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Develop on-line VSA/NIR & NMR measurement technologies for beef and sheep meat.

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Abstract

A long term vision for the red meat industry should include capturing in real time, information relevant to quality and production efficiency when converting muscle to meat (farm through to market). This is a key requirement for any all encompassing meat quality program and essential for providing feedback to maximise production efficiencies.

Fundamentally new measurement technologies are now being researched which may have application for online measurement at various points in the supply chain and the possibilities in this area is expected to grow. New developments which should be investigated include:-

1) Near Infrared (NIR) spectroscopy : Near Infrared (NIR) spectroscopy is a non-destructive and non-invasive measurement technology. Many studies have shown that NIR readings can be calibrated against accepted meat quality attribute measures; however, a key problem that has restricted the use of NIR in industry has been the inability to transfer calibrations. The KES instrument used in this work has the potential to transfer calibrations between machines.

2) New lower cost alternatives in NMR : Nuclear magnetic resonance (NMR) technology has been used to study red meat quality primarily in research applications. Development of one-sided low field (LF) NMR instruments by the New Zealand company Magritek offers the possibility that LF-NMR technology could be suitable for commercial “on-line” applications. This milestone reports on the ability of a Magritek LF-NMR MOUSE (Mobile Universal Surface Explorer) to measure meat tenderness. In addition, a Halbach LF-NMR instrument was tested on a limited number of samples.

The current program evaluates NIR and new NMR technologies as predictors of meat tenderness and other meat quality attributes. Part of this program will also develop the NIR hardware specifically for application to the meat industry (for example probe design, aperture size, number of reading).

While there are encouraging results for NIR technology as a predictor of pH and other quality traits, ongoing validation is required. NMR technology is undergoing rapid evolution in what could be a viable hand held tool for use in on-line applications. Improvements by Magritek to address temperature control and some other issues with LF-NMR equipment are currently proposed for the next phase of research.

Contents

1 Background

On line quality measurements are a critical component of the MQST objectives. Near Infrared spectroscopy has shown considerable promise as a technology to measure meat quality: the scientific literature has shown significant prediction of tenderness, fatty acid composition of fat and water binding capacity using this method. Recently, an objective within the MQST program has demonstrated that NIR can measure muscle glycogen content. NIR is therefore an important component to the programme objectives.

Near Infrared (NIR) spectroscopy is a non-destructive and non-invasive measurement technology. Many studies have shown that NIR readings can be calibrated against accepted meat quality attribute measures; however, a key problem that has restricted the use of NIR in industry has been the inability to transfer calibrations.

The following previously published articles were focused on the direct correlation of the Warner-Bratzler shear force (WBSF) tests against NIR measurements for beef: Mitsumoto *et al*. (1991), Næs and Hildrum (1997), Byrne *et al*. (1998), Park *et al*. (1998), Rødbotten *et al*. (2000), Freudenreich and Augustini (2001), Park *et al*. (2001), Rødbotten *et al*. (2001), Venel *et al*. (2001), Westad *et al*. (2001), Leroy *et al*. (2003), and Liu *et al*. (2003). Most of these studies were carried out on post-rigor meat. Typically the samples were cut out, placed in a cuvette, and scanned in a traditional NIR spectrometer. All the studies measured meat surfaces cut in a right angle to the fibre direction. A number of the studies showed that it is possible to predict the ultimate meat tenderness (with limited success) by looking at samples relatively early (usually 24 hrs) post mortem [Byrne *et al*. (1998), Freudenreich and Augustini (2001), Rødbotten *et al*. (2001), Shackelford *et al*. (2005)].

Rødbotten *et al*. (2000) also attempted to predict ultimate tenderness by pre-rigor measurements with limited success. Shackelford *et al*. (2005) obtained similar results when attempting to predict the shear force at 14 days from an NIR measurement taken 24 hours post mortem. A regression model with an R^2 of 0.22 was obtained. The results of these studies are unsurprising especially because the handling of the meat (both pre and post rigor) affects its ultimate tenderness to a very large extent.

Almost all the studies listed above indicate that spectra of tough meat have a higher absorption of light over the full spectral range (approx. 400 to 2500 nm). This could be due to different amounts of light scatter in meats with different fibre structures. Therefore, it is surprising that the correlations are still relatively low. For this reason, five of the studies (Næs and Hildrum (1997), Park *et al*. (1998), Freudenreich and Augustini (2001), Rødbotten *et al*. (2001), Liu *et al*. (2003)) also looked at how well the meat samples were classified into two or three tenderness groups. Næs and Hildrum (1997) have a good description of useful classification methods. This seems to be a much more successful approach.

