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Preliminary investigation of impedance spectroscopy for measuring meat traits

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Abstract

Measurement of meat traits using prediction tools is a challenge for the meat industry as the associated technology must be robust, produce accurate and reliable predictions and be relatively easy to use. These requirements have seen many technologies fail. In this study preliminary consideration has been given to the application of impedance spectroscopy for predicting the tenderness of lamb. As it stands the technology improves on previous impedance studies by using 4 current electrodes and by undertaking 3 scans in very high resolution at each measurement time. The results suggest that differences in tenderness between samples are reflected by changes in impedance. Thus the technology could be used to screen meat into say tough and tender categories at 1 day post-mortem, but the ability to predict 5 day shear force from 1 day impedance measures was not totally consistent.

Executive Summary

Previous research on impedance for measurement of meat has been limited to systems using 2 electrodes and this has the potential for polarisation of the electrodes to impact on measurement. The 4 electrode approach used by INPHAZE Pty Ltd provides opportunity to avoid this issue. Linked to this the INPHAZE Pty Ltd high resolution EIS technology enables scanning across a range of frequencies and the ability to undertake repeated scans on the same sample.

As a preliminary study INPHAZE Pty Ltd impedance technology was used to measure 12 samples of lamb (*longissimus*) at 1 day post-mortem. Half of the samples were then frozen and tested for shear force, cooking loss and pH. The other half of the samples were aged for a further 4 days and on the third and fifth day were remeasured with the impedance technology. Scans over 1 KHz showed a merging of impedance between samples from the same carcase, but Nyquist plots revealed that in the region above 1 KHz the peak frequency between 20 and 234 KHz was informative and changes in the real impedance at these peak frequencies mirrored changes in shear force. Also mean impedance values across the frequency range did show an association with shear force so that as shear force decreased, impedance also decreased and vice versa. However indications are that to develop prediction models for the technology further research is required based on a much larger sample so that the sources of variation can be taken into account and so the robustness of models can be tested.

Contents

1 Background

Flavour, juiciness and tenderness influence the palatability of meat. Among these traits, tenderness is ranked as most important for beef meat (Thompson, 2002), but has lesser influence in sheep meat (Thompson, Hopkins, D'Sousa, Walker, Baud, & Pethick, 2005). The three factors that determine meat tenderness are “background toughness”, the toughening phase and the tenderisation phase. The toughening and tenderisation phases take place during the post mortem storage, or ageing period (Hopkins & Geesink, 2009). It is now well-established that post mortem proteolysis of myofibrillar and associated proteins is responsible for tenderisation, but it is a matter of debate about which proteases are responsible for tenderisation and which muscle proteins are degraded (Hopkins & Thompson, 2002; Koohmaraie & Geesink, 2006; Ouali, Hernan-Mendez, Coulis, Beclia, Boudjellal, Aubury, & Sentandreu, 2006). Nevertheless as a consequence of this degradation of proteins the molecular structure of meat changes during ageing. Attempts to measure these changes and relate them to instrumental measures of tenderness have been extensive (Damez & Clerjon, 2008) and impedance is one of the technologies that have been studied.

Impedance is measured as the opposition to the flow of electricity and has resistive and capacitive components when measured in biological tissues (Damez & Clerjon, 2008). Previous work on impedance has shown that electrical impedance of meat decreases as ageing progresses and that after rigor mortis with changes in membrane properties impedance measures can be problematic (Damez, Clerjon, Abouelkaram, & Lepetit, 2008). These authors did however show that given meat displays anisotropic characteristics that if longitudinal and transversal measurements were made and an anisotropy index determined that this was strongly correlated to fibre strength in meat when measuring at a frequency of 100 Hz. Whether an alternative impedance spectroscope developed by INPHAZE (Chilcott, Halimanto, Langrish, Kavanagh, & Coster, 2010) has the resolution to give repeatable predictions of tenderness (measured as shear force), remains to be established.

2 Project Objectives

1. To conduct a preliminarily study on the measurement of lamb meat with an INPHAZE impedance spectroscope under laboratory conditions and relate the impedance measures to shear force and pH.

3 Methodology

3.1 Experimental design

Samples of m. *longissimus* from the lumbar section of 6 lamb carcases (both sides of the carcase) were purchased from a butcher and all external fat removed. Each loin piece was approximately 250g.

These samples were 1 day aged (3/08/2011). Each sample was cut in half and trimmed to 65-70 g with dimensions of approximately 60-70mm length, 40-50mm width, 20-25mm thick. The protocol for measurement of samples is outlined below. This allowed a comparison between samples aged for 1 and 5 days against impedance measures and the measurement at day 3 allowed the change in impedance measures with time to be captured.

