Final Report

AMPC
AUSTRALIAN MEAT PROCESSOR CORPORATION

Meat quality in live animals

Non-invasive measurement of meat quality in live animals using deep tissue Raman spectroscopy

Project Code
2017-1011

Prepared by
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Contents

1.0 Executive Summary

This Final (and 10th Milestone) Report summarises the overall progress of project 2017-1011. In brief, the objective of this project was to reduce the level of dark cutting (DFD) meat in Australia by developing sensor technology that can be used to screen cattle in real-time at the abattoir, either at receival, or immediately pre-slaughter. This will allow dark cutting susceptible animals to be diverted so they can better recover their levels of glycogen. To this end, we have investigated a fast, noninvasive, field portable Raman spectroscopic device called Spatially Offset Raman spectroscopy (SORS). The project was designed to test the potential of the SORS technique in a progression of controlled experiments, from the fundamental science of what information about beef composition can be obtained to field testing the instrument on 'real world' samples from carcasses at an abattoir and on whole cattle. Together, this series of experiments were designed to allow the feasibility of the use of SORS to detect and evaluate DFD dark cutting in cattle as a future tool for guiding lairage decisions to be assessed.

In Year 1, protocols for use of the SORS instrument to characterise the biochemical signatures of beef were established in laboratory tests on commercial meat samples. We found that:

a) SORS can detect signature bands for key recognised biomarkers that relate to beef quality and the occurrence of DFD dark cutting, these being lactate, glycogen, cortisol and glucose. From these initial experiments we established a library of spectral bands that allow detection and quantification of these four important determinants of beef quality. Though the SORS spectrometer used in this project was a commercial instrument optimised for use in warehouses rather than agriculture or abattoir settings, this phase of the project showed that detection of these biomarkers could be made non-invasively within beef cuts in 20-25 seconds. This work was described in the report for Milestone 2.

b) Dark cut (DFD) beef samples provided by our co-CIs David Hopkins and Steph Fowler (NSW DPI) were then investigated, and we established that SORS could also characterise the combination of these biomarkers to differentiate between DFD and non-DFD. These measurements were able to relate changes in SORS marker bands to the measured pH of the beef samples, a standard assay parameter for detecting dark cutting post mortem. This study established that SORS spectra can rapidly identify the DFD condition in cuts of beef and was described in Milestones 3 and 4.

c) Chemometrics (computational data analysis tools) are commonly required for detailed analysis of spectral markers in complex samples such as meat tissues. An important phase of this project was always expected to be the testing and development of chemometrics tools and computational models of the measured spectra in order to provide qualitative and quantitative analysis. In addition, any potential translation of the SORS technique into the meat processing and animal handling sectors will require widespread use by non-experts who would rely upon such chemometrics tools for interpreting instrument measurements into information about lairage requirements, detection of DFD or the characterisation of beef samples in general. During Year 1 we began to develop several chemometrics tools for addressing these questions, with the chemometric tools proving to be successful and important for many of the studies described in this report. These chemometrics tools were further developed in Years 2 and 3, and are highly suitable for further expansion and development in the future.

In Year 2 studies were conducted at an abattoir to determine the capability of SORS to detect biomarkers of dark cutting and build predictive chemometric model. In this phase of the project, we showed that SORS could:

d) Be successfully used at an abattoir, the JPS Hurstbridge Abattoir in Victoria, to collect SORS spectra within the operational constraints of carcass preparation. Though the chainline used for moving and supporting carcasses at this site presented challenges for handling the current SORS spectrometer, we were able to collect usable data from 118 carcasses, that were then successfully analysed to identify DFD samples.

e) Identify biochemical differences relating to the type of beef cut, with the following commonly marketed beef cuts being differentiated solely based upon their SORS spectra; rump steak, Scotch fillet, tenderloin, chuck, round and T-bone. These SORS spectral details were correlated with the measured pHs of these samples, further supporting our previous conclusions that the SORS spectra directly measure key characteristics of beef quality.

Year 3 studies were delayed by around 24 months because of Covid-related restrictions to travel and to abattoirs across Australia. Eventually, we were able to secure access to a cow that had died very recently of natural causes which should have meat characteristics that are very similar to that of live cattle, making it a reasonable test of the ability of SORS to obtain data with the level of sensitivity already demonstrated for meat samples in Year 1, and carcass samples at an abattoir, demonstrated in Year 2. While we were only able to gain access to one animal post mortem, this was sufficient to test and prove the principle that the SORS technique could obtain usable spectra of the meat tissues from a cow through the layers of hair, skin and fat present. While the design of the current SORS spectrometer limited our ability to collect data to around 8 mm beneath the surface, which therefore at present restricts deep tissue measurements to specific parts of cattle where the muscles lie relatively close to the surface of the skin, this was an important finding as it shows that the SORS technique can obtain the required information non-invasively in cattle. As will discussed later, this phase verified that the SORS technique can be further optimised for direct application to cattle as well as carcasses and beef samples.

In overall conclusion, in the first phase of this project we showed that SORS can obtain biochemically-specific information about beef composition that can be directly used to detect the dark cutting, or DFD, condition. The main selling point of the SORS technology is its ability to non-invasively and rapidly obtain chemical information beneath a surface, leading to its adoption in biomedical diagnostics as a possible alternative to biopsies and in homeland security scenarios for illicit narcotic or explosives detection at airports and shipping ports. Technology development of SORS in these fields involves the optimisation of the technology for different samples and building chemometrics databases for use by non-experts in the future, and this first phase also demonstrated that this development path should be equally feasible for future uptake of SORS by the meat handling and farming sectors. In the second phase, we found that reliable SORS spectra could be obtained from beef carcasses at an abattoir and that data collection could even be fit into standard operations. This indicates the potential for use of the technology on-site at abattoirs. We were also able to develop chemometrics analysis of SORS spectra to identify the subtle differences in biochemical composition that distinguish different cuts of beef. While outside the initial aims of this project, this finding raises the possibility of applying SORS to characterise beef samples for improving quality control and authentication, as well as for studying the effects of other environmental, animal care or storage factors on beef characteristics. Finally, in the third phase we were able to show that these SORS spectra of beef muscle can also be obtained through the hair, skin and fat layers of cattle. While currently limited to certain regions of a cow's body (namely the leg and loin regions) by the parameters of the instrument used, the instrument could be further optimised in collaboration with the manufacturer (Agilent) to expand application within the beef industry.

