Final report Phase 1

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Quantifying the benefits of developing a CT marbling solution (Phase 1)

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Abstract

This project has been conducted to gain an understanding on the feasibility of estimating total fat and lean from a hot boned out beef primal and the estimation of intramuscular fat in a primal.

A study on 1 primal was conducted to demonstrate that a significant difference ($P < 0.001$) existed in the amount of fat estimated between 2 and 15, 25, and 35 degs C. A further study using 6 primals also provided conclusive evidence that differences do exist between different temperatures. A mixture model provides greater accuracy when estimating fat e.g. the muscle and fat proportions at 35 degC had an R^2 of 0.99.

This study:

- ✓ Found that it is feasible to measure fat under hot-boning conditions and after evaluating several methods determined that an instantaneous method of estimating fat at hot boning could be developed.

- ✓ Confirmed the large variability that exists within primals for MSA grade versus chemical intramuscular fat (%). The correlation between MSA grade and chemical intramuscular fat (%) ranged from 0 to 0.94 across 20 primals.

- ✓ Developed an equation ($AdjR^2 = 0.84$) to estimate intramuscular fat in a primal but this equation still needs an independent evaluation.

Executive summary

This research project conducted by Agriculture NSW, NSW Department of Primary Industries, has investigated a "proof of concept of estimating intramuscular fat from CT images". To conduct this work Phase 1.1 determined if differences exist in the estimate of kilograms of total fat from CT images at a range of temperatures and Phase 1.2 determined the relationship between MSA marbling score, chemical intramuscular fat (%) and the intramuscular fat (%) from CT images and Phase 1.3 developed an equation that may assist in the estimation of intramuscular fat (%) in a primal cut.

Four studies within Phase 1.1 were conducted and the key results from these studies are outlined below:

Study 1

- Time taken to reach a specified temperature was determined (e.g., 160 minutes to reach 12 deg C)

Study 2

- A study on 1 primal demonstrated a significant difference ($P < 0.001$) in the amount of fat estimated between 2 and 15, 25, and 35 degs C.

Study 3

- Non-linear nature of greyscale values exist across a range of temperatures.
- The boundary method (grey scale threshold values for fat, lean, and bone) and a mixture model method need to be evaluated.

Study 4

- Mixture model provides greater accuracy:
    - Muscle pixel count at 2 degC:
        - R^2 = 0.71 and 0.96, respectively for boundary method and mixture model.
    - Muscle and fat proportions at 2 degC:
        - R^2 = 0.81 and 0.97, respectively for boundary method and mixture model.
    - Muscle and fat proportions at 35 degC:
        - R^2 = 0.41 and 0.99, respectively for boundary method and mixture model.
- Instantaneous method of estimating fat at hot boning could occur

Key results from Phase 1.2:

- Variability within primals for MSA grade and chemical intramuscular fat (%) demonstrated.
- R^2 relationship between MSA grade and chemical intramuscular fat (%) ranges from 0 to 0.94 across 20 primals.

Key results from Phase 1.3:

- Equation needs to be evaluated with an independent data set.

Conclusions

Differences in estimating fat at different temperatures do exist and the results from this project indicate that a method to estimate hot-boning is feasible. However, the estimate of intramuscular fat from CT scanning images remains a challenge. The difference between the subjective MSA grade of intramuscular fat and the objective chemical fat measurement clearly illustrates the variability that exists between these 2 methods of estimating intramuscular fat (0 to 0.94 R^2 across 20 primals). Future studies on estimating fat from CT images and mixture model techniques have the potential to develop a simple and reliable technique for estimating total fat and lean in boned out primals, hence estimate retail beef yield.

It is also feasible that a selection of 3 images (i.e., slices at 10mm apart) from a primal could be used to estimate intramuscular fat in the whole primal.

Recommendations

- Develop a series of standards of known densities for fat and lean to be run for a range of temperatures.
- Develop software that can be integrated into a hot-boning chain to estimate lean and fat in an individual primal.
- Organise a meeting to determine the next steps and determine if phase 2 now needs to take place.

