been shown to measure changes in meat related to tenderness, one must realise that there are large obstacles for the use of such a unit in a commercial system because only small pieces of meat can be placed in the centre of the magnet. The developments by magritek allow the placement of meat (or other samples) onto the top of the sensing unit (Figure 10), thus enabling the measurement of larger samples eventually eliminating the need for cutting a sample out. There is also the development of an NMR mouse that can be applied to the surface of samples (Figures 11 & 12). Other magritek units use the earth's magnetic field (Figure 7).

Even though these new developments are available, it is unclear whether they will be capable of measuring meat properties. However, some early studies of these bench top units can be examined and extrapolated to show whether measurements using other more portable instruments mentioned above are possible – they both have similar field strengths. There is also the difficulty in transfer of the calibrations from bench top to other units, so the use of the new devices needs to be examined as an entirely separate study.

Dr Andrew Coy of magritek indicated that it is not simple to predict what configurations need to be used and there is a considerable amount of work required to determine various relaxation times, or whether parameters related to diffusion or mobility are the most appropriate, especially when issues of signal to noise arise. In other words, a preliminary study really isn't cost effective or feasible and a full scale study is necessary to examine what mode the devices need to operate and how the various configurations can dissect out the attributes related to meat properties. It is also clear that an NMR study on meat properties cannot be undertaken without an integrated understanding of chemometrics, NMR procedures and meat science.

4 Examination of specific results from desk top NMR units to see whether there is information that can be used to measure meat properties.

Tornberg *et al.* (2000) investigated two chilling rates and three electrical stimulation treatments that gave differing rates of pH fall, and as a consequence gave different tenderising scenarios (Figure 1). The use of the various chilling rates and other processing conditions was based on studies by Wahlgren *et al.* (1997) which included author Carrick Devine. The varied processing conditions resulted in different levels of tenderness development and allowed these changes over time to be observed. It is highly likely the NMR is measuring something related to tenderness.

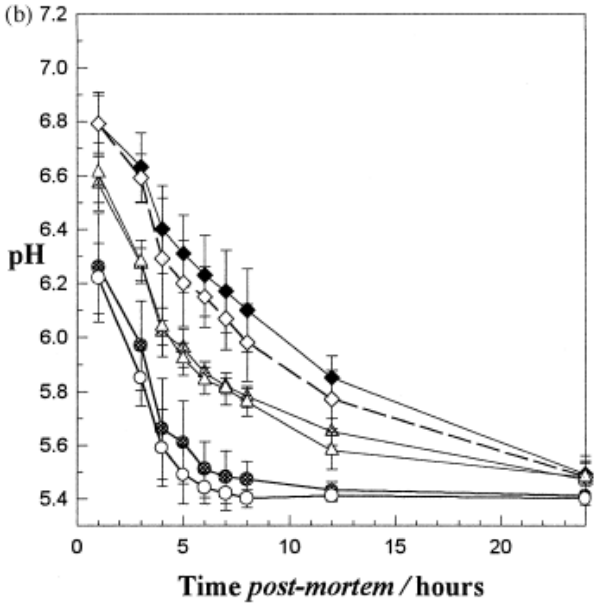

Figure 1.
The pH-fall as a function of time post-mortem for the fast (○,●) medium (Δ,▲) and slow (□,■) pH-courses. Open symbols are slow chilling (20°C, 5 h) and filled symbols fast chilling (12°C, 5 h). From Tornberg *et al.* (2000).

The changes shown in Fig. 1 are a consequence of several processing conditions that involve a fast chill (two regimes) three types of electrical stimulation. The figure shows that the pH falls vary under the different regimes, this leads to a difference in the rate and extent of meat tenderisation Wahlgren *et al*. (1997). Now tenderness (shear force) effectively starts at rigor (approximately 6 h after slaughter for electrical stimulation and 24 h without electrical stimulation). These effects on the rate of tenderisation would in turn be expected to be reflected in the NMR changes. These are shown in the Figure 2.

4.1 Changes in transverse relaxation time post mortem

Transverse relaxation times versus time post mortem are shown in Figure 2. Rigor mortis occurs at approximately 0.2 days post mortem. Tenderisation starts at rigor mortis and NMR T_{22} changes can be correlated with tenderness from that moment on.