These studies over the three phases of this project show that SORS can identify biochemical indicators of DFD dark cutting in commercial beef samples, in carcasses at an abattoir during processing and, potentially, within cattle.

We thank AMPC for not only funding the project but for their patience during the challenges of 2020 and 2021 presented by Covid impacts on the industry, and for agreeing to extensions to the initial project schedule.

The author should include in the Executive Summary an overview of the project objectives, approach, project outcomes and insights, conclusions and recommendations for further research/actions. This section should also include the project results and findings that can benefit members and the wider industry. This section should be a maximum of two or three A4 pages.

2.0 Introduction

The author should include the following information in the introduction:

- The purpose of the research project, including any background information
- The scope of the research, including any previous research that is relevant to this project
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- The scope of the research, including any previous research that is relevant to this project

3.0 Project Objectives

The author should outline the project objectives as specified in the research agreement.

3.1 Assess the usefulness of SORS spectroscopy as a noninvasive and chemical-free technology for predicting meat quality and assessing dark cutting in live animals (cattle) in real time.

3.2. Determine the sensitivity of SORS data to key biomarkers of beef meat quality.

3.3 Build predictive models for qualitative and quantitative analysis of beef meat quality in real-time from SORS data.

3.4 Work with both the livestock industry and instrumentation companies to optimize SORS for potential future use in abattoirs to guide better decision making on handling and lairage requirements and dark cutting outcomes.

4.0 Methodology

5.0 **Samples of commercial beef cuts**

6.0 The SORS system was first used to investigate various beef cuts including rump, Scotch fillet, round, chuck, tenderloin, and T-bone. This study has shown for the first time that monitoring of the changes in glycogen, fat and protein content of muscles can be investigated directly and, most importantly, non-invasively within various meat cuts. The spectral results reveal differences in structure-sensitive

bands from the amide I and III regions as well as from cysteine, glutamic acid and phenylalanine vibrations. Statistical analysis confirms these observations are based on strong clustering of SORS bands measured for the different cuts.

7.0

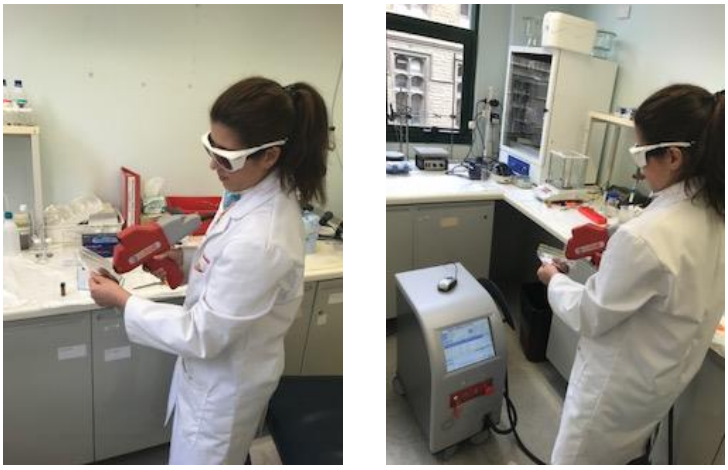


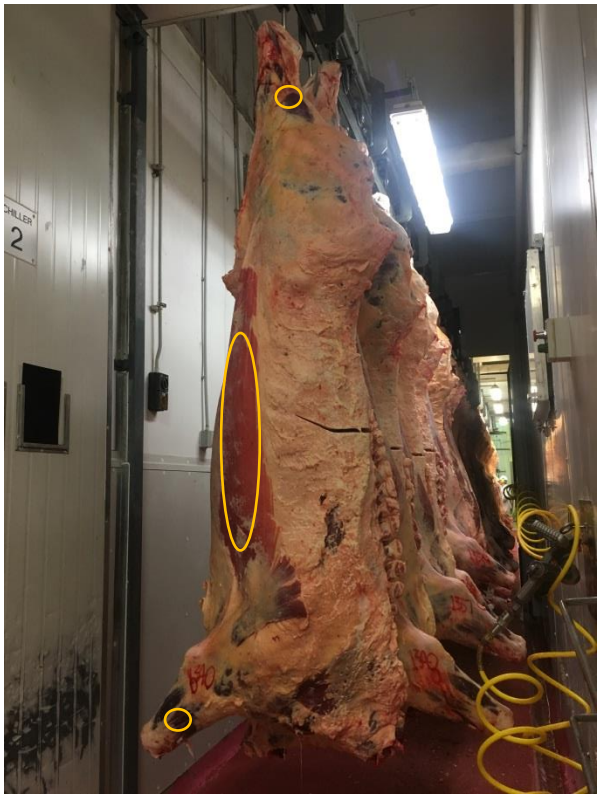
Figure 1. Example of use of the SORS spectrometer for measuring beef samples. The PDRA employed on the project, Dr Saeideh Ostovar pour, is pictured.

Figure 2: SORS measurements could be made on both plastic-wrapped and unwrapped samples. This is an advantage of the SORS design, enabling potential use in retail environments.

8.0 Further studies in Year 2 confirmed that these correlations between SORS marker bands and indicators of meat quality and dark cutting were maintained in spectra measured from beef carcasses at an abattoir. In Year 3 for Milestone 9, we have investigated if interpretable spectra of beef meat tissues could be obtained through the hair, skin and extra-muscular fat found in real animals, which would establish the viability of SORS for measuring markers of beef quality and dark cutting within animals.

9.0 **Samples from carcasses at an abattoir (JPS Hurstbridge, Melbourne VIC)**

10.0 Spectra were collected from 118 carcasses on the operating chain line, in coordination with Dr Steph Fowler (NSW DPI) and abattoir staff. Spectra were collected from 3 points on each carcass, as shown in Figure 3.



11.0 Fig 3. The site of SORS measurement for the experiments on beef carcasses (yellow ovals).

12.0 Spectra were collected as early post mortem as possible within the slow processing chain, through a slit made in each carcass at the grading site. The main measurement focused on collecting several spectra along the loin muscle and if time allows further measurement were performed on different areas of carcass where the subcutaneous fat was minimal, such as muscles in the leg area (semitendinosus) and around rib/abdomen areas. Immediately after spectra were collected, the pH at the same sites were measured. Carcass data including lot number and body number were recorded by abattoir staff.