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Background

If hot boning was to be a standard procedure in abattoirs then a method of grading primals needs to be implemented. Computed tomography (CT) scans is a potential method that could be used to grade primals and also determine retail beef yield on an individual primal basis and on a total carcass (i.e., summed across all primals). Therefore the question has been raised: if CT scans were used to estimate fat in primals are there differences in the estimation of kilograms of fat across a range of temperatures? A proof of concept of visual assessment versus estimate amount of intramuscular fat has been undertaken.
Four studies were implemented to determine if differences exist in the estimation of kilograms of fat across a range of temperatures and methodologies that could be implemented to estimate fat in primals. A study of subjective versus objective measurements of intramuscular fat was also undertaken to provide the industry with correlations between various techniques used to estimate intramuscular fat.

Objectives

Phase 1.

1. Determine if differences exist in the estimate of kilograms of total fat from CT scanning images at a range of temperatures.

2. Determine the relationship between the MSA marbling score, chemical intramuscular fat (IMF) (%), and the CT scanning image of IMF (%) of the 10 mm slices.

3. Develop equations to estimate IMF (%) in primal cuts

Phase 2.

Perform an economic cost benefits analysis to determine the profitability of implementing a CT scanning technology into an abattoir. Detailed costs of placing CT scanning equipment into the supply chain will be collected. Data by a senior economist will fully evaluate the cost benefit.

Phase 1. Proof of concept of visual assessment versus estimated amounts of intramuscular fat.

Phase 1.1 **Determine if differences exist in the estimate of kilograms of total fat from CT scanning images at a range of temperatures.**

Study 1

Introduction

To evaluate “if differences exist in the estimate of kilograms of total fat in a primal cut across a range of temperatures” an initial experiment to determine the time taken to reach 2, 5, 15, 25 and 35 °C temperatures was conducted.

Materials and methods

Three different sized boned out vacuum packed Angus striploin primals [small (0.76 kg), medium (1.54 kg) and large (2.15 kg)] were purchased from a local butcher in Armidale NSW. The 3 Angus striploin primals were used in a temperature experiment to determine the time taken to reach an internal temperature of 5, 10, 15, 25, and 35°C.

The 3 Angus vacuum pack primals were taken from the cool room at approximately 2°C and placed in a water bath. Prior to this, the water bath was placed in the cool room and filled with water overnight so as to chill the water to below 5°C. Due to the size of the water bath, the small and medium primals were trialled first. All primals had been vacuum packed and they remained in that state throughout the trial. The vacuum packed primals were sealed with a strip of duct tape over the desired probing positions. Probes attached to a Pico meter were used: 1 probe was inserted into the small primal and 2 probes inserted into the medium primal. All probes were inserted at approximately half of their depth and the outside was sealed with petroleum jelly to seal off the vacuum packed primals. The large primal had the same treatment but had 3 probes inserted at different positions along the primal. A 4th probe was used to monitor the temperature changes in the water bath. Primals were placed in the water bath and it was turned to 5°C. The Pico meter was monitored closely to determine when exactly the meat was at the same temperature as the water. When all probes reached 5°C, the water bath was then turned up to 10°C and the same monitoring continued in increments of 5°C until 35°C was reached. This was the case for the two smaller primals. The largest primal went from 5°C to 35°C in increments of 10°C**.**

Results

Figure 1 illustrates the temperature curve for small and medium primals. The time taken to reach a specified temperature was then used as an indicator of the internal temperature of a primal cut and was subsequently used to estimate the internal temperature in additional experiments.

