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Evaluation of eating quality attributes measured by TD-NMR

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Abstract

In this report we summarise the experimental results obtained in this study, and review the strengths and weaknesses of the NMR time-domain instrument and measurements as a tool for predicting quality. The primary strengths are: (1) the measurement is non-destructive, non-invasive, and non-contact, meaning that a grading cut need not be made in the carcase for measurement, and also that potentially carcase surface fat layer thickness could be determined with the same tool as part of an automated process; (2) the measurement can be rapid and hence amenable to automation for application on the process-chain; (3) the magnetic field strength employed in the measurement is low, and can be generated by permanent magnets; through good design there should be little concern over safety in application; (5) the NMR hardware is robust, the electronics is compact, suitable for an industrial environment and potentially being mobile; (5) relaxometry provides mechanistic information on the structural organisation of water in muscle/meat, which is strongly related to important meat quality traits. For example the so-called transverse relaxation time is a tool for measuring the changes in water distribution in muscles post mortem and it can be used to track the water transfer processes leading to rigor mortis; indeed, the mechanistic understanding of the NMR data provides strong incentive for its use in the direct determination of relevant meat quality parameters. (5) The method has benefits over other methods such as near-infra-red and Raman spectroscopy in that NMR is a bulk, as opposed to surface, measurement and does not require a freshly prepared meat surface.

Executive summary

The hypothesis of this project was that a simple time-domain nuclear magnetic resonance measurement could be used to provide indictors of several meat eating qualities, such as fat and moisture content, cooking loss and shear force etc. Milestone 1 focussed on a literature review concluding that NMR was a very promising technique for rapid and non-destructive determination of beef properties. In particular, NMR is able to distinguish different populations of water in meat samples, as well as being able to provide excellent measures of fat content; any property related to the water populations (drip loss for example) could therefore be measured and/or predicted with an NMR measurement.

Milestone 2 presented initial data and corresponding linear models built for the prediction of the various properties, with excellent results for fat and water content, drip loss, cooking loss (though with low sample number) and shear force. The sample pH could also be predicted, but only for pH below about 5.8 using a linear model, although a separate model for high pH samples might be possible. In Milestone 3 the dataset was expanded, retaining excellent predictive capability for fat (r=0.93) and moisture content (r=0.88) of the meat, as well as drip loss (r=0.79); the pH was also reasonably well predicted but over a limited range (pH<5.8). The shear force results were less convincing with the larger data set, possibly due to factors such as seasonal, breed and handling variations. The NMR tenderness model were not able to predict the very tough samples, but with those excluded, a cross-validated predictive correlation of r=0.58 was achieved which, in the context of the reported performance of other types of sensors trialled on meat, is still very good. Restricted to Angus samples, a linear model correlation of 0.95 was observed in Milestone 2, though with insufficient samples to test and cross-validate a predictive model. All in all these are very encouraging results.

In this report we summarise the experimental results obtained in this study, and review the strengths and weaknesses of the NMR time-domain instrument and measurements as a tool for predicting quality. The primary strengths are: (1) the measurement is non-destructive, non-invasive, and non-contact, meaning that a grading cut need not be made in the carcase for measurement, and also that potentially carcase surface fat layer thickness could be determined with the same tool as part of an automated process; (2) the measurement can be rapid and hence amenable to automation for application on the process-chain; (3) the magnetic field strength employed in the measurement is low, and can be generated by permanent magnets; through good design there should be little concern over safety in application; (5) the NMR hardware is robust, the electronics is compact, suitable for an industrial environment and potentially being mobile; (5) relaxometry provides mechanistic information on the structural organisation of water in muscle/meat, which is strongly related to important meat quality traits. For example the so-called transverse relaxation time is a tool for measuring the changes in water distribution in muscles post mortem and it can be used to track the water transfer processes leading to rigor mortis; indeed, the mechanistic understanding of the NMR data provides strong incentive for its use in the direct determination of relevant meat quality parameters. (5) The method has benefits over other methods such as near-infra-red and Raman spectroscopy in that NMR is a bulk, as opposed to surface, measurement and does not require a freshly prepared meat surface.

Disadvantages include that the NMR technique is at an earlier stage of evolution in terms of industrial application: there are only a limited number of reports of in-line NMR systems, and NMR has seen minimal use in industrial conditions generally; designing for appropriate IP (ingress protection) ratings would be necessary for example but certainly within the capacity of local expertise. A suitable magnet for on-line application would need to be developed, in which a sampling location can be controlled and provided with adequate field uniformity for the NMR measurement to be viable. Also the attendant RF electronics and software would need to be tailored to the application, but again all these issues are within the scope of expertise accessible to the current project team. The magnet system itself, being based on permanent magnets, is temperature sensitive, but this can be controlled, as can stray field from the magnet through appropriate system design.

Possible locations within a meat plant have been considered, with attendant footprint and facility requirements. Through feedback from external groups two potentially useful locations options have been identified, (1) directly before the chiller and soon after the slaughterboard, where meat would be measured pre-rigor, and (2) in the chiller where carcases could be graded in much the same way as manual MSA grading is currently performed. Ideally an in-line grading instrument would enable product classification, possible upgrading and filtering, and could also allow for feedback to producers, all bringing economic benefit although these aspects have not been investigated in this study (see for example North *et al*, 2008).

The report concludes with a discussion of the pathway to commercialisation of the technology; a pragmatic path from here includes running parallel tasks in two areas: (1) Maximising the outcomes from the existing benchtop equipment by extending the study to sensory trials (for example within the ALMTech programme) and by comparing with other measurement technologies such as NIR, Raman and hyperspectral imaging. (2) Completing paper-based design studies for the magnet (single sided versus donut-style, field strength, uniformity and magnet weight), console electronics (simplified for a single targeted measurement) and (3) in-plant design (environmental control and possible robotic automation). This information would substantiate a business case that could be pitched to an industrial partner for a project that builds a prototype.