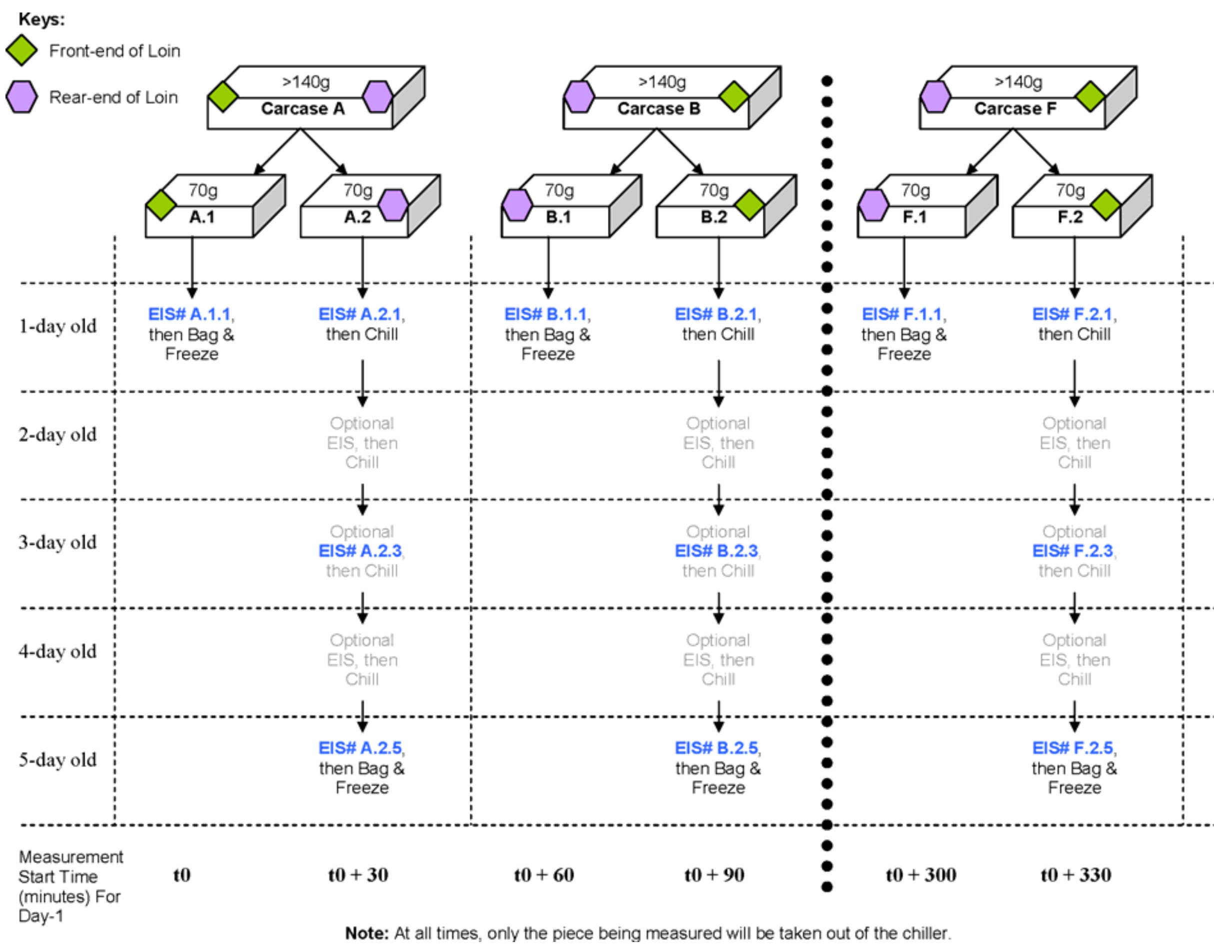
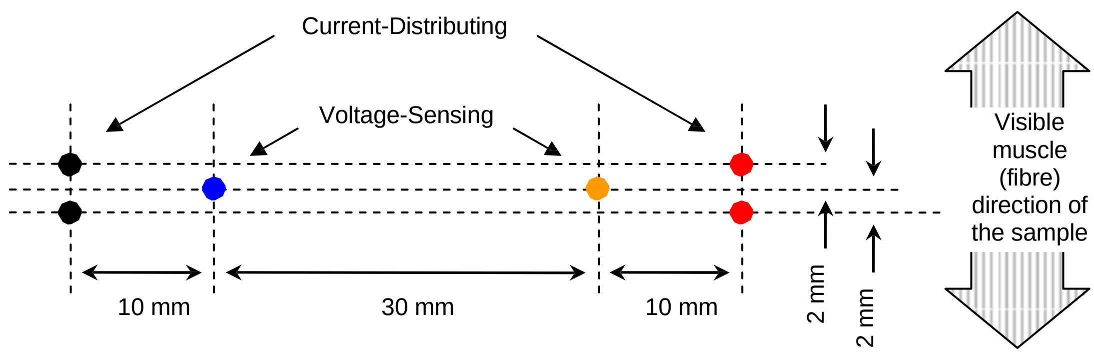


Figure 1. Protocol for measurement of samples by impedance

3.1.1 Electrodes & Geometry

6× Pure gold (99.99%+) segments, 0.8mm in diameter.



3.1.2 Measurement Protocol

Every sample was taken out of the chiller and dried with paper towel 3 min before measurement. Each measurement took just under 30 min which included the use of 4 electrodes (56milliHz ~ 1MHz, steps of 2, 3 spectra) then 2 electrodes (447milliHz ~ 1MHz, steps of 3, 3 spectra). After measurement, the samples were dried with paper towel before vacuum packing. One day old samples were frozen at -20°C and ageing samples kept chilled at 4-5°C. Three day old samples were measured as described and then held chilled until 5 days old when the samples were measured again and then frozen.