The fact that the different processing scenarios give different rates of tenderness development and T_{22} changes (Fig. 2), suggests that LF-NMR measures something related to tenderness and it is sensitive to processing conditions.

Some studies on pre-rigor meat have been undertaken by Micklander *et al.* (2002). In this study the changes as a result of high ultimate pH induced by adrenalin injection compared to stressed animals were examined. There was a clear effect on T_2 times (Figure 3) it is possible therefore that NMR may be used to monitor elevated ultimate pH meat.

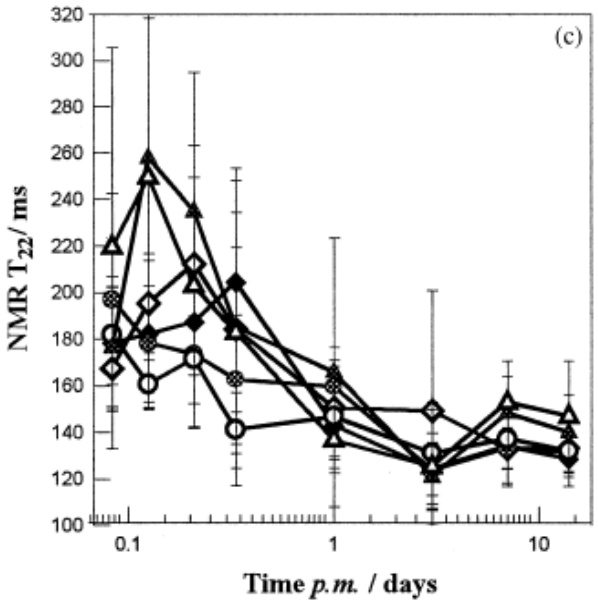

Figure 2. Change in T_{22} relaxation time constant as a function of time post-mortem (log-scale) for the fast (○,●) medium (Δ,▲) and slow (□,■) pH-courses. Open symbols are slow chilling (20°C, 5 h) and filled symbols fast chilling (12°C, 5 h). From Tornberg *et al.* (2000).

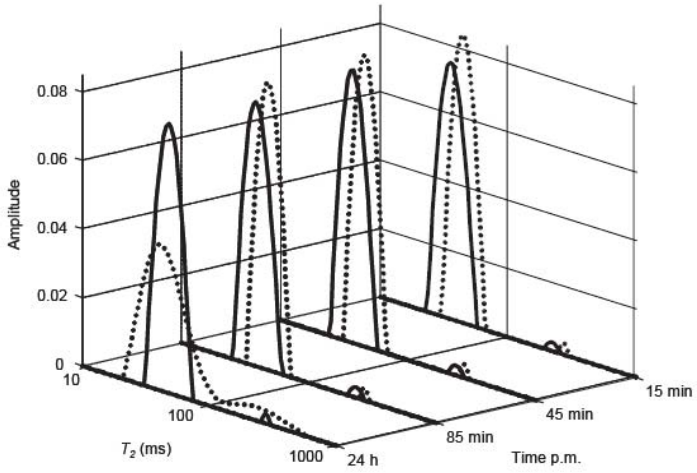

Figure 3. Distribution of T_2 relaxation times at 15 min, 45 min, 85 min and 24 h pm, estimated by distributed exponential fitting of CPMG curves obtained on samples from an adrenaline pig (solid line) and a stressed pig (dotted lines). From Micklander *et al.*, 2002.

There are other studies that show there are significant changes in meat properties related to tenderness (Bertram, *et al*., 2004b) (Figure 4).