Finally, most studies mainly focused on the spectral region below 1800 nm (even below 1100 nm) without any obvious difference in the results. This indicates that the long wavelength region is not very useful for tenderness determination. This is in line with the observation that tenderness is mainly detected by the amount of light scatter (which only has a limited wavelength sensitivity) and that it is important to get a representative spectrum of the meat sample (shorter wavelengths penetrate deeper into the meat surface).

The objective of this research was to investigate the ability for NIR spectra to measure the tenderness of meat aged to different tenderness levels. Hence, this milestone focuses on linking post-rigor NIR measurements with post-rigor tenderness measurements. A subsequent milestone will focus on forecasting meat aging rates from pre-rigor NIR measurements.

The current program will evaluate Near Infrared (NIR) and new low-field nuclear magnetic reasonance (NMR) technologies as predictors of meat tenderness and other meat quality attributes. Part of this program will also develop the NIR hardware specifically for application to the meat industry (for example probe design, aperture size, number of reading).

Critical to the success of the research is the demonstration of a pathway to commercialisation, for which an MLA/MNZ contribution was identified. The contribution will involve integration of the NIR measurements with the modelling concepts under development.

2 Project Objectives

The objectives of the research were :

- Report on the ability of NIR to predict tenderness and ageing rates when measured pre-rigor.
- Preliminary evaluation of NMR to measure meat quality attributes
- Evaluation of Nuclear Magnetic Resonance (NMR) for on-line detection of meat quality attributes.

The expected outcome of the research was development of i) NIR technological platform for measuring meat quality attributes and ii) use of new low-field nuclear magnetic reasonance (NMR) technologies as predictors of meat tenderness and other meat quality attributes.

3 Materials & Methods

3.1 The ability of NIR to predict tenderness of meat aged for different levels of tenderness.

A trial aimed at linking post-rigor NIR and tenderness measurements was performed on beef. During this trial, 90 muscles (*m. longissimus et lumborum*) from 45 beef animals (two muscles per animal) were removed 30 minutes after slaughter from steers shot with a captive bolt. In order to generate a range of quality outcomes, the muscles were processed and stored with varying treatments (i.e. some were wrapped to prevent shortening, different rigor temperatures were used, and some were electrically stimulated). The 90 muscles from 45 animals were organised into nine kill groups and Table 1 outlines the treatments for each kill group.

Table 1. Treatments for each kill group*.

Kill group	Sample numbers	Rigor temperature	Electrically stimulated?
1	101-110	15°C	No
2	111-120	35°C	No
3	121-130	35°C	Yes
4	131-140	15°C	Yes
5	141-150	5°C	No
6	151-160	15°C	No
7	161-170	35°C	Yes
8	171-180	35°C	No
9	181-190	15°C	Yes

*All muscles with an even sample number (half the samples in every kill group) were wrapped in polyethylene to prevent shortening.

After rigor was reached, all meat was aged at 15°C. For calibration purposes the following data was collected:

i) Pre-rigor

- NIR and pH readings every two hours until rigor was reached.

ii) Post-rigor

- NIR, pH and shear force readings at rigor and at six time points after rigor (nominally 7, 16, 30, 54, 60 and 80 hours after rigor was reached). Given the natural variability in time to rigor between samples, readings were not always taken at these exact time points, however the number of hours post-rigor was noted for each reading.

For each NIR reading, a freshly cut surface was created and measured. In order to measure the entire freshly cut surface, the meat surface was scanned twenty times by the KES NIR spectrophotometer equipped with a fibre optic probe (Figure 1).

Immediately after NIR scanning, the pH of the samples was measured. Then, for post-rigor samples, these were immediately frozen. The frozen samples were subsequently cooked in bags to an end-point temperature of 75°C in a 100°C water bath. From each sample, ten sub-samples with a 10 x 10 mm square cross section at right angles to the fibre direction were prepared. Each sub-sample was sheared using a MIRINZ tenderometer and the peak shear force was noted. The mean of these peak shear force values was calculated for each of the samples for comparison against the NIR readings.

All NIR spectra were standardised against the light source, an air measurement, and a white calibration tile measured at the beginning of each time point. The 20 individual scans for each meat sample at each time point were compared by the use of two PCA (principle component analysis) components and Mahalanobis distance. The five most extreme spectra were discarded. All remaining spectra were averaged and used in the subsequent data analysis.