In summary, NMR results achieved so far provide a strong foundation for undertaking further work in developing the technique into a valuable, potentially on-line, grading tool for the meat industry. Suitably designed, such an instrument can be robust, fast, and non-contact, bringing benefit both from the grading classification itself as well as through avoidance of a grading cut.

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1 Introduction

In this project, we have investigated correlations between NMR signals[1] and beef properties – fat content, moisture content, drip loss, cooking loss, pH and instrumental shear force. The objective was to determine whether time-domain NMR could be used to non-destructively provide indicators of the key beef properties. If successful this method could potentially replace several traditional chemical analysis and instrumental methods, which are time consuming and expensive (Pereira et al.).

The work of Pearce et al. suggested that meat-eating properties are strongly related to populations of water and fat within the meat. Time-domain NMR (or NMR – relaxometry) can be used to determine fat and moisture contents with high accuracy (Appendix 2), and is able to distinguish different populations of water within the meat, specifically: water bound to macromolecules such as protein, water located in highly organized protein structure (myofibrils) such as actin and myosin; and loosely bound water / free water (extra-myofibrils).

In our Milestone 2 report, analysis from initial measurements for the model-development phase of the project, showed very good predictive capability for total fat, total moisture and drip loss, and lesser (but still significant) correlations for pH below about 5.8, tenderness and cooking loss (at that stage with very limited sample numbers).

For the Milestone 3 report the dataset was expanded, retaining excellent predictive capability for fat and moisture content of the meat, as well as drip loss; the pH was also reasonably well predicted but over the limited range observed in Milestone 2. The shear force results were less convincing with the larger data set, possibly due to factors such as seasonal, breed and handling variations since the data collection was carried out over a period of several months, however in the context of other instrumental measurements on meat, the tenderness result would still be regarded as satisfactory. The work reported here included samples that were measured two days post mortem.

In this concluding report we review the results and look at the factors impacting on possible industrial application of the technology. We also outline the further work required to get relaxometry to a robust solution.

The significant number of, what we and others have shown, statistically significant correlations between relaxometry data and meat attributes warrants extension of these physiochemical parameters to sensory trials, in line with the work conducted by Pereira et al.

2 Summary results

The Milestone reports 2 and 3 cover the data and statistical analysis work conducted in this project. Here we limit commentary to summary remarks and areas of exception.

2.1 Prediction of meat quality parameters with NMR

Key results from Milestone 2 are presented in Table 1 below. Features of the work reported were:

[1] Appendix 1 provides information on the NMR technique.

- Approximately 50% of the beef samples were Angus.
- Samples were measured within 2 & 4 days post mortem. Ultimately we limited measurements to 2 days post-mortem as the shear force correlation degraded with increased time post mortem, and the day-2 data was expected to be more representative of results measured in the abattoir.
- A full range of 10 NMR parameters (T_1 longitudinal and T_2 transverse) were examined for statistical correlation with meat physicochemical measures.
- Excellent correlations were obtained.

Table 1 Correlations reported from Milestone 2.

Parameter	Predictive Correlation	NMR fit components employed
Fat content	r = 0.82	p_{2f}
Moisture content	r = 0.85	p_{2f}
Drip loss	r = 0.77	T_{2f}, p_{21},T_{21}, T_{22}
Cooking loss	r = -0.72 (linear correlation)	T_{22}
Tenderness (Shear force)	r = 0.81	T_{2f} and p_{22}
pH	r = 0.81	p_{2f}, T_{2f}, T_{21} and T_{22}

Key results from Milestone 3 are presented in Table 2 below. The full range of NMR parameters continued to be examined for statistical correlation with meat eating quality. Key differences from the work in Milestone 2 included:

- Dataset extended: additional samples were primarily non-Angus and included bull-beef and cow-beef of indeterminate breed, age and with varying weight.
- Additional seasonal variation possible with the extended measurement campaign.

Table 2 Correlations reported from the larger dataset of Milestone 3.

Beef property	Number of samples	T_2 variables	Linear model correlation	Prediction correlation	Prediction rmsep	Beef Property Measurement Mean Error
Total fat content	25	p_{2f}, T_{2f}	0.946	0.934	0.938 %	0.2 %
Total moisture content	21	p_{2f}, p_{21}, T_{21}	0.907	0.884	0.914 %	0.6 %
Drip loss	61	T_{2f}, T_{21}, p_{22}, T_{22}	0.790	0.786	0.120 g	0.01 g
Cooking loss	57	p_{2f}, T_{2f}	0.427	0.414	1.814 %	0.1 %
pH	172	p_{2f}, T_{21}, p_{22}, T_{22}	0.649	0.656	0.045	0.05
Shear force (linear model)	26	T_{2f}, p_{22}	0.333	0.169	1.971 kg	0.9 kg
Shear force (2nd order model)	26	T_{2f}, p_{22}	0.394	0.249	1.907 kg	0.9 kg

2.1.1 Fat content, moisture, drip

The results are statistically highly significant for total fat content, moisture, drip, but not for cooking loss. These results are obtained with linear models (correlations with linear combinations of T_2 variables) and are sufficient to make accurate predictions.

NMR is recognised as an excellent tool for moisture and fat quantification, so the strong predictive correlation results in these categories are not unexpected. In this context the result for cooking loss is disappointing however we cannot rule out experimental error introduced by the cooking method, and also through the small sample sizes employed in this case.

2.1.2 pH

For both pH and shear force it may be that non-linear models are required. The high pH (>5.9) data appear to belong to a different linear model and once removed, the linear correlations against NMR signal components improve significantly. It remains a possibility that a separate model could be developed for high pH samples but there were insufficient data in the present study to pursue this path.

As muscle converts to meat, the pH drops. The pH of normal muscle at slaughter is about 7.0 but this will decrease post mortem. In a normal animal, the ultimate pH falls to around pH 5.8-5.4. The degree of reduction of muscle pH after slaughter has a significant effect on the quality of the resulting meat. The rate of decrease in pH varies and is subject to other parameters such as animal stress, when the animal last ate, temperature change and electrical stimulation etc. (e.g. see figure 1 below). Notably, the path to the ultimate pH, and the ultimate pH level itself vary. At least with linear models it seems relaxometry can only be correlated over the higher-acid range.