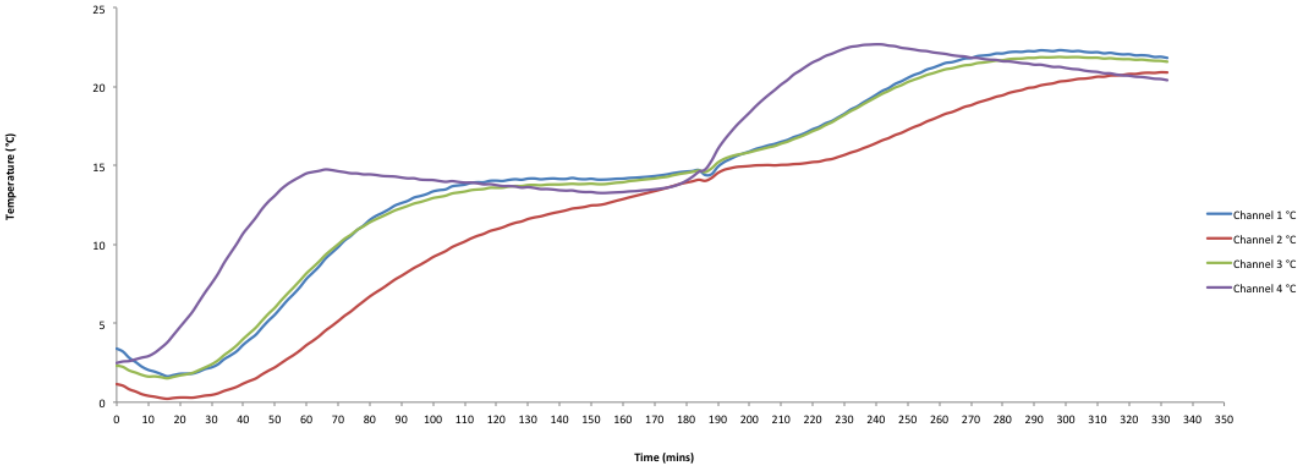


Figure 1. Time taken (minutes) to reach a specified temperature (deg C) using 4 channels inserted into the primals.

Conclusions

The results from this study demonstrated the time taken to reach a specified temperature in a water bath. The results from this study can be used in subsequent studies to remove a primal from the water bath at a specified temperature that could then be scanned for total lean and fat in the primal cut.

Study 2.

Introduction

This study set out to determine if differences in the estimation of fat using AutoCat exist at different temperatures on 1 primal. Therefore, the objective of study 2 was to estimate the amount of intramuscular fat (IMF) using the Autocat software in 1 primal cut at a range of temperatures.

Materials and methods

Two boned out vacuum packed Wagyu cube rolls were purchased from a local butcher; cube roll 1 (primal 1) weighed 2.84 kg and cube roll 2 (primal 2) weighed 2.76 kg. All primals were denuded (i.e. subcutaneous fat removed). Primal 1 was used to monitor temperature and primal 2 was used to determine if differences in the estimate of IMF existed at a range of temperatures.

The 2 primals were placed in a water bath; 1 primal used to monitor temperature and 1 primal used to estimate IMF. Details of the experimental procedure are outlined in study 1. Upon reaching the nominated temperatures (2, 5, 10, 15, 25 and 35°C) the 2 primals were then placed in a plastic container and carried to the UNE Meat Science CT (Philips Medical Imaging Australia, Sydney NSW) scanner so that scanned images could be used to determine the total amount of lean meat and IMF in 1 of the primals. The CT scanner was calibrated to take images at 1mm and 10 mm slices. Images at 1 mm slices were taken for primals at 2°C and 35°C and images at 10 mm slices were taken at 2, 5, 10, 15, 25 and 35°C. All results in this study are based on images at 10 mm slices. The primals were positioned in the same position, as marked on the scanner bed, for each scan so as to eliminate error in the images when comparing the amount of lean meat and fat at different temperatures. All images were converted to bmp files using ImageJ software (National Institutes of Health, USA) and the AutoCat and Calc (Neville Jopson, version 1998) software were used to estimate lean and fat components of the denuded primal at the temperatures mentioned above. The image diameter was set at 200mm and the grey scale values were set at 10 to 128 for fat, 129 to 210 for lean, and 211 to 255 for bone. All grey scale values were held constant across all temperature ranges. Software (McPhee, unpublished) in R code was developed to create a file so that fat and lean could be estimated in each slice. Comparisons across each slice and temperature were then made. The IMF percentage was determined as IMF (%) = (IMF in kg/(lean in kg + IMF in kg))*100

Results

Seventeen individual slices were obtained from the CT scans. At the same location on each slice of the image a section of muscle containing IMF was cut out using the elliptical tool in ImageJ (Figure 2).

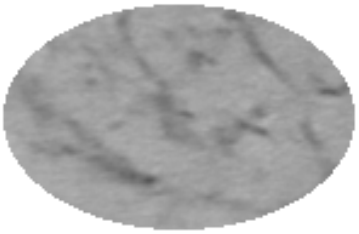


Figure 2. Image of a section of muscle containing IMF that was cut out of the image of an individual slice.