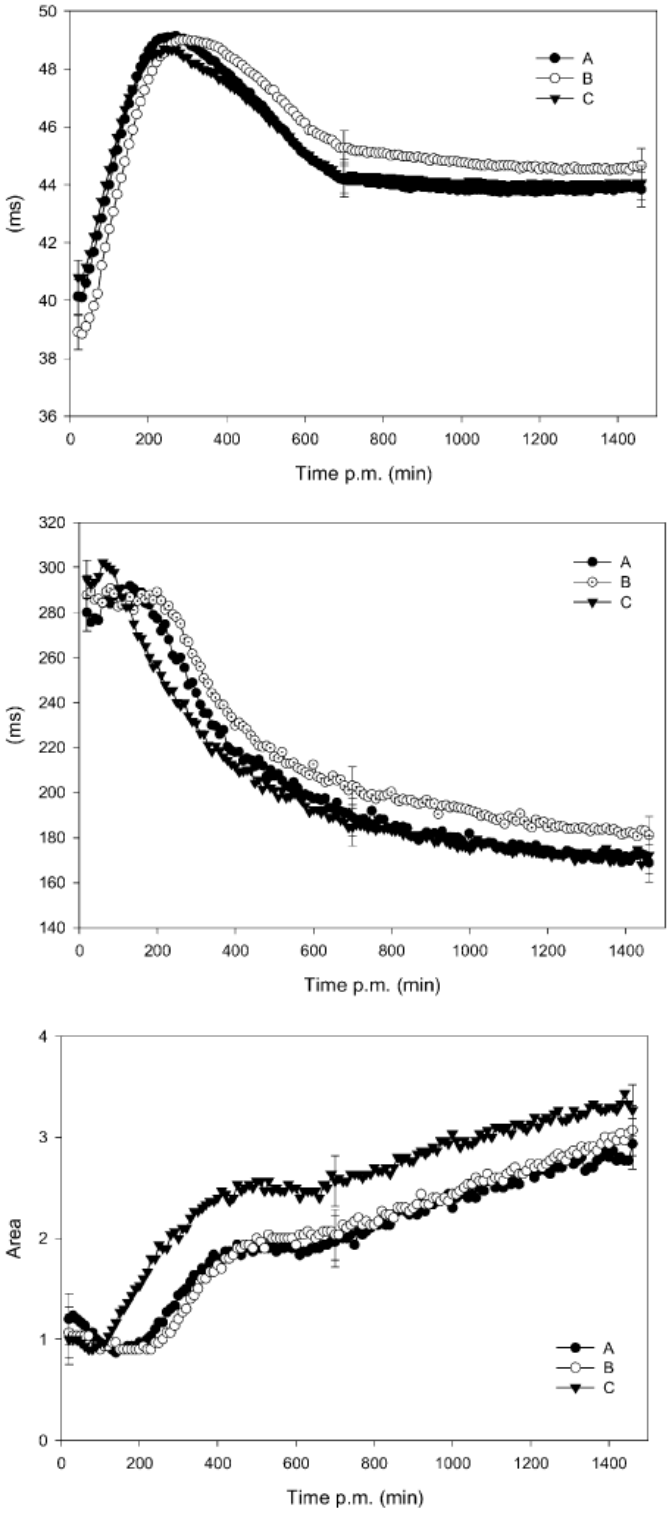

Figure 4.
Further examples of the time course in meat from pigs from Bertram *et al.* (2004b) study. Post mortem progress of T_{21} (top figure) T_{22} (middle figure) time constants and T_{22} (bottom figure) populations for pigs with different stunning treatments (A, B, C).

4.2 Changes in concentration of water pools post mortem

Concentration of intra-myofibrillar water (P_{21}) vs. time post mortem, also from Tornberg *et al*. (2000), is shown in Figure 5.

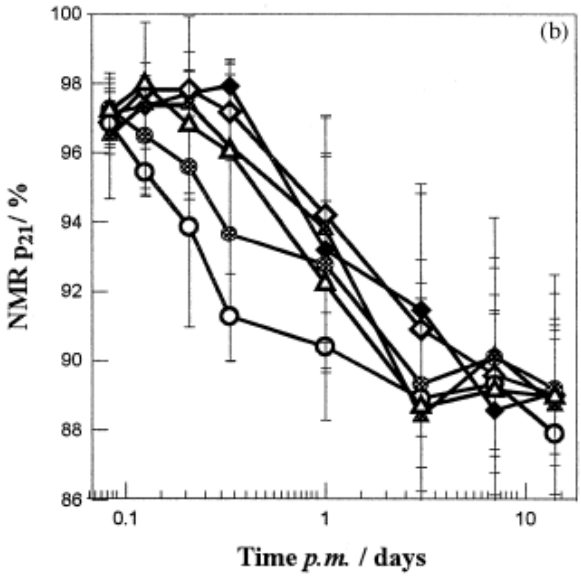

Figure 5.
Change in P_{21} (concentration of intra-myofibrillar water) as a function of time post-mortem (log-scale) for the fast (○,●) medium (Δ,▲) and slow (□,■) pH-courses. Open symbols are slow chilling (20°C, 5 h) and filled symbols fast chilling (12°C, 5 h). From Tornberg *et al.* (2000).