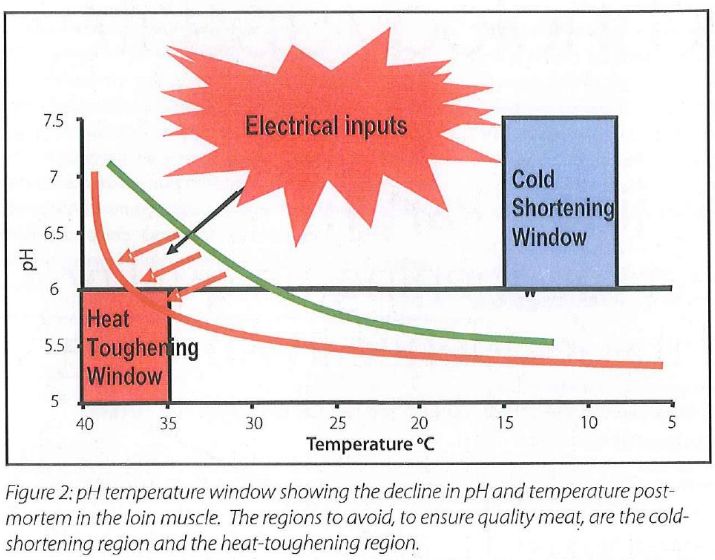

Figure 1 pH-temperature window for loin muscle post-mortem (from Meat Technology Update 2/11 – March 2011, MLA).

Building a more sophisticated model for pH prediction will need further work and is beyond the scope of this report.

2.1.3 Shear

Both the best linear model and the 2nd order polynomial model failed to predict shear force particularly well. A more detailed investigation with higher order models produced a model with correlation of 0.56 (where this model also included an Angus breed descriptor) but it was clear that there were high-shear-force data points not captured by the model as shown in figure 2 below. Excluding the two high-force samples yielded a model correlation of 0.69 (again with the Angus descriptor included), and a cross-validated predictive model correlation of 0.59.

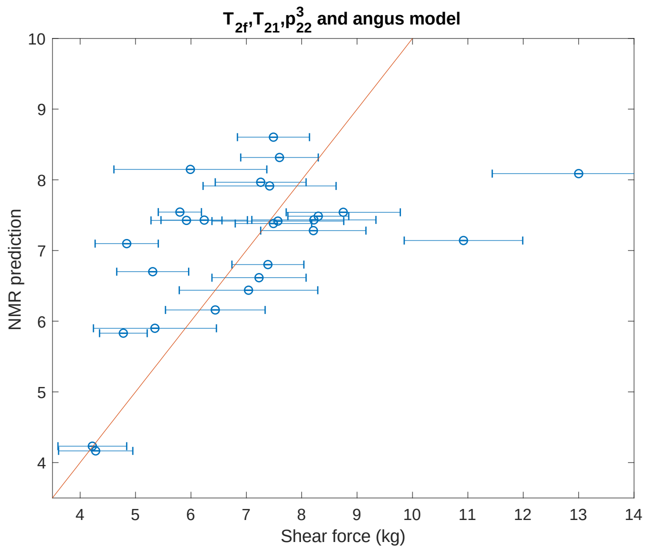

Figure 2 Higher order model for shear force, using Angus descriptor. The high shear force values are not captured.

One possible reason for the lower prediction accuracy is that there was a large seasonal variation in the beef samples because they were collected over the course of five months, and also included variation in animal breed and sex. Further, the tenderness measurement in the present study was carried out at day-7 post-mortem, whereas ultimate tenderness is known not to be achieved routinely until about day-21 post mortem (many tenderness measurements are in fact taken at day 21 post-mortem).

As noted in the Milestone 2 report, Angus beef seemed to belong to a separate dataset, which had high correlation with NMR data (r=0.95, albeit with limit dataset size). It is possible that different shear force models are required for categories based on beef type and collection seasons.

It would be useful to collect NMR data in parallel with tasting panel trials in order to investigate correlations with consumer tenderness rankings, since the relationship between consumer preference in tenderness with instrumental shear force may not necessarily be linear, as noted for example in a CSIRO report (, http://www.publish.csiro.au/an/Fulltext/ea07174) (figure 3 below) on the impact of hormone growth promotants on tenderness. The interesting point is the apparent roll-off of consumer ability to distinguish tenderness at the “tough” end of the scale, similar to the NMR response.