13.0 At 24 h post mortem carcasses were graded and boned out as per normal abattoir processes. Once graded, spectra, pH and wet chemistry were compared to grading data to determine what spectral differences exist between dark cutting and normal carcases early post mortem as well as identifying how this relates to pH, glycogen and lactate concentrations early post mortem. It would have been

beneficial to have carcass breed reported as well as the skin thickness differs greatly for different breeds, but unfortunately this information could not be provided.

14.0 Samples from a cow including representative hair, skin and fat layers

15.0 Samples were acquired from a dairy cow that had died the day previous to taking measurements (2nd June 2022). An approximately 20x20x15 cm block of tissue was removed from each of the neck, loin and leg regions and stored at 4°C. These samples had cross-section of different layers of tissue, these being hair, skin, subcutaneous fat, and muscle. Prior to spectroscopic measurements, samples were placed into a bag and heated to 30°C in a water bath to help simulate measurements on a live cow.

16.0 SORS Measurements

17.0 Measurements were performed using 0.20 seconds exposure time and 0.4 seconds offset exposure time with both zero and offset lasers were set to maximum power, which is 450 mW at the source with 5 mW at the sample. The SORS algorithm was set to baselined offset and the baseline type set to none. The laser excitation wavelength was 830 nm with a spectral resolution of $1cm^{-1}$ over a 200-1800 cm^{-1} region of data being collected. Samples were wrapped in commercial cling film to protect the SORS probe from damage or contamination.

18.0 10 measurements were collected across the surface of each tissue layer of each sample. 5 of these measurements were tightly clustered in an approximately 2x2 cm square, with the other 5 measurements widely scattered over the remaining approximate 20x20 cm surface of the tissue sample layer. This was done to determine if there were any heterogeneity in the sub-surface measurements. **Error! Reference source not found.** shows an example of a tissue sample, and the locations where the different measurements were taken; clustered measurements were taken within the green box and scattered where taken within the yellow. Each tissue section was analysed with the hair on, then the hair removed, followed by the skin being removed, and lastly the subcutaneous fat being removed. **Error! Reference source not found.** summarises the measurements taken by region and tissue layer that was removed.

19.0 Spectral processing

20.0 The resulting spectra were processed using Unscrambler 11.0. Smoothing was performed using a Savitzky-Golay algorithm (polynomial order: 1, 5 symmetrical points). Detrending and baselining were applied to the spectra before being normalised by peak area.

21.0 Project Outcomes

As this project outcomes are directly linked to the question of SORS data interpretation for detecting DFD dark cutting, this is detailed in Section 22.0 Discussion, below.

22.0 Discussion

The author should include a full interpretation of the results.

Principally the SORS instrument used in this project was designed for identification of hazardous materials, explosive and raw pharmaceutical compounds. In order to adapt this instrument for biological samples in this case muscle foods, several optimizations procedure have been performed to minimize the variation in the data. The samples was measured trough plastic wrapping to minimize the chance of contamination. It should be noted that clear plastic wrapping is not going to interfere with the actual signal from meat as this instrument initially was designed to do measurements through barriers such as plastic, glass, paper, etc.

SORS spectra had previously been measured for normal beef samples, and were now measured for the provided dark cut beef samples. Over 50 measurements were made for different parts of the beef loin cut (provided by Dr Stephanie Fowler). The chosen areas had minimal fat content in order to make sure the signal from fat doesn't interfere with the actual signal from the meat tissue.

Figure 2: Example SORS spectra of normal (red line) and dark-cut beef (black line). Spectral measurements typically took less than 30 seconds for each chosen area.

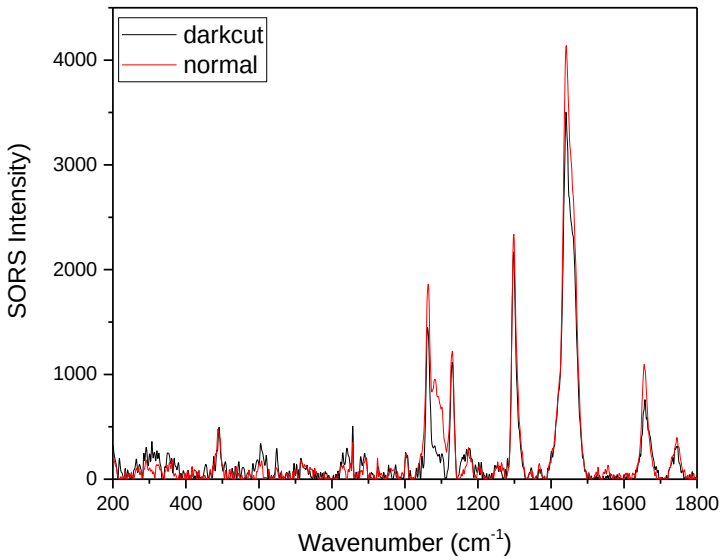


Figure 4: Examples of raw SORS spectra highlighting typical differences measured for DFD dark cut and normal beef samples.

As has been previously established by other researchers, the occurrence of dark-cutting is usually associated with depletion of glycogen from muscle stores prior to slaughter. The amount of glycogen in beef muscle plays a crucial role as it drives the extent to which the post-mortem muscle acidifies. Example SORS spectra of normal and dark cut meat are shown in Figure 2. The spectra show very similar features to each other apart from the intensity change in the 100-1200 cm^{-1} region that was reported previously by us in our Milestone 2 report is responsible for glycogen content in the meat samples. As shown above, good quality SORS spectra were readily obtainable for both normal and dark cut beef. As band differences between normal and dark cut beef are clearly above signal-to-noise levels, the spectra are able to readily discriminate dark cutting.

Figure 3 shows a zoom-in of the data in Figure 1 of the 900-1200 cm^{-1} region that we previously identified as containing marker bands for glycogen (Figures 3-5 represent the glycogen marker bands in the beef samples that were reported previously). A clear decrease in the SORS bands intensity associated with dark cutting at 1064, 1082 and 1128 cm^{-1} confirms that the glycogen content has been decreased.