Figure 1. Meat being measured with KES NIR spectrophotometer.

PLS calibrations were developed for pH and shear force for all time points where data was available. The full wavelength range (576-1695 nm, where the signal-to-noise ratio is reasonable) was used for the calibration. For various reasons the data set for kill group 1 was not complete and it has therefore been omitted from the data analysis.

The following pre-processing methods were tested:

- No pre-processing
- Standard Normal Variate (SNV)
- Multiplicative Signal Correction (MSC)
- 1st and 2nd order detrend
- 1st and 2nd order derivative (by use of the Savitsky-Golay algorithm)

The models were validated using both cross validation and independent test set validation with the following splitting of the data sets:

- Two thirds of the 80 samples (all time points for each sample) were used for calibration with cross validation for finding the optimal number of PLS factors.
- The remaining third of the samples (all time points for each sample) were used for independent validation of the resulting model.
- The calibration and independent validation samples were selected systematically according to sample number in order to ensure that all kill groups were equally represented in both data sets.

3.2 Preliminary evaluation of NMR to measure meat quality attributes.

This milestone reports on the ability of a Magritek LF-NMR MOUSE (Mobile Universal Surface Explorer) to measure meat tenderness. In addition, a Halbach LF-NMR instrument was tested on a limited number of samples.

A two-factorial experiment was designed using electrical stimulation and wrapping to create a sample set of lamb loins (n = 24) that varied in meat tenderness between samples and over time after slaughter. Data on shear force measured day 1, day 2 and day 3 post-slaughter were compared to LF-NMR data. Measurement repeatability using the Magritek MOUSE was poor and, due to the low signal to noise ratio, prediction of different shear force values caused by treatment differences or ageing was not possible. However, from the measurements on the Halbach NMR instrument shear force could be predicted at the three specific time points (day 1, day 2 and day 3 post slaughter). It should be noted that the sample set used for the Halbach measurements was small (n = 4).

It is suggested that the results on the Halbach NMR instrument are repeated on a larger sample set to verify the promising result obtained. Furthermore, the signal to noise ratios of the NMR-MOLE and the Halbach NMR instrument are comparable. This indicates that the NMR-MOLE may be suitable for prediction of meat quality attributes if the previously reported issues of temperature instability are resolved (refer to Appendix 1).

3.3 Evaluation of NMR for on-line detection of meat quality attributes.

A trial was conducted to evaluate the ability of a Magritek open-topped LF-NMR device to measure key meat quality attributes. Such a trial cannot be undertaken without an integrated understanding of chemometrics, NMR procedures and meat science. Therefore, a collaborative team of meat scientists was formed with additional assistance Magritek technicians to set up the LF-NMR device and assist in data interpretation.

The left and right loins of 70 lambs that had undergone different processing treatments (to purposely create sample differences) were removed and held at 15°C. Measurements over a range of meat quality attributes including pH, glycogen concentration, water holding capacity and tenderness were taken over the pre-rigor and ageing periods and these data were compared to LF-NMR readings.

4 Results

4.1 The ability of NIR to predict tenderness of meat aged for different levels of tenderness.

The repeatability of the reference method for pH measurements was 0.17. The repeatability of individual shear force measurements (tenderometer) was 1.9 kgF, but since the reference shear force values used here are averages of ten measurements the expected error associated with the shear force references is approximately 0.6 kgF.

A preliminary study showed that cold shortened and DFD meat produced different signatures in the NIR spectrum, hence for the shear force predictions, all spectra from kill group 5 (samples 141-150) were removed because most of these samples showed signs of cold shortening. Additionally, all samples with a pH profile indicating DFD meat (samples 119, 120, 121, 122, 177, and 178) were removed.

The results from the pre-processing method that produced the lowest prediction error (RMSEP) are shown in Table 2. For pH, the Multiplicative Signal Correction (MSC) pre-processing method gave the best results. For shear force predictions, pre-processing did not improve prediction error, so no pre-processing was applied. The accuracy of the pH calibration is almost on the same level as the repeatability of the pH reference measurement.

Therefore, the NIR measurement may be used for monitoring the pH of meat (both pre- and post-rigor). The accuracy of the shear force calibration was not as good as the shear force test but similar to other works in the field. The value of the shear force results lies in the fact that the calibration appears to be transferable across the different data sets that were collected at different times.

Table 2. Summary of data and results from the trial.