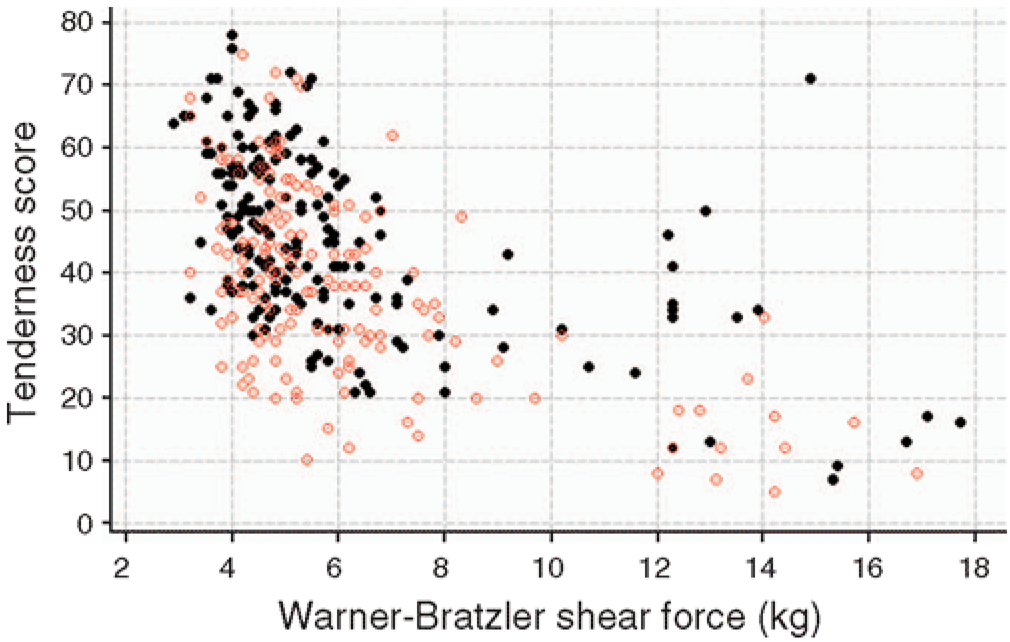

Figure 3 Consumer tenderness score against instrumental shear force (from CSIRO study on impact of hormone growth promotants; black points: natural, pink: with HGP treatment).

As noted in the literature, there are discrepancies between various studies as to correlations between tenderness and the different water populations. For example Tornberg (2000) reported the most tender beef samples were those with larger extra-cellular volumes (T_{22}, p_{22}), whereas Pearce et al (2011) reported that a high amount of intra-myofibrillar water and low extra myofibrillar) may be associated with more tender meat from a study on lamb loin. However in reviewing similar work they note inconsistencies in the relationship between water populations and mobility and tenderness across species and within breeds, which could reflect differences in intramuscular fat or glycogen content. In the present study, the results are more in line with those of Tornberg, where the more tender meat is associated with higher populations of extra-myofibrillar water.

3 Relaxometry – strengths and weaknesses

3.1 Strengths

3.1.1 Mechanistic information

The application of Time Domain NMR or relaxometry to meat parameters has been reported in the literature with correlations similar to and better than those reported here.

Relaxometry has been proven to be an excellent technique for obtaining knowledge of the structural organisation of water in muscle/meat, which is highly related to important meat quality traits. This has provided many mechanistic explanations behind the correlations. We have not entered into discussion on the mechanistic attributes that have been shown to explain the correlations. Others report on this, where conclusions/discussion includes:

- The T_{22} time constant provides a direct measure of the amount of mobile water that can be expected to be potentially lost in drip loss. This is consistent with the observation of water channels between fibres and fibre bundles by microscopy (Bertram 2001a). This is also consistent with experimental observation in the present study where a linear correlation coefficient of 0.75 was found between drip loss and T_{22}, although we produced a slightly better model correlation by including also T_{2f}, T_{21}, p_{22} components (r=0.79).
- Correlations with T_{21} and sarcomere length have been demonstrated and are suggestive of spatial distances in the myofilamentous matrix (Bertram 2002).
- T_2 relaxation is a tool for measuring the changes in water distribution in muscles post mortem and it can be used to track the water transfer processes leading to rigor mortis. (Bertram 2001b).
- It has been shown that T_2 relaxation provides information about structural features in meat including:
  - X-ray studies have demonstrated that pH affects the myofilamentary lattice due to electrostatic repulsion and there is a demonstrated relationship between myofilament lattice and the T_{21} time constant. Thus, a correlation between the T_{21} time constant and pH should be expected. In the current work we found the strongest correlation of pH was with T_{22}, along with the relative populations p_{21} and p_{22}.
  - The T_2 relaxation is not characterised by a single relaxation but rather a continuous spectrum of relaxation times, and that denaturation of proteins result in a broader distribution of relaxation times.

Although in our work we have simply focused on correlations, the mechanistic nature of the measures being made strengthens the argument for the use of relaxometry as a tool for measuring attributes of meat.

3.1.2 Rapid Measurements

Through the duration of this work we have refined our measurement method.

We progressively resolved and confirmed through statistical analysis the best correlations with attributes of the relaxometry measurements. We showed that the best correlations sit with the T_2 (transverse) relaxation measurement. This was consistent with the findings of others.

Notably, of the two standard time-domain NMR measurements (giving longitudinal and transverse spin relaxation times, T_1 and T_2 respectively), a complete T_1 measurement would take of order 7 minutes, which is perhaps problematic in a high through-put environment, but the T_2 measurement could be completed in 10-20 s. A measurement of this duration opens the door to automated in-line testing.

3.1.3 Non-destructive, non-invasive, non-contact

In this work we took samples of meat and put them into the magnet with a specialised holder. This approach would be equivalent to using a benchtop instrument and would be regarded as a minimally invasive approach. Such an approach may provide an interim step on the path to commercialisation (see later discussion). Ultimately however it is feasible that a bespoke instrument be designed to 'project' the magnetic field into the meat such that the measurement would be non-contact.

A potential spillover benefit of 'projecting' the magnet field is that as a 'sensing probe' is moved toward the carcase the depth of fat could be ascertained.

Others have designed and tested prototype 'projected' field devices for assessing meat attributes. However, to project 30 mm below the animal hide (in work on live animals) a very large magnet was required; i.e., 43 kg. We have the opportunity to improve on this by using the expertise of our extended team. Our extended team includes magnet and NMR instrument designers.

A non-contact method has the benefit that a grading cut to carcases could potentially be eliminated. This is a particularly attractive when considering automated measurement and processing of carcases. The grading cut can cause distortions to the carcase such that the carcase is no longer acceptable for automated processing.

A further benefit of a non-contact method is removal of the chance of contamination.

3.1.4 Low magnetic field strength

Our work was conducted at a magnetic field strength of 0.29 T. Others have used similar field strengths (e.g., 0.23 T, Pereria). Field strength and weight of the magnet go hand in hand, the higher the field strength typically the heavier the magnet. Being able to conduct measurements at low field strength will be an advantage to design aspects of any instrument. With that said however there will be other magnetic design considerations, and NMR signal to noise ratio increases with field strength. Work will be required to ensure an optimisation of field strength, weight, field homogeneity and signal to noise (see also later discussion).