The amount of glycogen is important because it drives the extent to which the post-mortem muscle acidifies and, below a pH of 5.7, myoglobin can maintain its oxygenation and confer abright red color to the meat

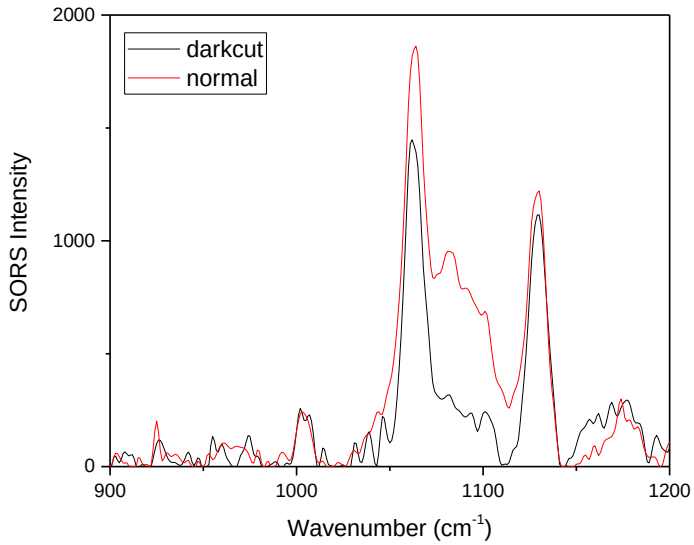


Figure 5: Zoom-in of SORS spectra of normal (red line) and dark-cut beef (blackline) from 900-1200 cm^{-1} region

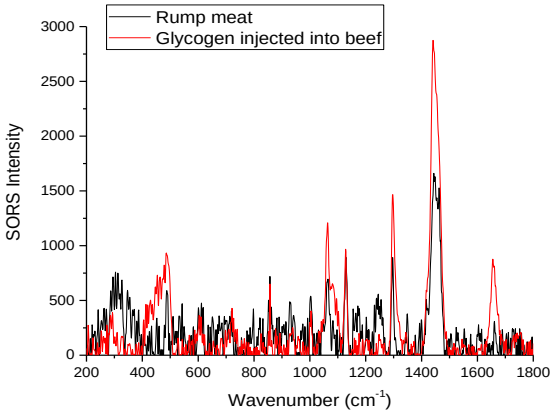


Figure 6: SORS spectra of rump beef (black line) and beef sample which was injected with glycogen solution

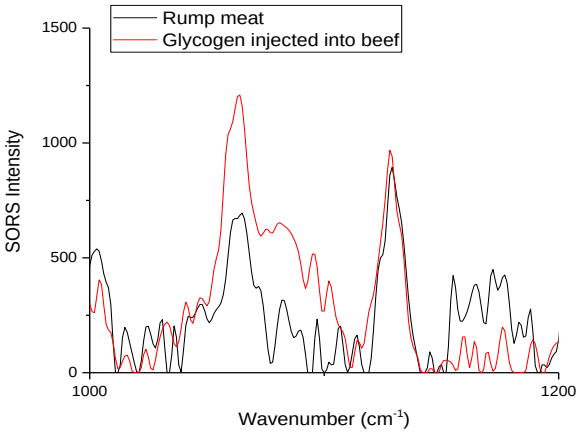


Figure 7: Zoomin of 1000-1200 cm^{-1} region of Figure 4

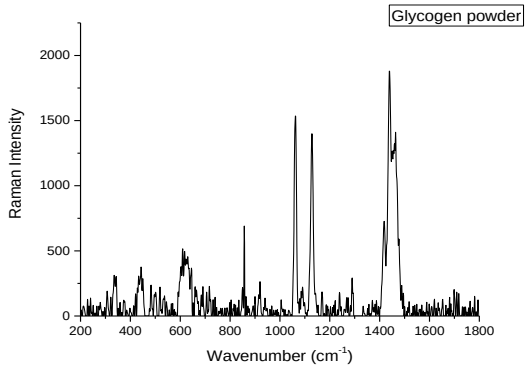


Figure 8: SORS spectra of glycogen powder

3. Chemometric models for prediction of dark-cutting

To further validate these spectral results for normal and dark-cut beef, chemometric analysis has been applied. Principal component analysis (PCA) is commonly used as a statistical tool to reduce the dimensionality of a data set which consists of a large number of interrelated variables (such as Raman spectra of meat samples), while retaining as much as possible of the variation present in the data set. This is achieved by transforming the original data to a new set of variables, the principal components (PCs), which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables. There was no need to remove background signals as SORS provides fluorescence free spectra in contrast with conventional Raman spectroscopy. An intensity normalization procedure only (minimal data pretreatment) has been performed prior to data analysis. Figure 7 presents the principal components analysis (PCA) of SORS spectra measured for both normal and dark-cut meat beef (50 spectra) that reveals clear differentiation of normal and dark-cut beef into separated groups.

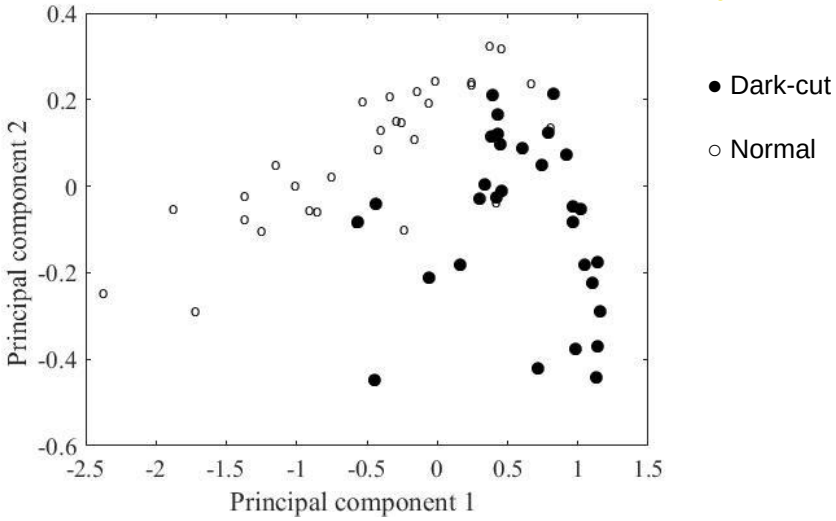


Figure 9: PCA analysis of SORS spectra for dark-cut and normal beef for principal components 1 and 2. Distinct clustering of the spectra is observed, confirming that the SORS data measured are able to reliably identify biochemical signatures of dark cutting.

4. Examine the potential for the spatially offset Raman spectroscopic device to measure carcasses early post mortem

Spectra were collected as early post mortem as possible within the processing chain through a slit in the sub-cutaneous fat at the grading site. SORS was successfully capable of collecting spectra after 24hrs post mortem. The differences between fresh meat sample and dark cut meat can be observed in Figure 8. Further chemometric analysis revealed that normal, dark-cut meat and fresh sample can be differentiated into three different groups.