Property	# of samples	Range	Calibration samples			Independent samples	
			#PLS factors	R^2	RMSEP	R^2	RMSEP
pH	785	5.15 - 7.17	9	0.82	0.21	0.79	0.22
Shear force	533	1.9 - 27.0 kgF	11	0.65	2.64	0.60	2.97

Figures 2 and 3 show the calibration and independent prediction results against the reference methods for pH and shear force.

According to the results, cold shortened and DFD muscles cannot be handled by the same calibration models as “normal” meat. This is may not be a problem, since DFD samples can be detected by using the pH prediction and preliminary investigations suggest that cold shortened meat may be identified by the use of temperature and/or rigor calibrations. Once identified by these methods, it is likely that the shear force of DFD and cold shortened meat could be predicted using alternative NIR calibrations.

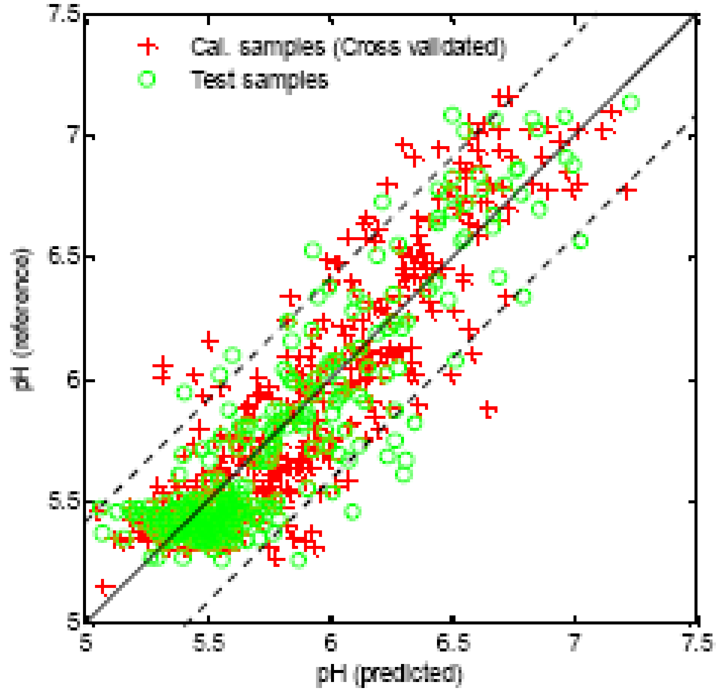

Figure 2. Comparison of predicted (NIR) and measured pH.

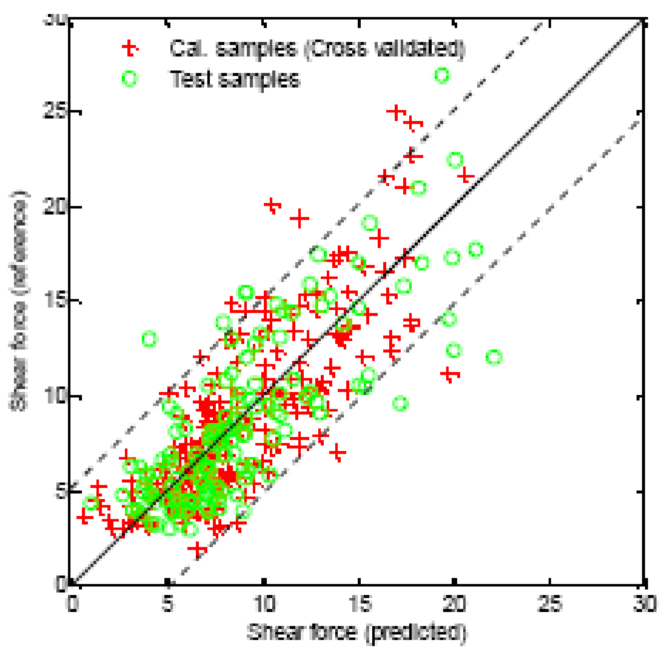

Figure 3. Comparison of predicted (NIR) and measured shear force.

Further work will focus on linking the ageing rates of meat to pre-rigor NIR measurements.