3.1.5 Benefits over existing/other techniques

Some other authors (Bertram 2004) have gone so far as to suggest that relaxometry may well be a better technique than some existing measures"

> *"With regard to relaxation, it can be concluded that the technique has proven successful in determination of WHC (water holding capacity) in fresh meat, and promising results with regard to other meat eating quality traits involving water properties such as cooking loss and technological quality are presently achieved. A problem when assessing a new method for determination of WHC is that it can only be compared with existing methods, and accordingly a better result than the inter-correlation coefficient of this method can never be obtained….The fact that LF-NMR relaxometry gives superior information compared with existing methods must be considered a strong argument in favour of this method in the characterisation of water-holding."*

Other techniques in use include Infrared spectroscopy and, new to the application, hyperspectral cameras. All are surface analysis methods requiring examination of a cut surface. In addition, infrared requires significant calibration.

Considering whether relaxometry could be used before the chiller and soon after the slaughterboard, one point of note is that Tornberg (2000) conducted pre-rigor studies comparing relaxometry with NIR, finding that relaxometry was able to resolve very early post-mortem events whereas NIR was not. Another consideration in working in working at this location in an abattoir is the temperature; the pre-rigor relaxometry work by Tornberg was from 35 to 5°C, so it's fair to assume temperature of the carcase will not be an issue[2].

3.2 Weaknesses

3.2.1 Stage of technology evolution

NMR is extremely advanced for high-end laboratory applications but is less advanced in terms of industrial applications. Some reference points include:

- Single sided NMR has been used industrially for many years for measuring the likes of water content in concrete.
- Benchtop instruments, spectroscopic NMR and relaxometry are now commonplace laboratory tools. There are numerous suppliers of benchtop instruments e.g., Bruker, Magritek, Oxford Instruments, Minispec. These are now commonly used for relaxometry measurements of fat and water in food products. The systems are reasonably priced subject to features. Note, the benchtop relaxometry equipment used for Milestones 2 and 3 included a bespoke magnet and an off the shelf Kea console (from Magritek). We used this equipment in our workshop and crated it to conduct work at a second lab facility. The

[2] In the work reported in Milestones 2 and 3, samples were thermalised in a water bath at 16°C prior to NMR measurement, and temperature increase over the measurement time was typically within 1-2 degrees.

system shipped well and was set up within hours of being unpacked and with minimal calibration. Thus the equipment is reasonably robust in this form factor.
- Extensive high-end laboratory grade instruments; with an associated high price-point.
- NMR mobile surface device (also called NMR-mouse) in food analysis indicates that further development of the hardware is required to make an on-line method a reality.
- There are however only a limited number of reports of in-line NMR systems. On line monitoring of a paste, which does not require spatial resolution and at-line monitoring of fat content in meat emulsions has been demonstrated as has on-line stop and flow analysis of seeds.

As NMR has seen minimal use in industrial conditions, designing for appropriate IP (ingress protection) ratings would be necessary. These same requirements would have to be considered with the magnet.

3.2.2 Design considerations

Bertram (2004) has completed a review on the use of NMR, both relaxometry and spectroscopy, for meat analysis. He formed a very favourable view on the technology but noted that further work was required on the hardware to make on-line methods a reality, including:

- The construction of appropriate magnets, equipment for signal excitation and rapid methods for data collection and analysis.
- Magnets that can polarise the sampling location within an appropriate scale and with satisfactory magnet homogeneity

Progressing this work requires experienced magnet and instrument designers. Note, the benchtop console unit used for our work in Milestones 2 and 3 has significant flexibility designed into it. This would not be required for the proposed specific application to meat. Therefore, we believe that we can design an instrument with an acceptable price point.

3.2.3 Magnets in meat works

Temperature control of the magnet will be important. The magnet we used has been operated in the Antarctic, so the low temperatures in abattoir chillers is not a problem, the temperature of the magnet however is best kept at a stable temperature approximately 5°C warmer than ambient. To ensure accurate measurements the magnet would need to have a period of equilibration after initial turn-on so this would need to be factored into the operating regime. This will have to be a design consideration.

Magnets in an environment where knives are used may be seen as a concern. Any stray magnetic field can however be managed and or designed for, e.g. through the use of an exclusion zone with guarding and/or ferromagnetic yokes that contain the magnetic field.

Both of these matters are manageable through good design.

3.2.4 Calibration

Periodically the system would need to be calibrated. This can be easily conducted by using a reference sample e.g., vegetable oil or water samples or similar.

4 Next Steps

In this work it has been shown that there are several statistically significant correlations between relaxometry measurements and parameters known to impact meat eating quality i.e., water, fat, drip loss. In addition, for pH values less than 5.8 there was a significant correlation with pH showing that relaxometry could track the conversion of muscle to meat. The weakest correlations were with tenderness where at best we had a correlation of 0.59.

The correlations were all generated with the meat at 16°C. We know they will work equally well at the temperatures in a chiller and we are optimistic, based on the work of others, that measurements could be made pre-rigor.

These correlations combined with the fact that the there is potential to:

- automate,
- make a non-contact measurement,
- include a measure of fat depth,
- conduct pre-rigor

make relaxometry an attractive potential tool for meat property measurements. Therefore we believe there is a strong case for progressing to an industrial tool.

We believe a pragmatic path from here includes running parallel tasks in two areas:

1. Maximise the outcomes from existing benchtop equipment,
2. Complete paper-based design studies.

This information would substantiate the build of a prototype.

4.1 Maximise the outcomes from bespoke benchtop equipment

4.1.1 Sensory properties

The work reported here focused on physiochemical properties. It would be good to extend this to sensory trials, for example those run within ALMTech programmes. It would be good to include relaxometry as a measurement tool in such work, including undertaking measurements pre-rigour and in the chiller.

4.1.2 Comparison with other methods

From a parallel programme we expect to have analyses that compare relaxometry with Hyperspectral imaging, Raman and Near Infrared spectroscopies. In addition the strengths and weaknesses of these methods will also be known.