3.2 Meat quality measurements

All frozen samples were shipped to Cowra and a 1-2g sample removed from each sample for measurement of pH. This was determined using an iodoacetate method adapted from that described by Dransfield, Etherington, & Taylor (1992). The 1 g sample was added to 6ml of cold iodoacetate (at pH 7) and homogenised at 19,000 rpm for two bursts of 15 seconds. Samples were held on ice for 30 seconds between each burst. Following this the samples were incubated in a water bath at 20°C and the pH was measured.

Shear force samples were weighed prior to cooking (mean 64.5 g). The shear force samples were cooked for 35 min in plastic bags at 71°C in a 90 L water bath with a thermoregulator and a 2000 W heating element (Ratek Instruments, Boronia, Victoria, Australia) and measured using a Lloyd Texture analyser as previously described (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010). The cooking batch was made up to 20 samples using 8 non-experimental samples. Samples used for shear force determination were weighed pre and post cooking (mean 53.0 g) to measure the amount of cooking loss. After cooking the samples were cooled in running water and patted dry using paper towelling before weighing. Cooking loss percentage was calculated from the following formula;

Cooking loss (%) = 100 x (1 – cooked weight/fresh weight)

Association between the meat quality traits and impedance was determined by graphical interpolation, given the small numbers of samples tested in this preliminarly study.

4 Results and Discussion

The meat quality results for each sample are outlined in Table 1. The shear force results indicate that the samples were tender compared to what may have been expected particularly for 1 day aged samples (Warner, Jacob, Hocking Edwards, McDonagh, Pearce, Geesink, Kearney, Allingham, Hopkins and Pethick, 2010) and would be considered to provide good eating quality (Hopkins, Hegarty, Walker, and Pethick, 2006) being at the lower end of the shear force distribution. This created less range than ideal for developing relationships as it is preferable to cover the range seen commercially, however as shown in Table 1 there were differences between samples aged for 1 and 5 days in shear force as expected (on average 4.5 N) and this was reflected in differences in impedance also. In agreement with other studies impedance values were lower as shear force decreased which Damez *et al.* (2008) suggested reflects a change in conductivity, due to the degradation of membranes. The impedance measures given in Table 1 are mean values over the frequency range scanned from 56milliHz ~ 1MHz based on the use of 4 electrodes. Previous studies have used 2 electrodes (e.g. Damez *et al.* 2008) and in others the number was not specified (e.g. Byrne, Troy, & Buckely, 2000), but the use of 4 electrodes should reduce artefacts from polarisation of the electrodes.

In the current study for 2 carcases the shear force after 5 days of ageing was higher than at 1 day of ageing and impedance measures increased, whereas for the other 4 carcases as shear force declined with ageing so did impedance measures (Table 1). This outcome reflects the effect of positional influences within muscle on shear force (e.g. Hopkins & Thompson, 2001) and is why the position was rotated to ageing treatment across the 6 carcases. Sampling of more carcases would allow this factor to be accounted for in analysis. However the result does verify that changes in shear force will be reflected in impedance independent of ageing period which is a positive outcome. The relationship between mean impedance measures and shear force is shown in Figure 2 and this indicates a linear trend for measures taken on samples aged for 1 day and then frozen. By contrast there is no apparent trend for samples measured after 5 days of ageing and then frozen. This is in agreement with other studies that have shown a weakening correlation between impedance and shear force as ageing progresses (Byrne, Troy, & Buckley, 2000).

Table 1. Mean shear force (SF) in Newtons for each sample (aged for 1 or 5 days), pH (1 and 5 days), cooking loss (CL; %; 1 and 5 days), percentage change in SF during ageing, impedance (Z) in ohms (at 1 and 5 days) and percentage change in impedance.

Carcase	SF (1 day)	SF (5 days)	% SF Change	pH (1 day)	pH (5 days)	CL (1 day)	CL (5 days)	Z (1 day)	Z (5 days)	% Z Change
A	28.8	20.2	-30	5.66	5.94	19.8	15.7	430	310	-28
B	24.5	16.1	-34	5.71	5.75	18.6	18.4	225	170	-24
C	21.6	27.5	+27	5.72	5.56	19.5	20.0	155	210	+36
D	30.3	23.4	-23	5.58	5.58	17.8	17.3	460	370	-20
E	25.6	27.6	+8	5.56	5.76	16.6	16.2	275	315	+15
F	29.3	18.0	-38	5.59	5.63	16.5	18.6	390	170	-56

A particular point of interest was to compare impedance measures taken at 1 day of ageing on samples against the shear force of those samples that were then aged for 4 days. This indicates the potential to use the technology for prediction purposes. As indicated in Figure 2 for 4 of the samples there is a linear trend that as impedance values measured on day 1 increased the shear force of the samples at day 5 increased. However 2 samples (C) and (A) did not follow this trend with sample C being the real exception.