4.2 Preliminary evaluation of NMR to measure meat quality attributes.

- There is evidence that low field nuclear magnetic resonance (LF-NMR) can measure changes in water compartments in meat associated with rigor, water binding and cooking. Such changes are important also for near infrared (NIR) measurements of meat tenderness, suggesting the detailed work already undertaken for NIR will underpin LF-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 LF-NMR over time in concert with factors potentially related to tenderness.
- Existing bench top units are impracticable.
- The development of new LF-NMR 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 LF-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 LF-NMR study indicates that there is potential for the development of a successful LF-NMR meat measurement system, it will require considerable financial investment and involvement of the meat industry for its acceptance.
- A trial of the ability of Magritek LF-NMR devices to measure key meat quality attributes is proposed in collaboration between AgResearch MIRINZ and Murdoch University.

4.3 Evaluation of NMR for on-line detection of meat quality attributes.

The literature shows evidence that low field nuclear magnetic resonance (LF-NMR) can measure changes in water compartments in meat associated with rigor, water binding and cooking. For example, LF-NMR benchtop units can measure changes in cytoskeletal proteins and other water compartments that are associated with tenderness and other meat quality attributes.

However, existing benchtop LF-NMR instruments are impractical for use in a meat processing environment as they would require samples to be removed from the product and analysed. The development of an open-topped LF-NMR instrument by the New Zealand company Magritek offers the possibility that LF-NMR technology could be used in a commercial “on line” application on whole carcasses or cuts.

As expected there were significant differences between samples and over time for the measured meat quality attributes. This confirmed that the experimental design was suitable for testing the LF-NMR instrument. Correlations between T2 relaxation times and meat quality data were poor due to the noise apparent in the LF-NMR readings. Evidence suggested that the noise was likely to be caused by temperature fluctuations. However, averaging the data from all samples to reduce noise yielded a trend in T2 relaxation time over time, in particular giving a drop in T2 near rigor.

This is consistent with previously published data on benchtop instruments. The conclusion was made that refinement of the Magritek open-topped low field instrument is required before further work should be undertaken for an “on line” meat quality measurement application.

5 Impact to Industry

The knowledge gained from the determination of proof of concept tenderness measurements using the NIR will provide the opportunity to manage quality and predict product performance in the market, while also being used as a method to provide robust data which can be fed back to farmers as a decision support tool.

Critical to the success of the research is the demonstration of a pathway to commercialisation, for which an MLA/MNZ contribution was identified. The contribution will involve integration of the NIR measurements with the modelling concepts under development.

6 Conclusion

This is an ongoing project to develop and use on-line NIR equipment to assist in the management of meat quality during processing, to predict product performance in the market and to provide robust data which can be fed back to farmers and used in decision support. Recent work has focused on measuring the tenderness of post-rigor beef and predicting the ageing rate of pre-rigor beef. During these trials it was shown that NIR can also measure the pH of pre- and post-rigor beef to the same level of accuracy as the reference method (a pH meter).

The direct calibration studies of data from the time point trial have shown :

- pH can be predicted pre and post rigor by NIR with an accuracy similar to the error of the pH meter. The best results were obtained when the spectra were not log transformed prior to calibration.
- It was possible to establish a direct model between NIR and shear force. The accuracy is similar to other works in the field. The shear force model does not cover cold shortened and DFD meat, alternative calibration models would need to be developed for these circumstances.
- The number of unexplained outliers was generally very small, indicating that the NIR data set is good.
- Further work will focus on linking the ageing rates of meat to pre-rigor NIR measurements.

Low field nuclear magnetic resonance (LF-NMR) has the potential to manage quality during processing and predict product performance in the market as well as providing robust data which can be fed back to farmers and used in decision support. There is evidence in the literature that LF-NMR can measure fat levels in meat and changes in water compartments in lean tissue associated with rigor, water binding and cooking. This indicates potential to measure key meat quality attributes using LF-NMR. However, existing LF-NMR bench top units are impracticable for on-line use in meat plants.

7 Recommendations

6.1 On-line NIR measurement

The next phase of research proposed will aim to develop calibrations against glycogen content in pre-rigor muscles in order to predict the ultimate pH of hot-boned beef, an early indicator of meat quality. NIR has the potential to be taken on-line and would represent a cheaper and potentially automated alternative to existing ultimate pH predictors (such as the AgResearch MIRINZ 'Rapid pH' laboratory method). The outcome will be to develop correlations against the glycogen content and pH in pre-rigor beef muscles at various temperatures using the proof-of-concept NIR spectrophotometer developed previously. Test the ability of the correlations to predict the ultimate pH of beef muscles measured pre-rigor in a commercial situation.