4.2 Design studies

The following paper-based design steps would be required:

4.2.1 Design a magnet

Complete a paper-based design study using modelling software. Design considerations would include: Single-sided versus solenoidal, magnetic field homogeneity, strength to project the magnetic field below the fat layer, sufficient signal to noise and a manageable weight.

Our extended team has significant experience in the design of specialist magnets.

Two options of form factor would be considered in the design of the magnet, either:

- a single sided or unilateral magnet that can be attached on a robot end effector controlled by a vision system, or
- a donut magnet design that partly encircles the carcase where it is likely the carcase would have to be brought to the magnet.

The needs of automation will be considered along with magnet specifications and cost to implement, to select which of these options is most practical.

4.2.2 Design a console

Using the knowledge gained from this work, a simplified console that conducts a specific T_2 relaxation measurement using a CPMG pulse sequence (described in the Milestone 2 report) would be required.

The Magritek console used in this work to generate the RF signal, control the pulse sequence and collect the response, is designed as an experimental instrument with an array of test options. A simpler, specific console would be needed for an industrial device. Also, designing in robustness for an industrial application, e.g. electrical noise management, water ingress etc., would also need consideration.

Software for data analysis would be needed such that the raw relaxometry data would be fitted to three exponential functions to obtain time constants and populations. In this work, data analysis was conducted using simple in-house developed software. The extension of the analysis using algorithms that provide meat properties should not be a big task. The application of machine learning would however be beneficial and this would require some development.

4.2.3 In-plant design

Subject to the outcome of the work noted in Section 4.1, models would need to be designed for a robotic system being located at either:

- the end of the slaughter line and before the chiller, or
- in the chiller.

Services required for installation would only extend to single-phase power for the NMR equipment (magnet temperature stabilisation, NMR RF electronics), plus whatever is required for the robot.

Design work would be best completed with a specific customer.

4.3 Commercialisation

We propose that work that supports the build of a prototype is conducted. Steps to be taken include:

- Connect with further trials as appropriate.
- Site trials using a bench top NMR.

Also of note:

- In principle the robot could be mobile since the NMR electronics and associated computing hardware can be made very compact.
- A machine learning approach could usefully be investigated to improve predictive models over time.
- None of the hardware (permanent magnet, NMR RF electronics and probe) is expensive so does not present a barrier to implementation.

5 Conclusions and recommendations

This Milestone 4 report concludes the current project to investigate the application of NMR relaxometry in the grading of beef-eating qualities. We have shown that NMR is able to provide excellent measures of total fat and moisture contents in meat, as well as an excellent predictor of drip-loss. We have been able to build reasonable linear models for pH, at least for pH<5.8, and over a limited range also for tenderness.

We have mapped out options for machine design for application in industry, as well as identifying a path to achieving a prototype machine. In parallel, the data models would benefit from additional trial data, especially for tenderness where sensory panel data would also be a very useful addition. Since NMR is able to distinguish different populations of water in the muscle, we believe cooking loss should also be predictable with suitable changes to the experimental method used in the current study. We are optimistic that machine learning can be applied to improve the individual quality predictions, and that these measures could be combined into an overall consumer acceptability grading score, all based on data from a single instrument which is able to take a non-contact measurement within several seconds.

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7 **Appendix 1** What is NMR?

Nuclear magnetic resonance spectroscopy (NMR)[3] was first developed in 1946 by research groups at Stanford and M.I.T., building on radar technology developed in WW2. Over the next 50 years NMR developed into the premier organic spectroscopy available to chemists to determine the detailed chemical structure of the chemicals they were synthesizing.

Many atomic nuclei possess permanent magnetic moments (or “spin”, like a gyroscope) and, when placed into an external magnetic field, tend to align themselves along the field. The most often-used nuclei in NMR are hydrogen-1 and carbon-13, although certain isotopes of many other elements nuclei can also be observed. The magnetic moments of all nuclei present in a sample sum up to a macroscopic vector quantity called nuclear magnetization. In equilibrium, nuclear magnetization is aligned along the magnetic field and, being tiny and static, is almost impossible to detect against the main field background.

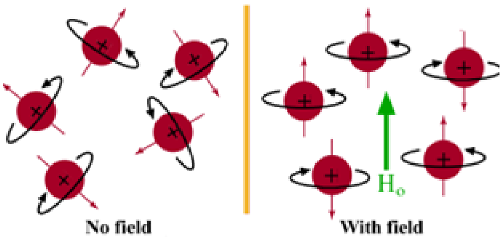

Figure A1.4 Alignment of nuclear spins in an external magnetic field

Spin alignment

In an NMR experiment the magnetic alignment is perturbed using a radio-frequency pulse, where frequency is proportional to the external field strength for a given nuclei. In returning to equilibrium the nuclei re-emit radio frequency energy which can then be detected by a nearby receiver coil.

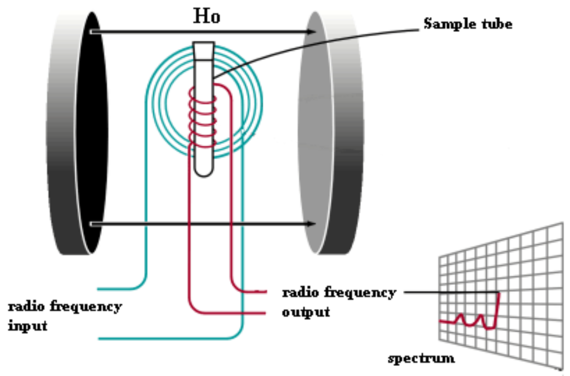

Figure A1.5 Schematic of an NMR experiment in which a radio frequency input (blue coil) perturbs the nuclear magnetisation produced by the external field H_0, the relaxation of which can be detected (red coil).