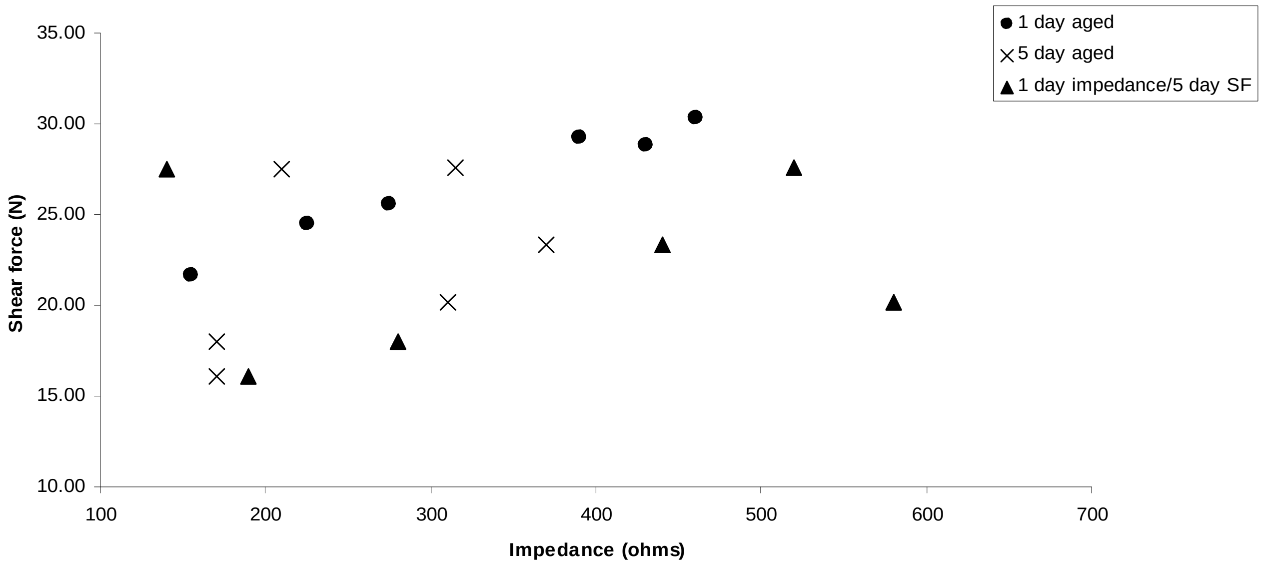


Figure 2. Relationship between impedance (ohms) and shear force (Newtons) for 1 day aged samples (●), 5 day aged samples (×) and impedance measured at 1 day on samples aged for 5 days (▲).

The pattern of impedance for changes in frequency is shown in Figure 3. This clearly shows that after 1 kHz there is a merging of impedance values between samples from the same carcase. This could be important for by restricting the frequency range, measurement time can be reduced, although measures at high frequencies are fast, whereas those at low frequencies take longer to complete. As shown in Figure 4 the general pattern of change in impedance across frequencies is similar during ageing, although the range in values decreased by 50% and this greater range for day 1 values explains why there is a stronger relationship with day 1 shear force measures. It is also apparent that the 6 samples measured at day 1 were in 2 broad groups for impedance (Figure 4) and this persisted during ageing. Thus in this case, the:

$$Average = \frac{\sum Z_1 \ldots Z_6}{6} \approx MidRange = \frac{Z_{\min} + Z_{\max}}{2}$$

The mid range values also decreased with ageing as shown in Figure 3.