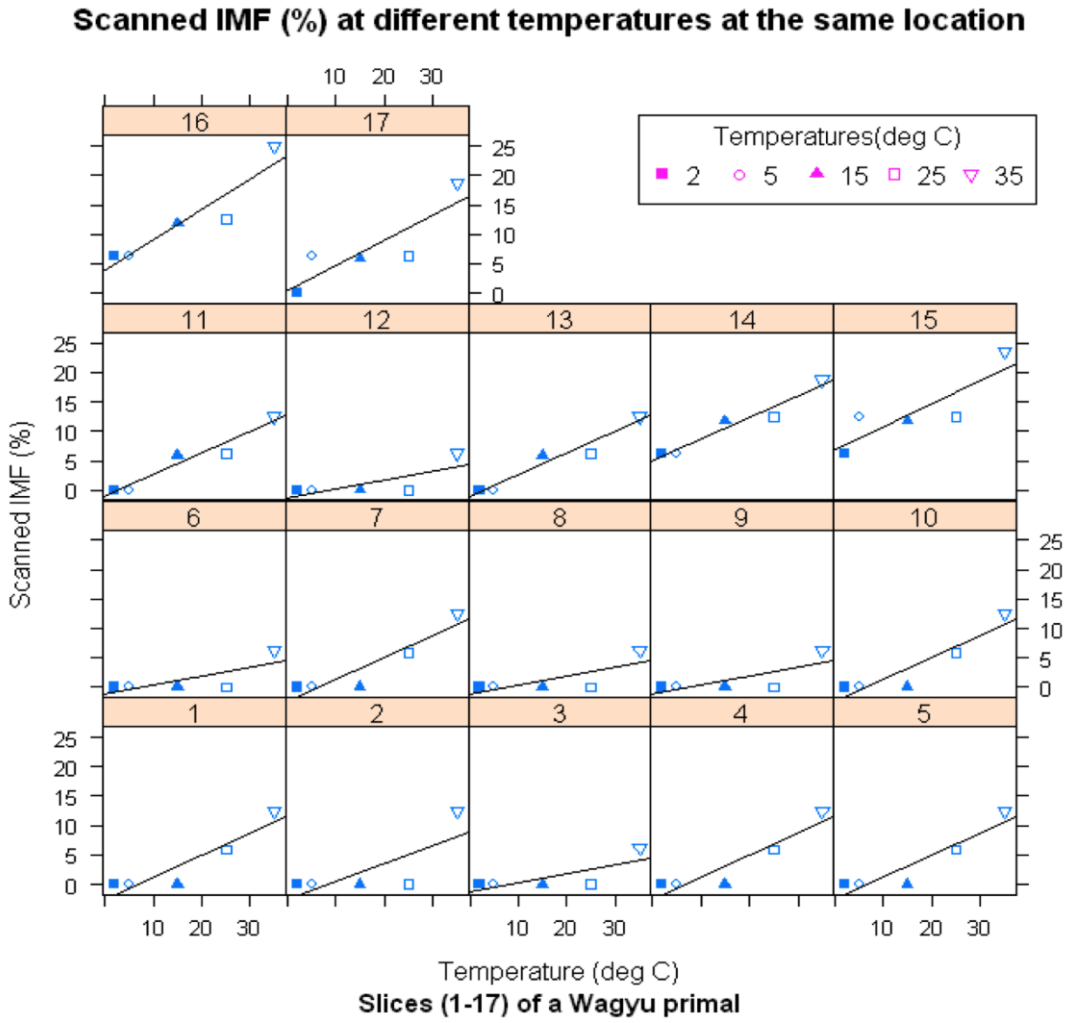


Figure 3. Scanned intramuscular fat (IMF; %) at different temperatures at the same locations along the Wagyu cube roll for individual slices 10 mm apart

AutoCat and Calc were then used to determine the amount of IMF in each of the slices with image diameter and grey scale values as mentioned above. Intramuscular fat percentage was then calculated. Differences in the amount of IMF (%) at different temperatures for individual slices were detected (Figure 3).

The mean total amount of IMF in grams of fat and standard error (s.e.) at each of the temperatures across all slices (n=17) are illustrated in Figure 4.