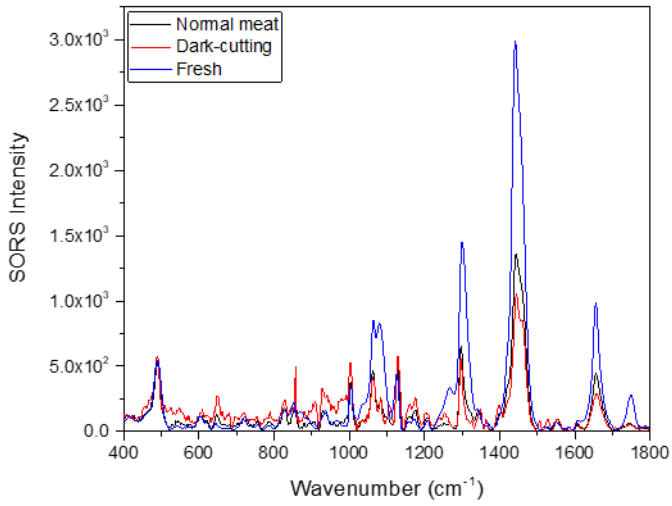


Figure 10: SORS spectra of normal (black line), dark-cut meat (red line) and 24hrs post mortem meat (blue line).

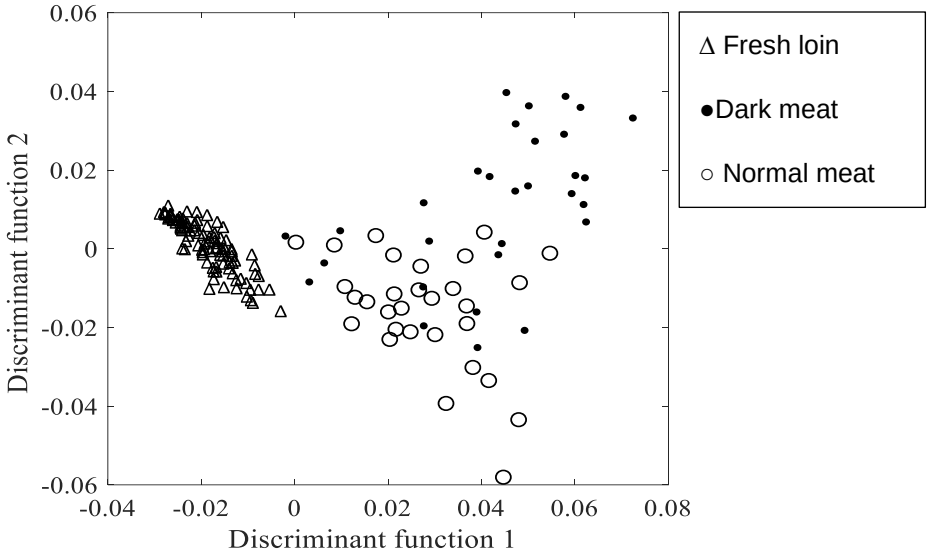


Figure 11: PC-DFA analysis of SORS spectra for fresh loin, dark-cut and normal. Distinct clustering of the spectra is observed, confirming that the SORS data measured are able to reliably differentiate each group.

5. Demonstration of successful use of SORS at a commercial abattoir

The measured spectra from carcasses that was collected on the day of killing were compared with carcass data which was provided later, and predictive model was build according to the pH, meat and fat color. Figure 10 shows the PLS modeling of 118 carcasses which used to predict the pH values according to the measured SORS spectra. This figure clearly shows the DFD meat can be predicted at early postmortem by predicting the pH values as most DFD cuts got pH above 5.6.

The same principle was applied for building chemometric models of meat and fat color. The Data in figure 11 clearly shows that the presence of DFD meat is predictable according to the meat color (meat color above 4 usually classified as DFD) in combination with the SORS spectra, however, the results from fat color are not consistent (figure 12) as DFD meat can have fat color varying over ranges of 1 to 3.