The time required for the nuclei to return to equilibrium after excitation is the “relaxation time” and relaxometry NMR (or time-domain NMR) involves the measurement of these times, and is typically done at relatively low applied magnetic field strengths. T1 relaxation, also called spin-lattice or longitudinal relaxation, relates to energy dissipated to the surrounding molecular framework; T2 relaxation, or spin-spin or transverse relaxation, relates to energy dissipated to neighbouring nuclei. Nuclei in different environments have different T1 and T2 relaxation times, hence the NMR

[3] This description is adapted from http://www.ebyte.it/library/educards/nmr/OnePageMrPrimer.html and other on-line sources.

relaxometry method has the ability to discern different populations of the given nucleus (^{1}H in water and fat relevant examples in the present case). Analysis of these two relaxation times are discussed further in Appendix 2.

This response is also exploited in magnetic resonance imaging (“MRI”) and also in high-field spectroscopy where chemical structures can be elucidated by transformation of the time-domain signal into the frequency domain, in the latter case the small differences between the magnetic fields experienced by individual atomic nuclei due to their chemical environment, including those produced by the presence of electrons and those due to interactions with close-by nuclides of the same or different kind and which may be mediated by chemical bonds, are detected . Being very small, these field variations are measured *at most* in parts per million (ppm) with respect to the external field, down to tiny fractions of a ppm.

The NMR system employed in the present research consisted of a temperature stabilised permanent magnet array of Halbach configuration giving a uniform field transverse to sample entry direction (called “Oscar 2.0” as seen in Figure A1.3 below). The sample container consisted of a common plastic bottle with screw cap attached to a suspension rod (Figure A1.3). The sample space was kept chilled via the use of a thermoelectric cooler mounted above the magnet. A Kea spectrometer developed by Magritek (Wellington, NZ) and personal computer completed the NMR instrument.

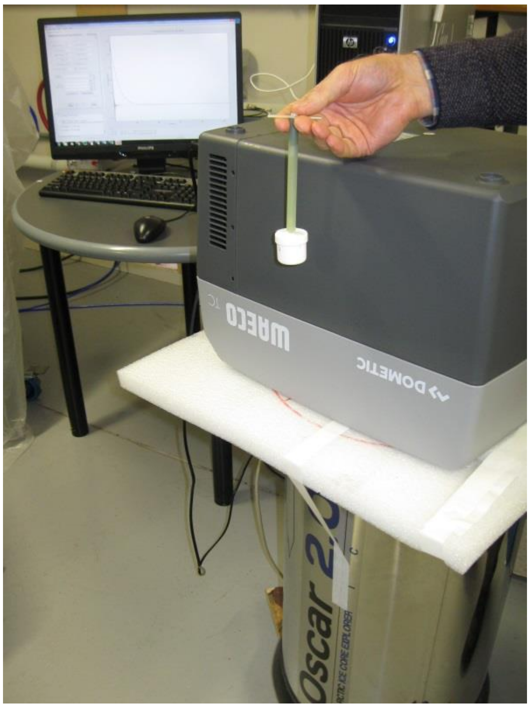

Figure A1.3: the NMR system with sample holder in front of thermoelectric chiller sitting on the magnet (“Oscar 2.0”).

8 **Appendix 2** Analysis of relaxation times

There are two types of NMR relaxometry measurements: the longitudinal relaxation time (T_1) and the transverse relaxation time (T_2). The T_2 relaxation signal can be resolved into three main components related to distinct water populations as shown in figure A2.1.

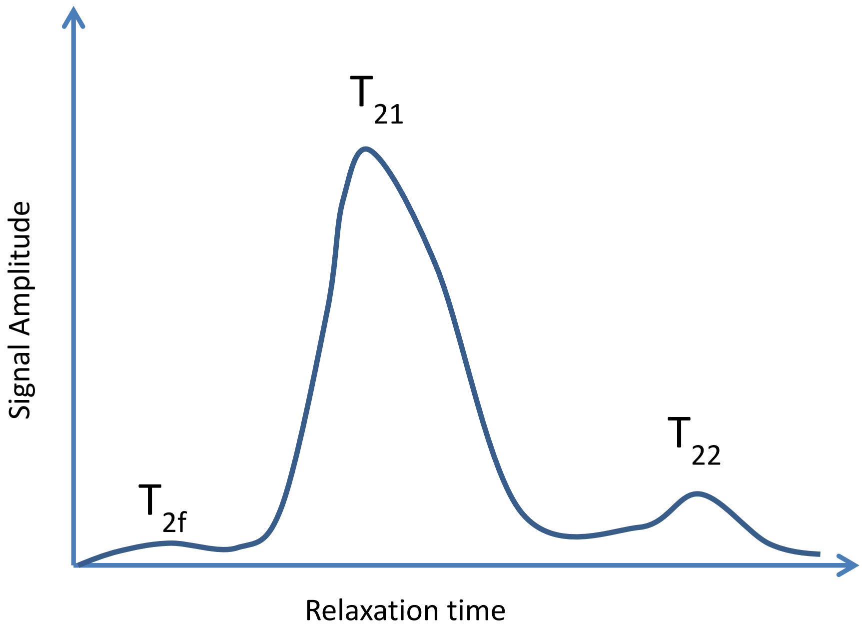

Figure A2.1: T2 relaxation time distribution showing three peaks. T2f: water bound to macromolecules, T21: water located in myofibrils, T22: free water

T_{2f} is associated with water bound to macromolecules such as protein. T_{21} is related to water located in highly organized protein structure (myofibrils) such as actin and myosin. T_{22} is related to loosely bound water / free water (extra-myofibrils) (Bertram et al., 2001a; Bertram et al., 2002). These parameters change with ageing, storage and cooking condition. They also reflect changes in pH.

All correlations of beef parameters were with T_2 components, and because the T_2 measurement speed is significantly faster than that for T_1 (seconds vs minutes for elapsed time, respectively), this indicated a high-throughput industrial instrument might be realisable.

Nevertheless we both T_1 and T_2 were recorded during Milestone 3; analysis showed that the inclusion of T_1 information in the predictive models did not improve the prediction accuracy substantially.