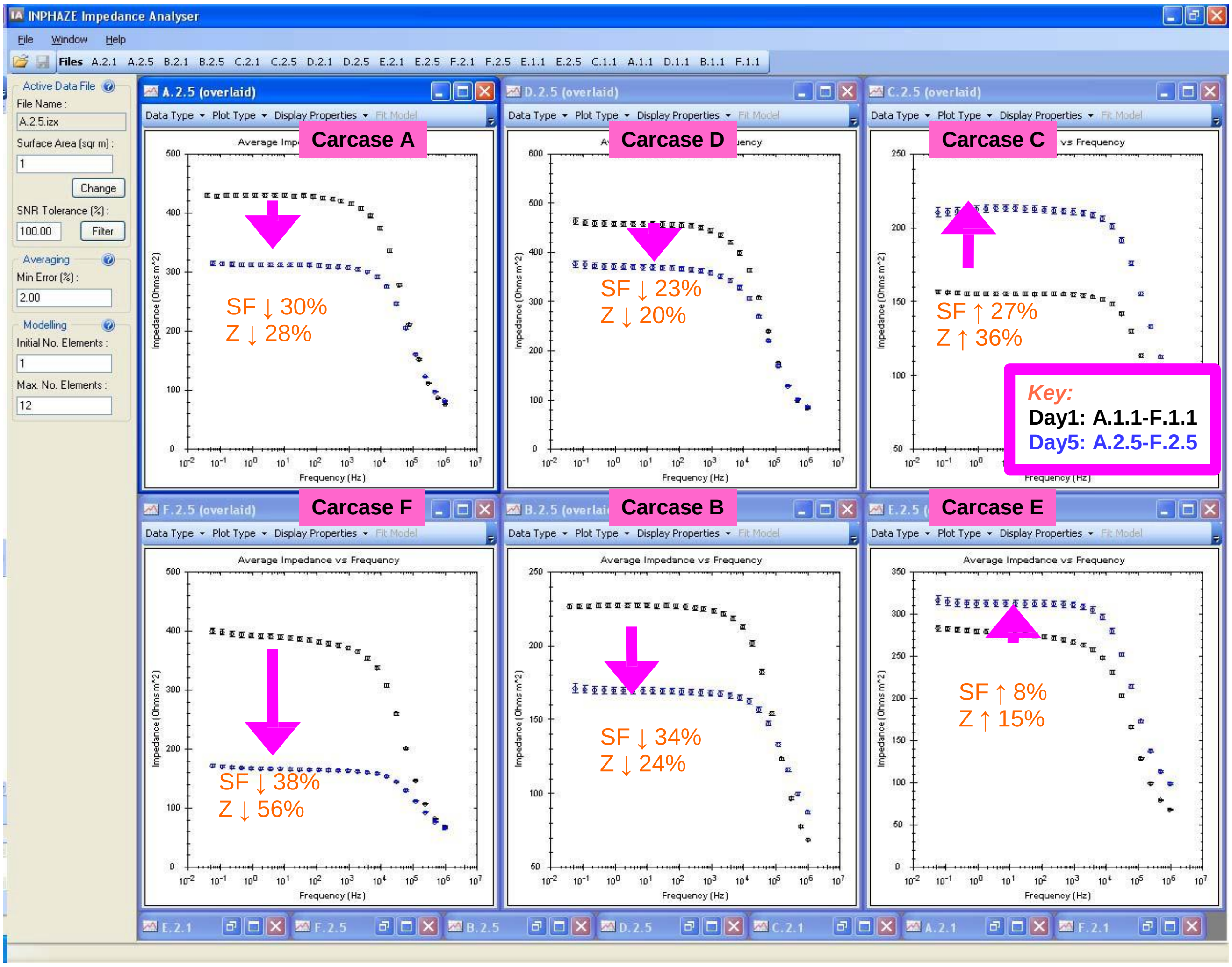


Figure 3. Day1 to Day5: Correlation with shear force (SF).

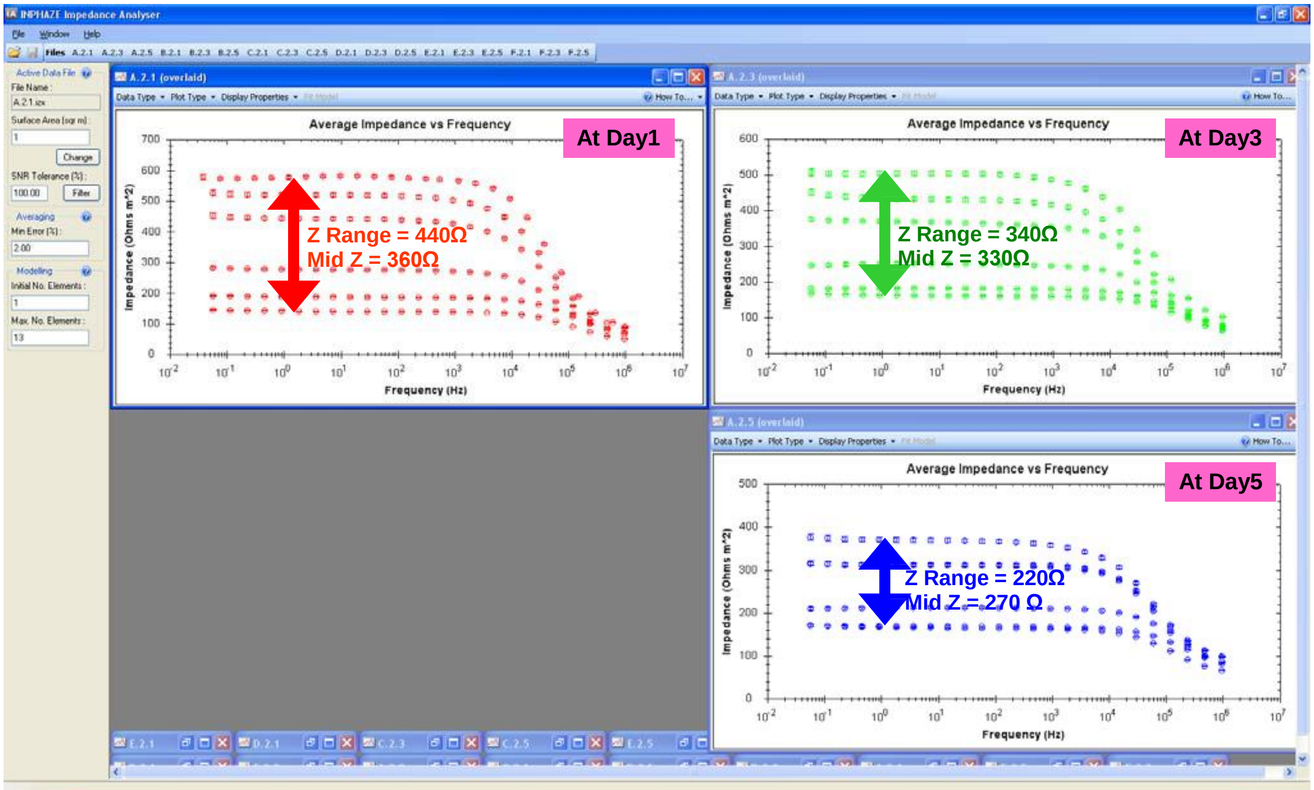


Figure 4. Impedance pattern of 6 samples across ageing days for portions A.2-F.2 which were aged for up to 5 days.

It is of interest that the impedance of samples measured on day 1 which were subsequently frozen on day 1 (Portion 1) generally fell within the range of samples also measured on day 1, but then aged for 5 days (Portion5) given there was allocation across portions to ageing period (Figure 5). Thus portion 2 samples (A.2-F.2) had an impedance range of 140Ω to 580Ω and this was used to determine the mid-range impedance and the variation amplitude shown in Figure 4.