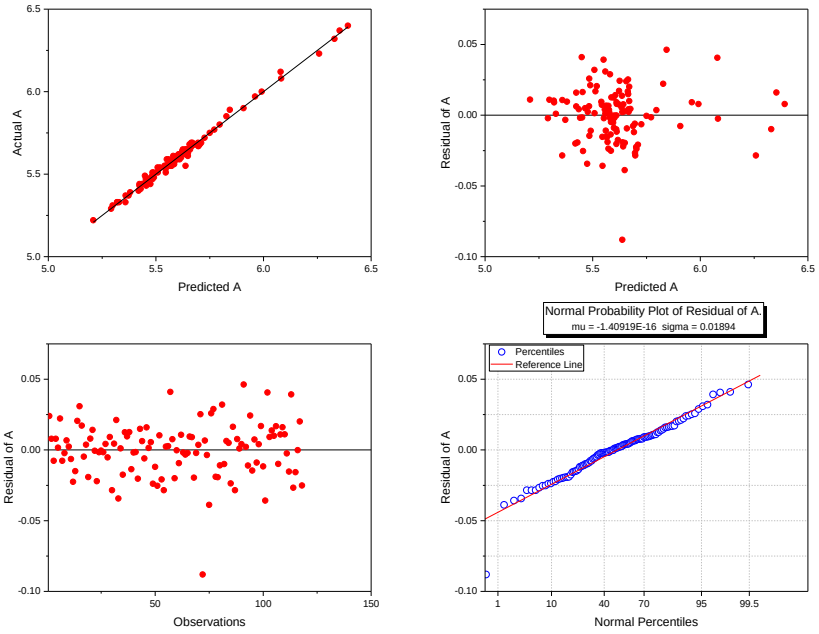


Figure 112: PLS modelling of 118 carcasses using the SORS spectra and pH value

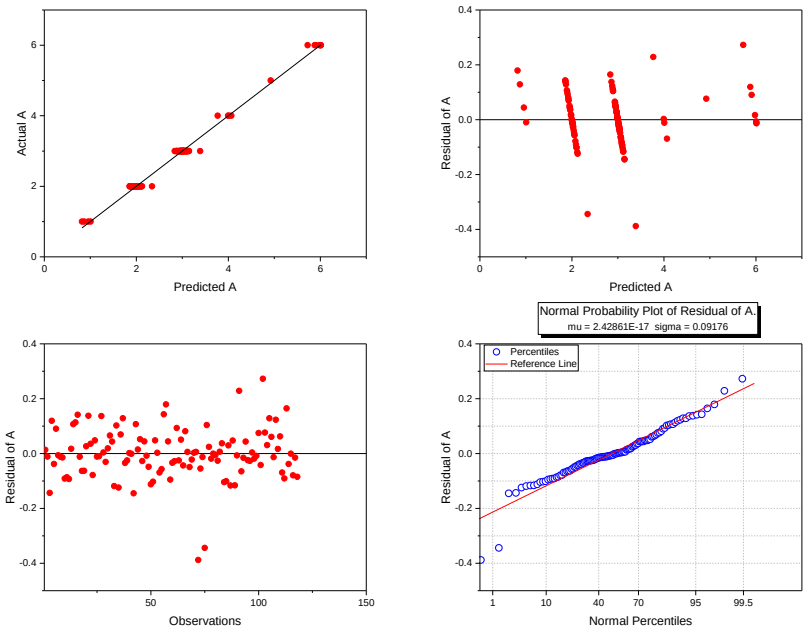


Figure 13: PLS modelling of 118 carcasses using the SORS spectra and meat color

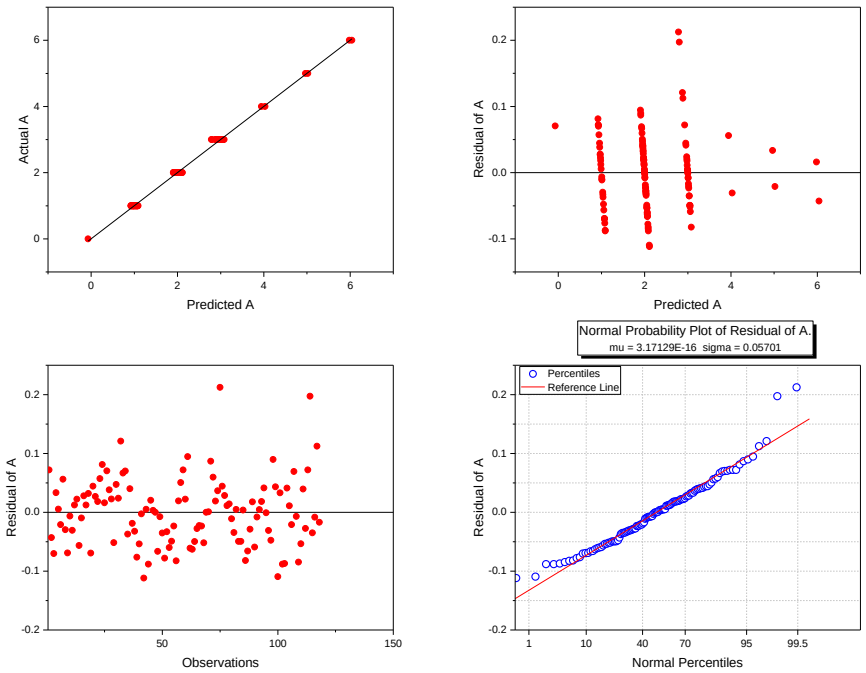


Figure 14: PLS modelling of 118 carcasses using the SORS spectra and fat color

6. SORS data collected for different muscle type in various beef cuts

The main objective of this study was to apply deep tissue Raman spectroscopy, SORS, so as to identify spectroscopic signatures for different cuts of beef. The sensitivity of this method for differentiating between very closely related muscle types within the same species was assessed. In this study six different common beef cuts (rump, Scotch fillet [rib eye], tenderloin [eye fillet], chuck, round and T-bone) were examined using SORS to differentiate between each cut by probing 5 millimetres beneath the surface of meat tissue. Chemometric methods were then applied to identify relationships existing between each sample allowing us to differentiate them into distinct groups.