The fact that P_{21} values vary with different tenderising situations further suggests that we are measuring changes related to tenderness. It is possible that these changes are related to protein denaturation. If so, it may be possible to relate these to tenderness knowing that as meat increases in tenderness, drip also increases.

5 NMR analyses are complex!

The NMR analysis is not simple. The established method for analysing nuclear magnetic resonance relaxation decay profiles is exponential curve fitting, resolving the decay into a number of mono-exponential functions equal to the number of resolved factors. The curve fitting is performed by minimising the squares of the residuals. However, this is not trivial, one of the problems being that exponential fitting involving a high number of exponential functions is ill posed in a mathematical sense. The linearization approximation in multi-exponential fitting algorithms has severe problems with the non-linear exponential functions, and the solutions found may not be unique. Typically discrete and distributed exponential fitting is combined with chemometric data analysis to retrieve all information from data. While chemometrics gives overview and is the best approach for calibrating regression models for prediction of parameters, exponential fitting aids interpretability. Number of exponential components, relaxation times and concentrations of each of the components, which are estimated with exponential fitting, give qualitative and quantitative information about the system.

A brilliant idea was put forward by Windig and Antalek (1999) when studying first order reaction kinetics by high-resolution NMR. This method called Direct Exponential Curve Resolution Algorithm (DECRA) takes advantage of the fact that exponential decay functions (time´intensity), when translated in time, retain their characteristic relaxation times while only their relative amounts or concentrations change. By such simple translations (slicing) it is possible to create a new “pseudo” direction in the relaxation data (time´intensity´slice) and thus facilitate application of trilinear (multiway) data-analytical methods, which in turn provide mathematically unique recovery of the underlying T_2 components.

6 Comparison of NMR with NIR work

There have been recent studies using near infrared spectroscopy that showed one can use this technology to measure meat properties. However, NIR is a correlative technology and this means that extensive calibration has to be used and there are difficulties of transference from one instrument to another in the case of diode array systems (needed for rapid data capture). Much of the present work gone into understanding what NIR is actually measuring provides a background that is of relevance to NMR. We believe that we are measuring changes in the cytoskeletal or structural proteins.

When meat tenderises, the free water (as measured by centrifugation) starts from a base level at *rigor mortis*. The base level is most likely that coming from denaturation of the actomyosin components. From this point the free water increases as the meat tenderises – at the same time the bound water decreases and this most likely comes from the cytoskeletal components (Devine, *et al*., 2004) (Figure 6).

For NMR and NIR, therefore, we are most likely measuring water compartment changes in the meat - it might be possible to separate the various sites with appropriate data analysis, i.e., a contractile protein compartment and a structural protein compartment to give valuable information on various aspects of meat quality by examining drip and tenderness as two separate components (Wahlgren, *et al*., 1997). In other words, while the total final water comes from two different sites, the protein changes responsible are independent. Meat is not very homogeneous with variability being a hallmark of meat quality measurements and the variability within a given piece of meat as well as variability between various cuts needing to be established (Devine, *et al*., 2006).