6.2 On-line LF-NMR measurement

Improvements by Magritek to address temperature control and some other issues with LF-NMR equipment are currently proposed for the next phase of research. The proposed approach of future work is to firstly evaluate the improvements to the Magritek LF-NMR device to ensure that it copes with temperature fluctuations and that the new signal to noise ratio is high enough to identify differences between meat samples and over time. Secondly, we will test the ability of the improved device to measure key meat quality attributes including pH, tenderness, glycogen content, water holding capacity and intramuscular fat content. Thirdly, the feasibility of the Magritek device as a potential on-line measurement system in a meat processing environment will be assessed, particularly in regard to which attributes the system can measure, how accurately they can be measured, the required measurement time, and the practicality of use in meat plants.
The development of new LF-NMR devices by Magritek that allow meat to be placed on top of the device may enable on-line measurement of meat properties. Current work, carried out alongside Magritek, is focusing on examination of the Magritek LF-NMR devices to test whether key meat quality attributes show measurable responses.

Assuming that the current LF-NMR study indicates that Magritek devices can measure key quality attributes, further work will be required to develop accurate measures for specific attributes. This work is proposed for the next phase of research.

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Appendix –

Some Types of NMR equipment available.

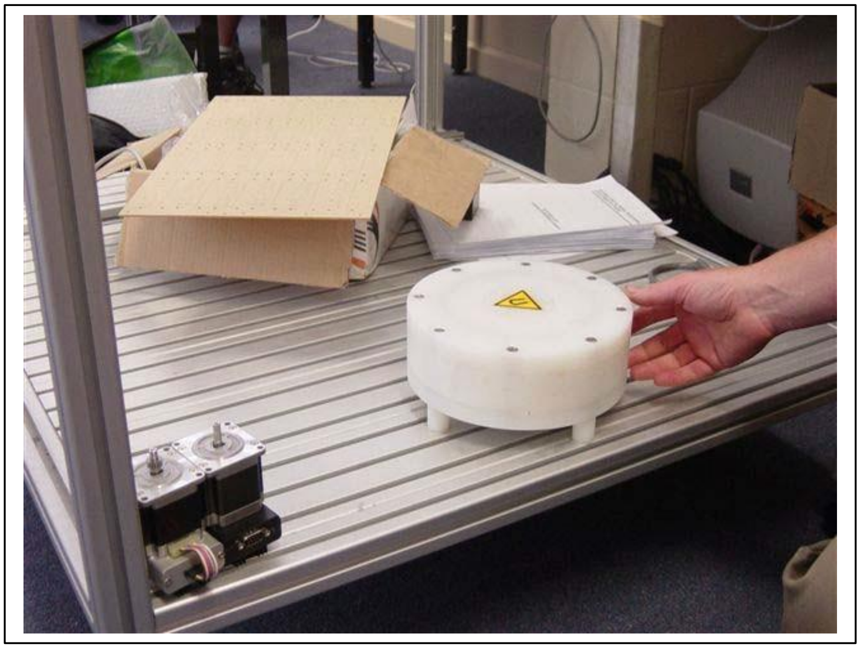

Figure A1 - Magritek development. Field is at some distance from the device. This could be used on a table where material moves over it, or could be applied robotically to a carcass

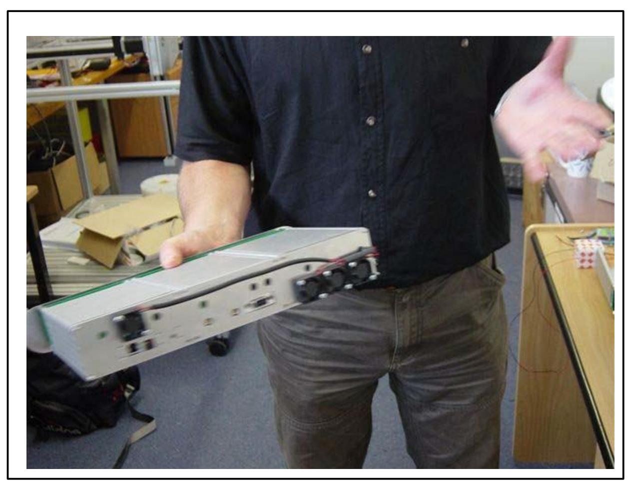

Figure A2a - NMR mouse. Field is at the surface. In other words this could be applied to meat cuts. **Figure 2b** - Electronics for NMR mouse. The size of this suggests a universal acceptability of a mouse concept. I think that the electronics for other devices can be small also

Figure A3 – High resolution NMR