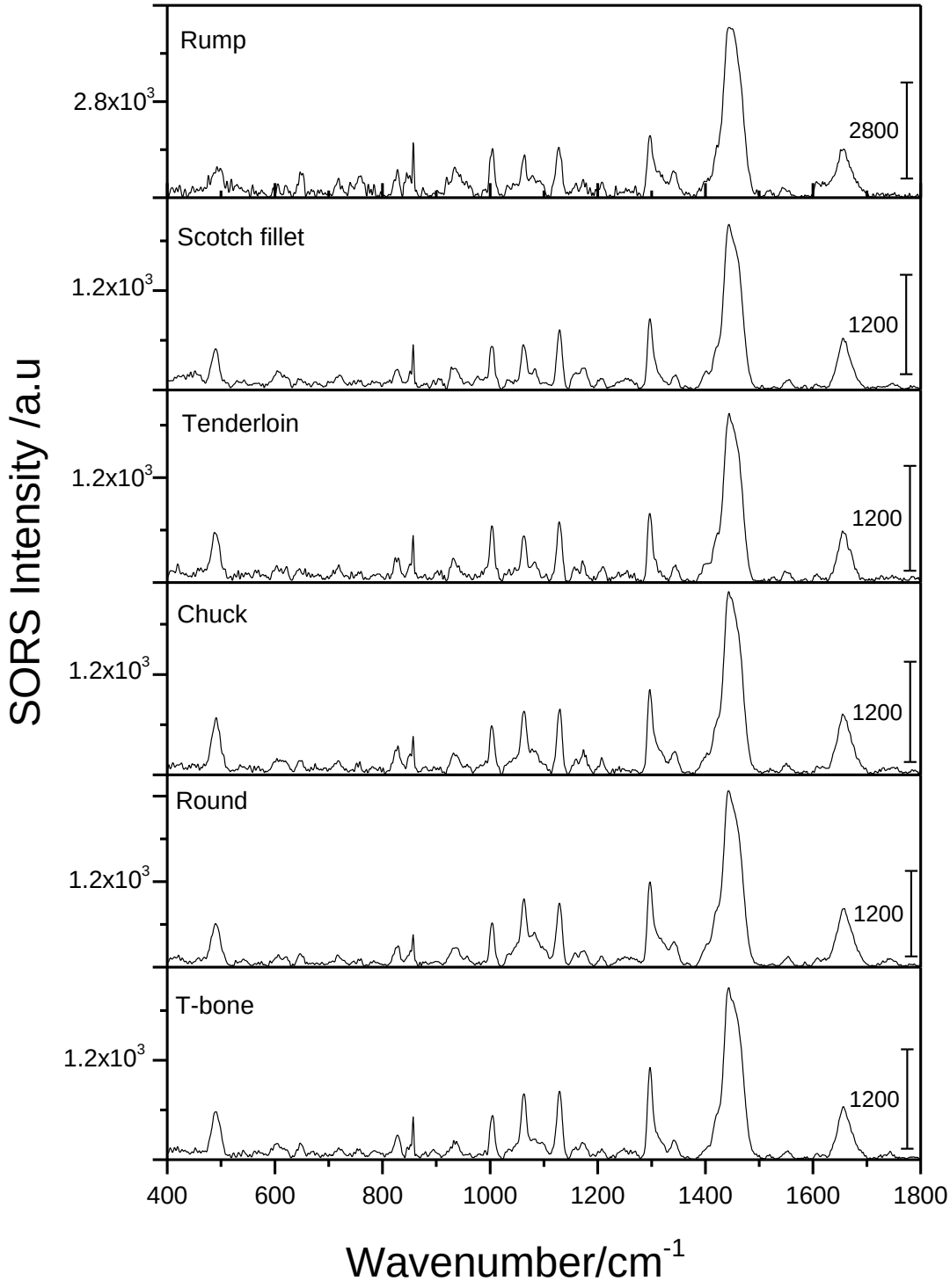


Figure 15. SORS average spectra (n = 200) of rump, Scotch fillet, tenderloin, chuck, round and T-bone cuts of beef. All were measured at 830 nm with an accumulation time of 30 seconds.

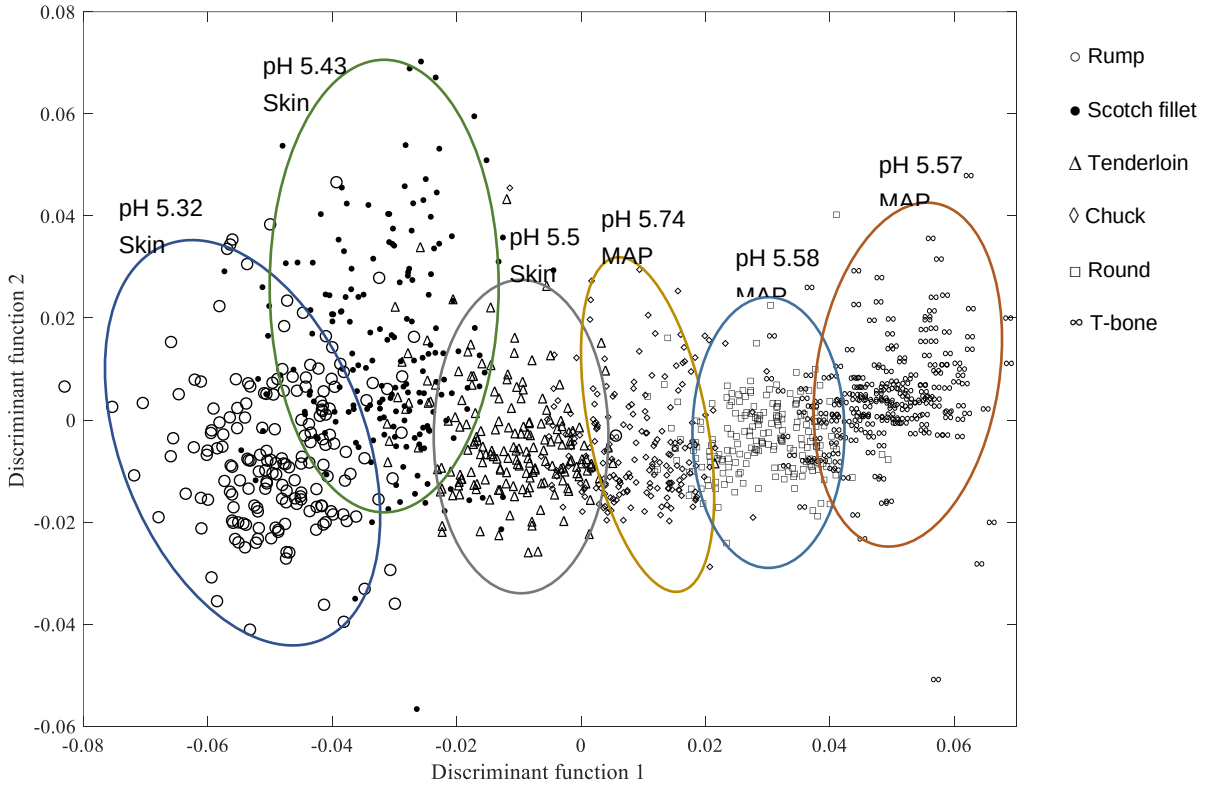


Figure 16. 2D scores plot of PCA-DFA for rump, tenderloin, Scotch fillet, chuck, round and T-bone using PCA scores. Ovals are used as guides to the reader to more clearly show clustering of data points, but are not intended to imply defined limits for each grouping.

Year 3

Results and interpretation of the measured SORS spectra

Figure 15 shows the average of both clustered and scattered spectra from each section by the layer of tissue on which the measurements were performed. Preliminary observation of the resulting spectra shows a number of peaks from each layer, however little detail can be seen in the hair on spectra taken from the neck region. Hair on spectra from both the loin and leg region show similar features to hair removed spectra, suggesting that SORS measurements in these regions may pass through the hair layer and into the skin to some degree. These features in the spetcra from the loin and leg regions also correspond to features in our previously measured spectra of beef samples, indicating that they are sensitive to the biochemical makers of beef quality and the presence or absence of dark cutting characteristics.