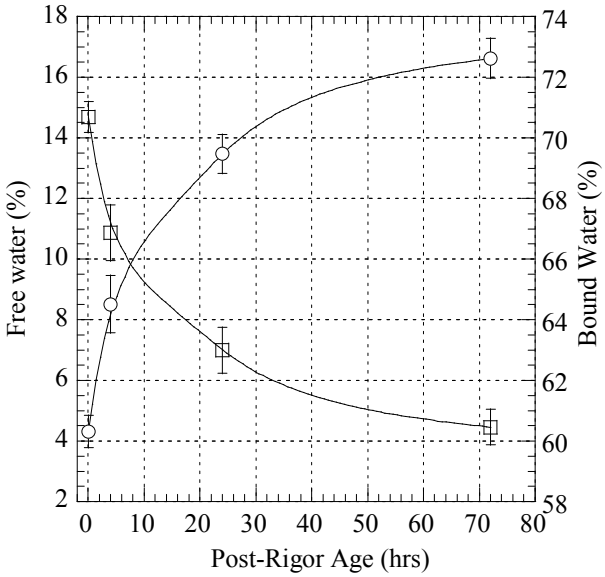

Figure 6.
Mean percentages of free (o) and bound (•) water plotted against post-rigor age. Error bars represent ± 1 standard error of the means. Now the free water is a consequence of the water released during the rigor process PLUS the water that is released during tenderisation. The bound water becomes less as the meat tenderises. These changes are significant and could affect both NIR spectra and NMR signals.

7 Types of NMR equipment available

Figure 7.
Terranova unit by magritek. This unit uses the earths magnetic field and may be useful for preliminary studies - in its present form, it would not be suitable for wide scale meat measurement because of the small size of the bore.

Figure 8.
Bruker minispec is a laboratory instrument and clearly not suitable for on-line purposes. It was a similar instrument to this that has been used in the studies analysed above.

Figure 9.
High Field NMR device with superconducting magnets. It is great for detailed studies but impractical for large meat samples

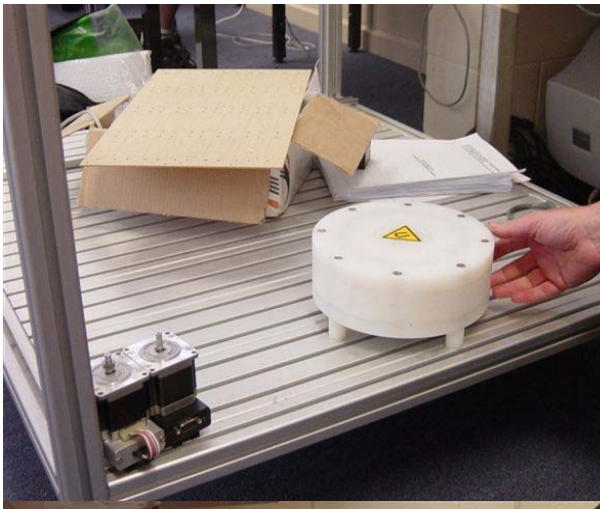

Figure 10.
One of the magritek developments. The field is effectively at the top of the device. This configuration could be used on a table where material moves over it, or could be applied robotically to a carcass or cut.

Figure 11.
NMR mouse. Field is at the surface. In other words this could be applied to meat cuts.
NMR-MOUSE® is a Registered Trademark of RWTH-Aachen. Because of a different configuration, the data acquired from this instrument is different to the unit described above. The most important difference being that the strength of the magnetic field is significantly lower.
A hand-held NMR sensor, the **MO**bile **U**niversal **S**urface **E**xplorer or the NMR-MOUSE® allows measurement of NMR relaxation and diffusion parameters in surface-near volume elements of arbitrarily large objects.

Figure 12.
Electronics for NMR mouse.

8 Conclusions

There is evidence that NMR can measure changes in water compartments in meat associated with water binding and cooking. Such changes are important also for NIR measurements of meat tenderness, suggesting the detailed work already undertaken for NIR will underpin NMR studies.
Tenderisation of meat also involves changes in cytoskeletal proteins and other water compartments.

Examination of existing literature shows that there is a change in NMR over time in concert with factors potentially related to tenderness.

Existing bench top units are impracticable.
The development of new devices by magritek that allow meat to be placed on top of the device will enable changes associated with tenderness and other meat properties to be determined. These devices consist of a flat bed design as well as a mouse unit.

There needs to be a detailed examination of the various NMR parameters in terms of noise, time of acquisition and preparation of the meat in order to obtain known and defined tenderness levels and other meat property relationships.

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Assuming that the NMR study indicates that there is potential for the development of a successful NMR meat tenderness system, it will require considerable financial investment and involvement of the meat industry for its acceptance.

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