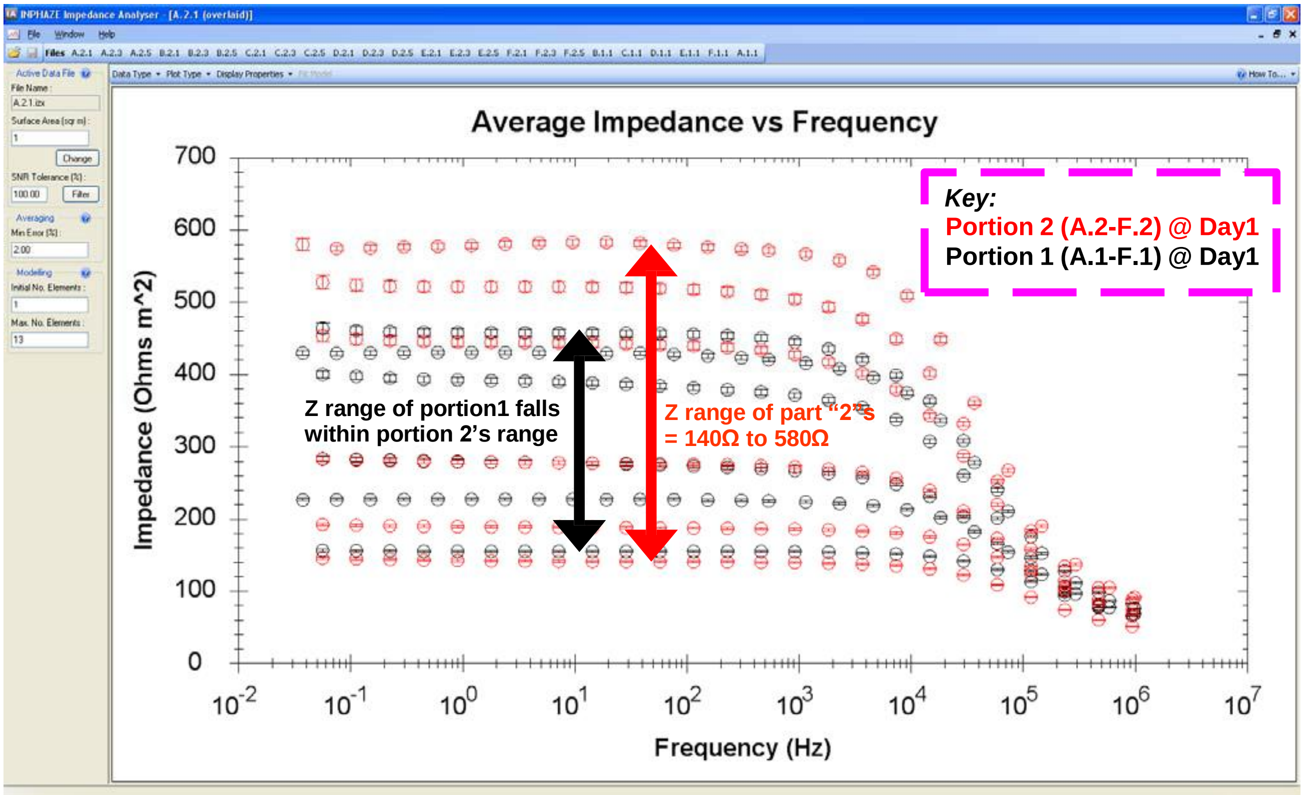


Figure 5. Relationship between impedance and frequency for Day 1 samples according to portion (A.1-F.1) vs. (A.2-F.2).

Meat is electrically anisotropic, such that the electrical properties of meat are influenced by the direction of the electrical fields and for this reason Damez *et al.* (2008) applied current to samples both longitudinally and transversally. This showed that measurements along meat fibres give lower impedance values than those taken across fibres (transversally) and this was attributed to the fact that current flowing across fibres must pass through cell membranes, whereas current flowing along fibres is mainly carried by ions in the extracellular space. In the current study measurements were performed across the fibres which should relate more strongly to changes in protein structure and thus tenderness, whereas it could be speculated that measurement along fibres would relate more strongly to changes in pH. Additionally in the current study the electrodes were 1 cm apart, which according to the results of Damez *et al.* (2008) should produce smaller changes in impedance across the frequency range. Others have used 1.5 cm (Byrne *et al.* 2000).

Another aspect which was examined in the study was the change in impedance measures over time on the same sample as shown in Figure 6. This shows that sample C had increasing values which was in line with the shear force results and sample D exhibited a rise in impedance values at day 3 and then declined again at day 5, with sample B showing the opposite effect.