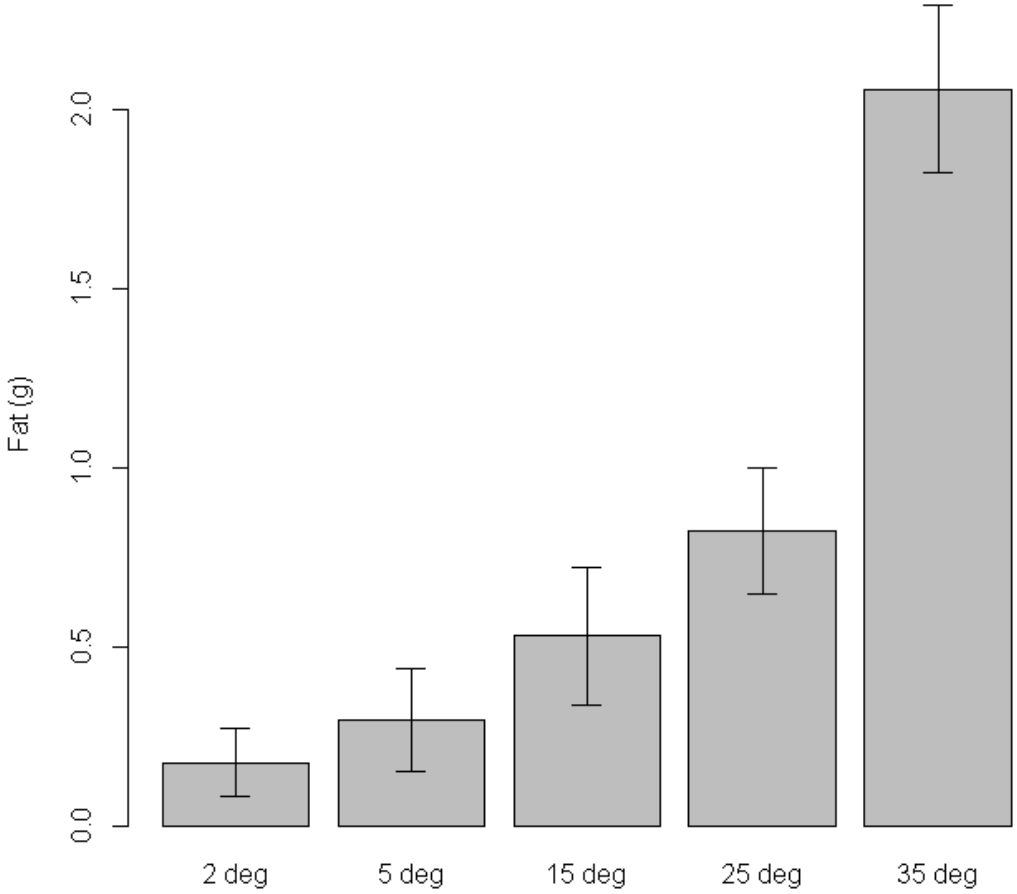
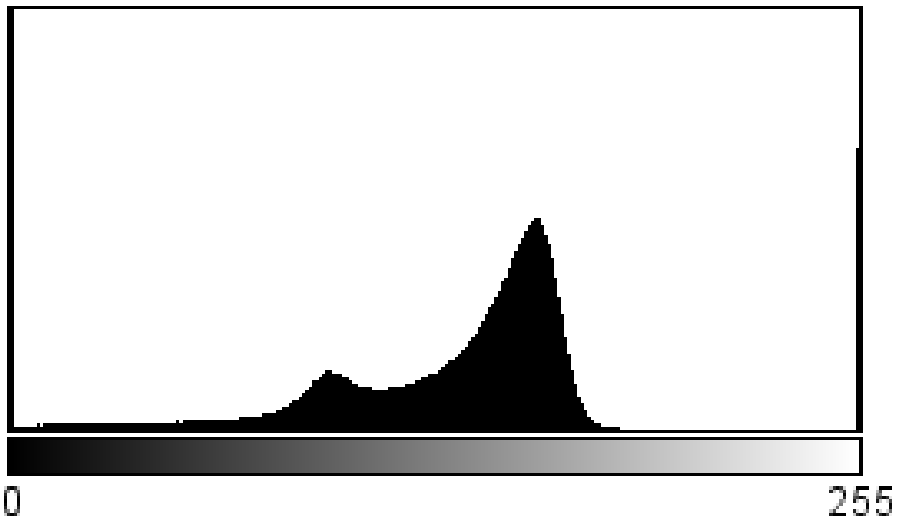


Figure 4. Mean (± se) grams of intramuscular fat from individual slices of a cube roll primal (n=17) across 5 temperatures (2, 5, 15, 25, and 35 deg C)

Figure 4 illustrates differences in the amount of fat at different temperatures. A students t test indicates a significant difference ($P < 0.001$) in the amount of fat detected between 2 and 15, 25, and 35 degs C.