In this project, we mainly use linear regression to explore correlations between the NMR signal components and the measured beef properties, although for shear force we also explored non-linear models. The criteria for selecting the best predictive models were high linear model correlation (r^2) and low multi-co-linearity among the NMR variables. This could be done effectively using the data analysis software package R (software for statistical computing and graphics).

For the analyses presented in this report, the NMR T_1 measurement (inversion-recovery) data is fitted to a bi-exponential function, yielding components T_{11}, T_{12} with respective populations p_{11} and p_{12}. Note that the raw NMR data was normalised to 1 so that the fitted results were independent of sample size. The NMR CPMG measurement data for T_2 is fitted to three exponentials, yielding fit components T_{2f}, T_{21}, T_{22}, with respective populations p_{2f}, p_{21} and p_{22}. See figures A2.2 and 2.3 below.

The total number of NMR signal variables is p = 10 (p_{11}, T_{11}, p_{12}, T_{12}, p_{2f}, T_{2f}, p_{21}, T_{21}, p_{22} and T_{22}), but the good predictive models are possible using on the T_2 variables.

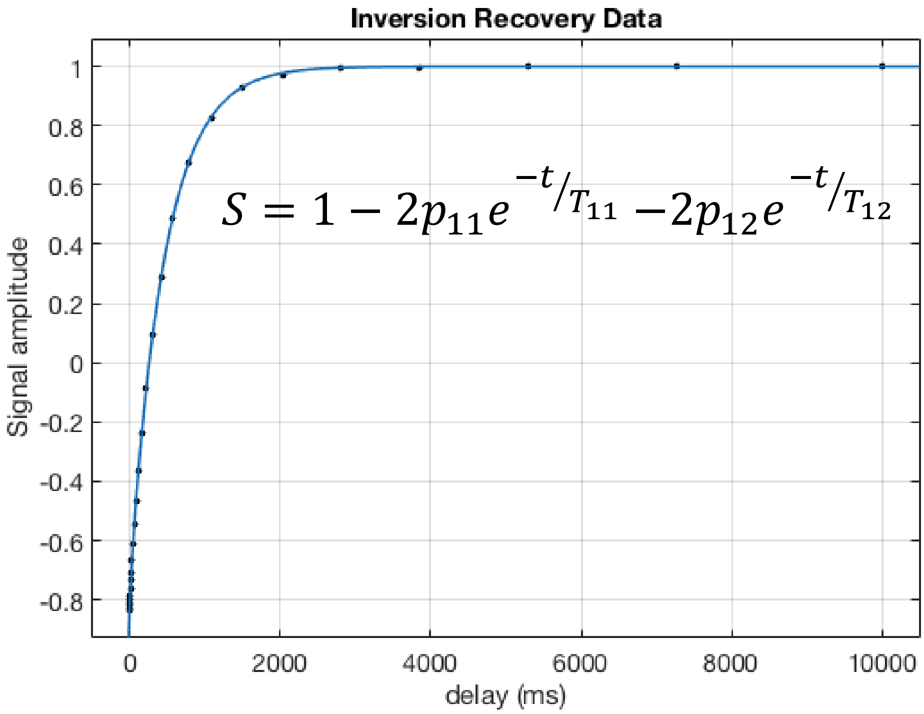

Figure A2.2: Bi-exponential curve fit on inversion-recovery relaxation data.

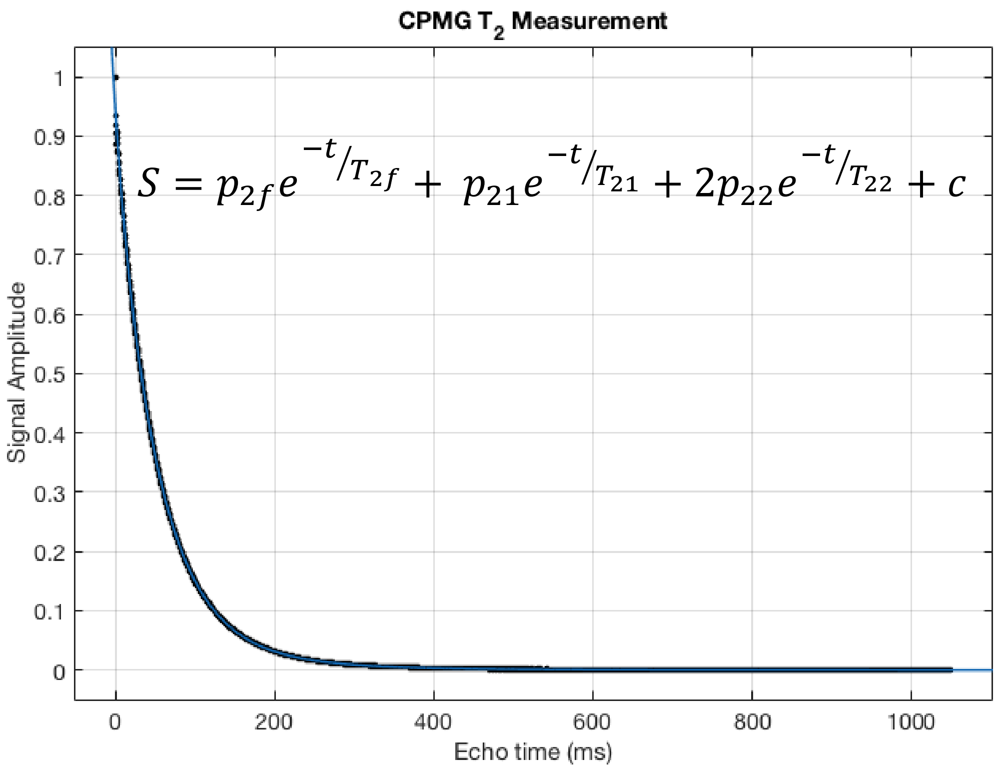

Figure A2.3: 3-exponential curve fit on CPMG relaxation data