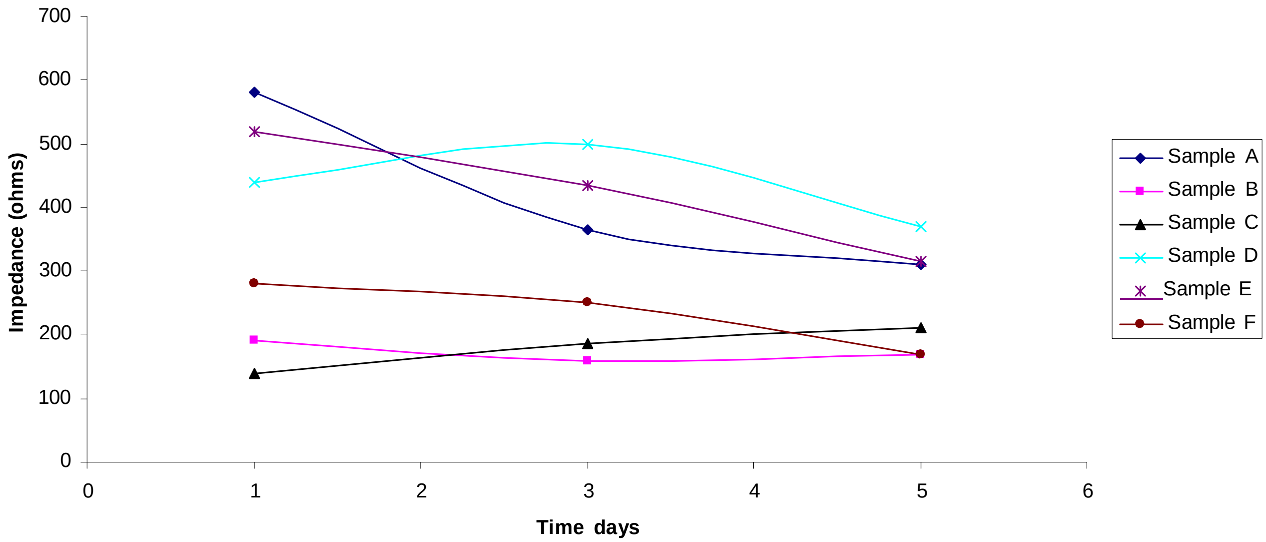


Figure 6: Impedance trend over post-mortem time of each sample (A-F; portion 2).

To further explore the relationship between impedance measures and shear force, nyquist plots were derived for each sample. Nyquist plots shows basically 4 things at the same time: (1) on the X-axis is the real impedance (2) on the Y-axis is the imaginary impedance (3) the distance (vector) between the origin and a point is the magnitude of impedance for the frequency that the point represents and (4) the angle of the vector shows the phase lag the sample will cause at that particular frequency. A plot for sample A is shown in Figure 7. The frequency at which the peak of the semi-circle occurs is the characteristic frequency and it directly relates to the characteristic time-constant of the sample. The time constant of a sample is determined by the dielectric (resistive and capacitive) property of the sample. If the sample has many distinct layers then you will see many semicircles. In this study, all samples just had 1 semicircle, hence 1 characteristic time-constant (a particular dielectric resistive and capacitive combination).