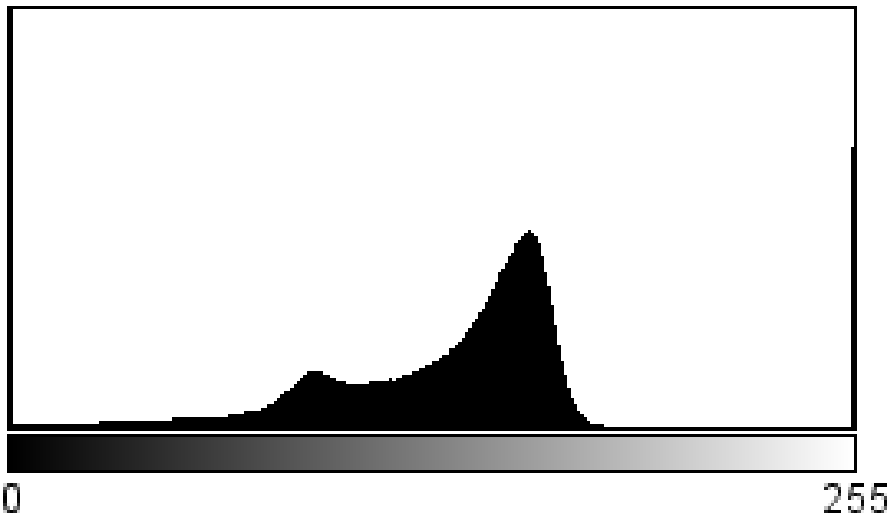
Bimodal distributions (Figure 5), developed in ImageJ, of each of the primals at different temperature were then visually assessed for the grey scale cut off points. The visually assessed grey scale cut off points are reported in Table 1.

Primal at 2°C



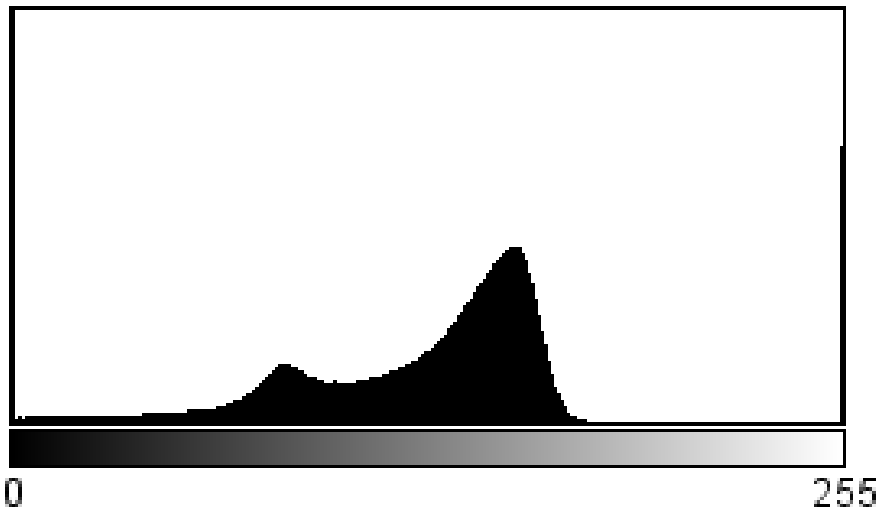
Count: 6029312	Min: 0
Mean: 39.441	Max: 255
StdDev: 65.671	Mode: 0 (4278670)

Primal at 5°C



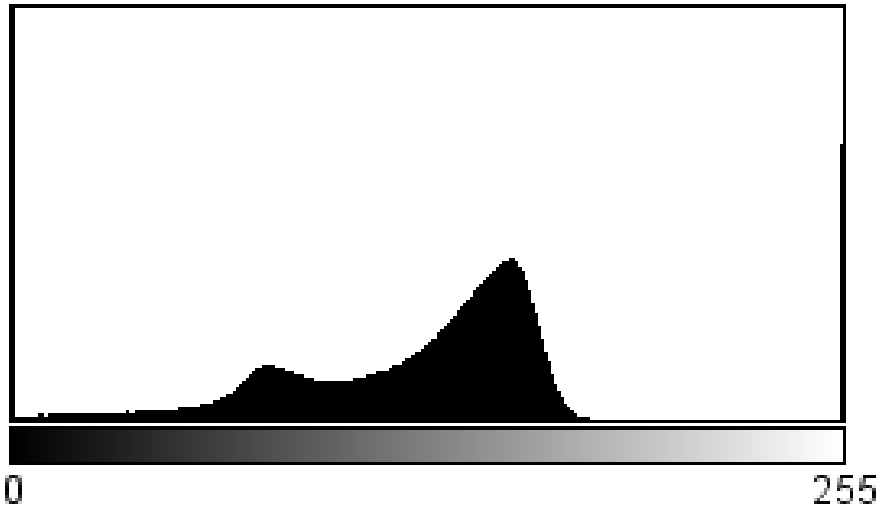
Count: 6029312	Min: 0
Mean: 38.830	Max: 255
StdDev: 64.709	Mode: 0 (4279646)