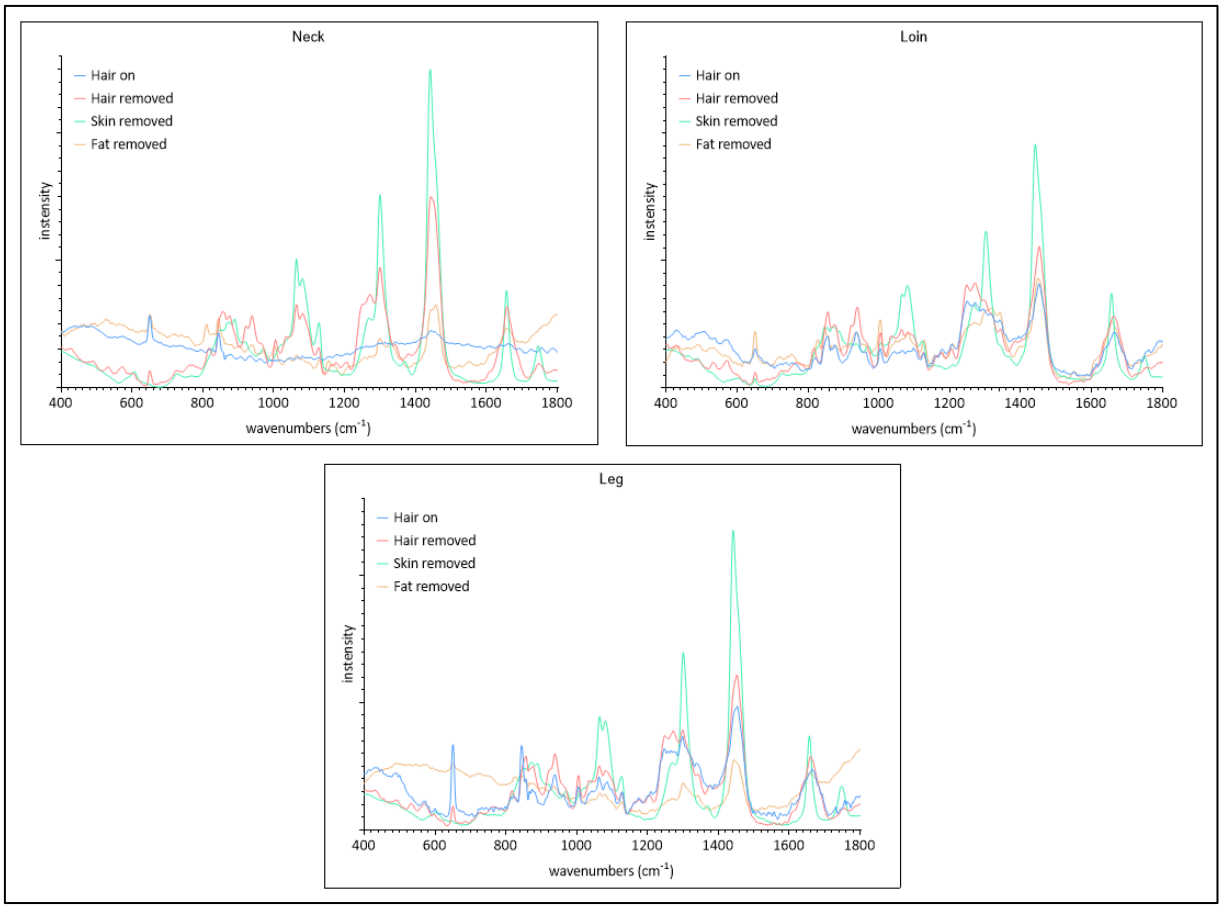


Figure 17. Average SORS spectra from each section of the cow samples (neck, leg and loin) by tissue layer (Hair on, Hair removed, Skin removed, Fat removed).

Therefore, these experiments indicate that the leg and loin regions of cattle offer good opportunities for collecting relevant data from live cattle.

23.0 Conclusions / Recommendations

The aim of this project was to evaluate the potential for SORS spectroscopy to detect the biochemical changes that relate to DFD dark cutting in cattle that could then be used within the beef industry to guide informed lairage decisions. This would then be of potential use within the meat processing and farming sectors, reducing the current financial losses that DFD leads to, with other possible future users being the food retail sector and consumers of beef products who would benefit from more evidence based decisions when purchasing beef. Studies progressed from the lab under controlled conditions to situations fundamental to the beef industry (an abattoir and a cow). We have shown that SORS is sensitive to the biochemical information required to DFD dark cut meat from normal meat, and this can be done within the working practices of a commercial abattoir, and the required data can be obtained through the hair, skin and fat layers of cattle. Therefore, we conclude that SORS is a noninvasive and chemical-free technology for predicting meat quality and assessing dark cutting in live animals (cattle) in real time. The repeated border and abattoir closures have prevented our access to live cattle with known histories and with an statistically useful proportion of carcasses. Therefore, while the proof of principle of SORS as a tool for lairage decision making has been demonstrated, further work is required to help translate the technology into the meat processing sector. This should consider:

i) Working with the SORS instrument manufacturer (Agilent) to improve focal length further than the current 8 mm and improve fieldwork compatibility. Agilent's international headquarters for spectrometer development is in Melbourne, and the company encourages academic collaboration where there is a potential for translation to new sectors (such as agriculture and food), and Agilent and CI Blanch have previously discussed the potential of SORS in this respect.

ii) Now that Covid-restrictions are reduced, working closely with NSW DPI and selected abattoirs to formalise protocols for collecting data under diverse conditions relevant to the meat processing sector.

iii) Expand the database with data collected from many more cattle with known histories in order to develop the analytical tools that can be used by non-experts across the industry.

Together, these would help to establish SORS as a practical tool for the meat processing industry.

Furthermore, our studies have also shown that SORS spectra are sensitive to subtle changes in biochemical composition and pH that are characteristic of common beef cuts. This indicates that the acknowledged advantages of SORS (fast, non-invasive, able to measure spectra beneath a surface, chemical free) could be used to develop new tools for detecting other changes in beef quality due to animal breed, authenticity and history, as well as other environmental factors. The high sensitivity of SORS spectra to biochemistry coupled with it's now demonstrated wide applicability to beef raises possibilities for exploring its potential to address other challenges in the beef industry.

24.0 Bibliography

The author should include all references used in the report or referred to for background information. This must be done using the Harvard Referencing Style Guide.

Scientific papers directly produced from this project

Ostovar pour, S., Fowler, S.M., Hopkins, D.L., Torley, P.J., Gill, H. and Blanch, E.W. 2019, 'Investigation of chemical composition of meat using spatially off-set Raman spectroscopy', The Analyst, doi:10.1039/c8an01958d

Ostovar Pour, S., Fowler, S.M., Hopkins, D.L., Torley, P.J., Gill, H. and Blanch, E.W. 2019, 'Investigation of chemical composition of meat using spatially off-set Raman spectroscopy', Analyst, vol. 144, no. 8, pp. 2618-2627

Ostovar Pour, S., Fowler, S.M., Hopkins, D.L., Torley, P., Gill, H. and Blanch E.W. 2020, 'Differentiating various beef cuts using spatially offset Raman spectroscopy', Journal of Raman Spectroscopy, vol. 51, no. 4, pp. 711-716.

25.0 Appendices

The author should any supporting documentation which has been referenced in the report. Each Appendix must be named and numbered.

No appendices are included.