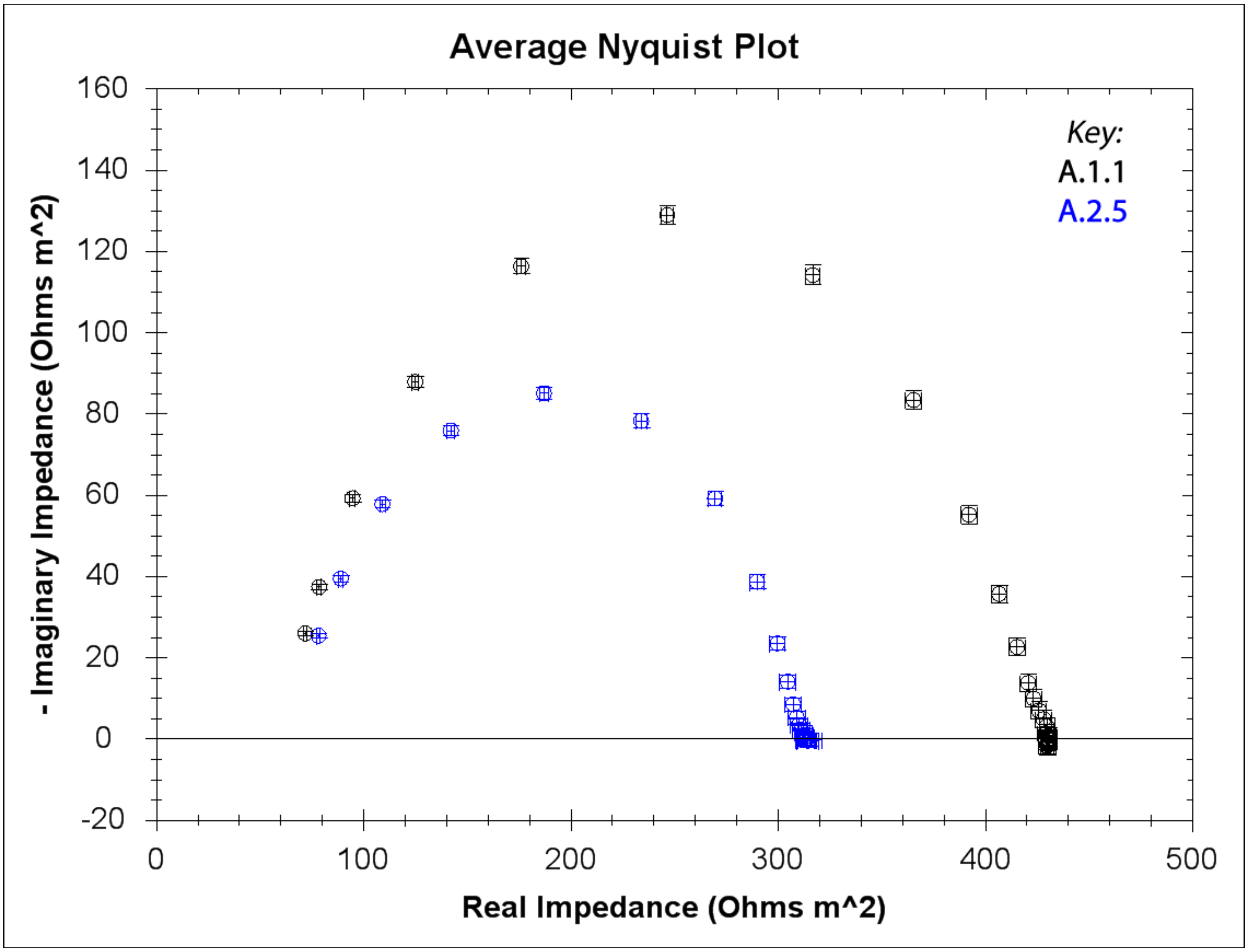


Figure 7: Nyquist plot for sample A (A.1.1 and A.2.5).

Nyquist plots revealed that in the region of 1 kHz as shown in Figure 3 where the impedance starts to drop that the peak frequency between 20 and 234 KHz was the most informative and changes in the real impedance at these peak frequencies (Table 2) mirrored changes in shear force (see Table 1). This shows that as the peak frequency increases within a carcase this corresponds to a reduction in shear force. Interestingly in samples where the shear force increased with ageing (C and E) there was no change in peak frequency. This requires more investigation as to what characteristics of muscle structure are causing this effect. Consistent with the mean impedance results (Table 1) as shear force increases with ageing (samples C and E) the real impedance at the peak frequency also increases and vice versa for the other samples.

Table 2. Results from the Nyquist plots of each sample.

	Peak Frequency [KHz]			Real Impedance of Peak [Ohms]			-Imaginary Impedance of Peak [Ohms]		
Carcase	**Day 1**	**Day 5**	**change**	**Day 1**	**Day 5**	**change**	**Day 1**	**Day 5**	**change**
A	36	58	61%	250	190	-24%	130	85	-35%
B	73	234	221%	142	110	-23%	58	32	-45%
C	117	117	0%	107	150	40%	35	43	23%
D	29	58	100%	280	200	-29%	132	100	-24%
E	58	58	0%	150	200	33%	72	80	11%
F	29	117	303%	237	105	-56%	115	36	-69%

From this preliminarily study it has been shown that a narrow range of frequency could be applied for the measurement of tenderness and that impedance can detect the changes in tenderness (measured by shear force) with ageing. As previously mentioned there appears scope to reduce the frequency range applied for measurement and any reduction at the low end will decrease measurement time. Thus based on the results here it is recommended to start with 30Hz rather than 56milliHz and to scan up to 1MHz in future work. It is of note that absolute changes however in shear force are not mirrored by the same scale effect in impedance changes for example see carcase F vs. carcase B data (Figure 3) or the data in Tables 1 and 2. Also the ability of impedance to predict shear force of aged meat was not totally consistent across all samples (Figure 2).

This study has focussed principally on tenderness, but previous research has shown that impedance does have potential for measuring fat levels (Damez & Clerjon, 2008) and so the current technology could be useful for predicting intramuscular fat although the suggestion is that this would need to occur before rigor (Damez *et al.* 2008). Related to this the potential for fat to interfere with measurements would need to be considered in any application and use on-line for tenderness prediction would probably necessitate placement of electrodes into muscle free of subcutaneous fat. There was no apparent relationship between either pH or cooking loss and impedance measures.

5 Success in Achieving Objectives

As a preliminarily study it has been demonstrated that the technology has *potential* for the prediction of tenderness in lamb and thus by default also in other red meats. The agreed milestones were met and the study has laid the groundwork for further research and development.

6 Impact on Meat and Livestock Industry – now & in five years time

This project has demonstrated that the impedance spectroscope developed by INPHAZE Pty Ltd does have potential. However the technology requires further development/research as outlined below, before it could be considered for commercial application.

7 Conclusions and Recommendations

Further work on the impedance technology is required to;

1. Validate the potential of impedance for predicting tenderness by undertaking a designed experiment that includes 8-10 replications with 15-20 samples per replication measured over time. In such a study other traits such as pH and intramuscular fat should be measured.
2. Contingent on point 1 produce a measurement head incorporating the electrodes that can be applied to muscle/meat.

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A.MQT.0057 - Preliminary investigation of impedance spectroscopy for measuring meat traits

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A.MQT.0057 - Preliminary investigation of impedance spectroscopy for measuring meat traits

9 Appendices

Appendix 1

The financial support provided by Meat and Livestock Australia and NSW Department of Primary Industries is greatly acknowledged. The technical assistance of Matthew Kerr and Tracy Lamb (NSW DPI) with the laboratory testing is gratefully acknowledged as is the assistance with impedance data collection by Tino Kausmann and Mariam Darestani from INPHAZE PTY LTD. Thanks also to Penny butchers, Mosman for providing the lamb samples.