Primal at 15°C



Count: 6029312	Min: 0
Mean: 37.963	Max: 255
StdDev: 63.325	Mode: 0 (4257525)

Primal at 25°C



Count: 6029312	Min: 0
Mean: 37.599	Max: 255
StdDev: 62.702	Mode: 0 (4246928)

Primal at 35°C

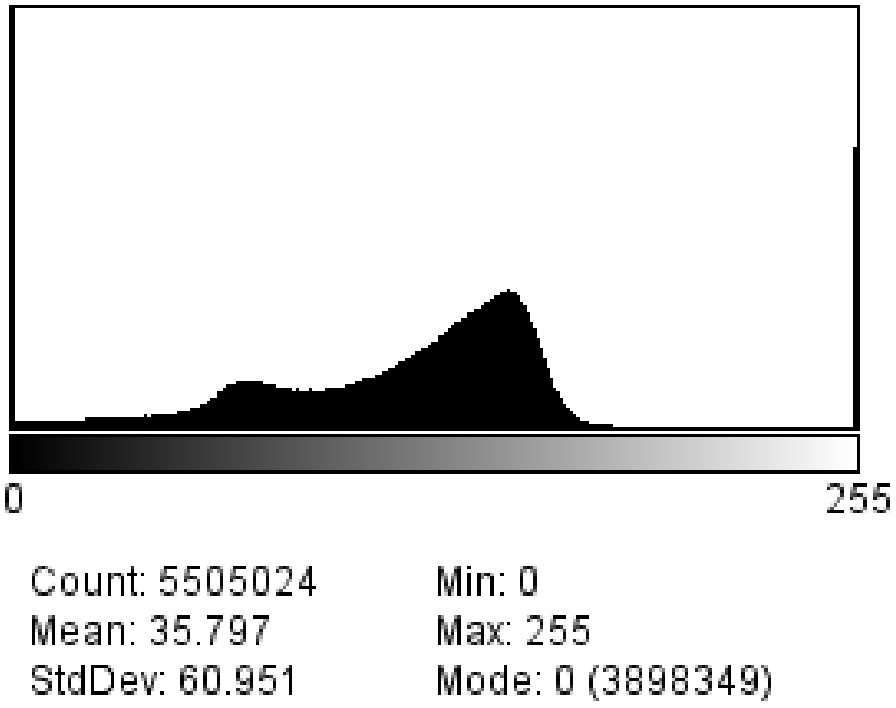


Figure 5. Bimodal histograms of grey scales that represent fat and lean meat at different temperatures.

Table 1. Minimum and maximum grey scale values at different temperatures

	Fat	
Temp deg C	Min	Max
2	10	110
5	10	106
15	10	104
25	10	100
35	10	99

Conclusions

Differences in the amount of fat, when grey scale values were held constant, did exist between a range of temperatures (Figure 4); a significant difference ($P < 0.001$) between 2 deg and 15, 25, and 35 deg was found. This study was only conducted on 1 primal therefore an additional study with 6 primals was conducted to: (a) determine grey scale values across a range of temperatures and different primals; and (b) investigate a technique that could be used to estimate fat at different temperatures.

Study 3

Introduction

Having established from Study 2 that differences in the estimation of fat exist at different temperatures a more conclusive study on 6 primals was conducted and the techniques of estimating fat were to be evaluated. Therefore the objectives of study 3 were to conduct a detailed experiment using 6 primal cuts to: (a) determine grey scale values across a range of temperatures; and (b) investigate a technique that could be used to estimate fat at different temperatures.

Materials and methods

Six striploins (primals 1 to 6) were purchased from a local Armidale butcher. All primals were denuded and immersed in a water bath until the desired temperature range was reached (Table 2) before being CT scanned. A 7th primal of similar dimensions to the other 6 was used to indicate primal core temperature. Three probes attached to a Pico TC-08 eight channel thermocouple data logger (Pico Technologies Ltd, Cambridge UK) were inserted (far left, middle, far right) into the primal and temperatures recorded (as described above in study 1)

.

Table 2. Mean internal temperature (n = 6) of primals (±SE) at time of CT scanning

Temperature	Mean ± SE
1	2.5 ± 0.3
2	5.2 ± 0.3
3	16.1 ± 0.3
4	26.1 ± 0.1
5	35.3 ± 0.2

All primals were scanned at the UNE Meat Science facilities using a recently purchased second-hand GE HiSpeed QX/i gantry CT scanner (GE Health Care, USA). The field of view was set at 300 mm thickness and each slice set at 10 mm. Images obtained were analysed using ImageJ software (National Institutes of Health, USA) to remove the scanning bed and save files as bmp files. A histogram of pixel values (grey scales) showing a bimodal distribution for each primal at each temperature was produced (Figure 2). A UNE CT scanning technician (personal communication) indicated that these bimodal distributions were viewed to assess the cut off points of the grey scale upper and lower threshold limits for fat, lean, and bone (note: primals used in this study were boneless). However, it is feasible to use ImageJ to assist in determining the threshold values for fat and lean. In this study we have investigated a technique that could be used in a processing plant that would have the scanning of primals and the estimation of fat and lean automated.

The steps to determine the threshold values in ImageJ are outlined in Table 3. After conducting the steps in Table 3 values were saved to an excel sheet and a weighted average using the area calculated. All weighted values are standardized values. The lower limit for lean= upper limit for fat + 1 and the lower limit for bone= upper limit of lean + 1.

Table 3. Steps required in ImageJ to analytically determine the threshold

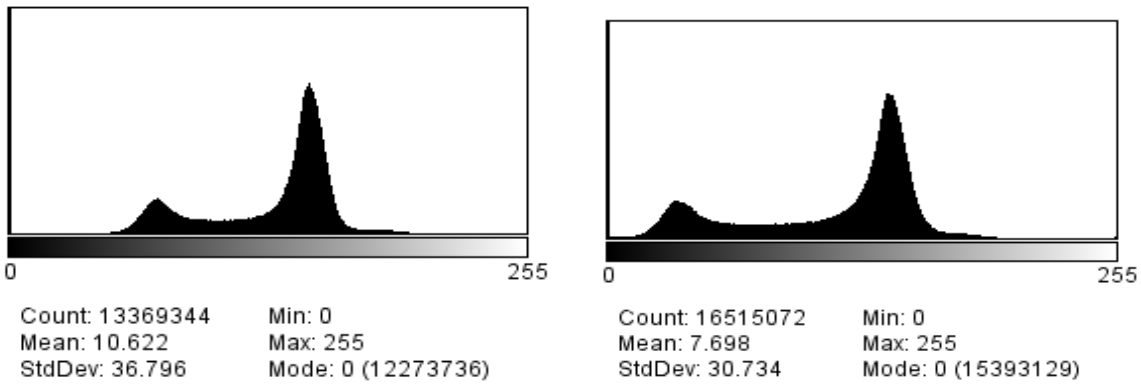
Steps	What to do or name of tab to select		
1	Import the sequence		
2	Image		
3	Adjust		
4	Threshold		
4.1		Auto	
4.2		Apply	
4.2.1			Tick box for calculation of threshold for each slice
5	Again Import the sequence		
6	Image Calculation		
6.1		Subtract	
7	Analyse		
7.1		Set measurements (Area, min, max grey scales)	
8	Plugins		
8.1		Stacks	
8.1.1			Measure Stack

Results

The images of each of the primals were loaded onto ImageJ and analysed. Figure 6 illustrates a histogram of pixel values (grey scales) showing a bimodal distribution for each primal at each temperature.

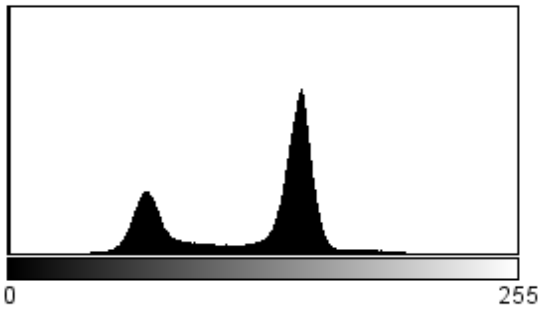
Primal #3

2.5 ± 0.3 °C	35.3 ± 0.2 °C

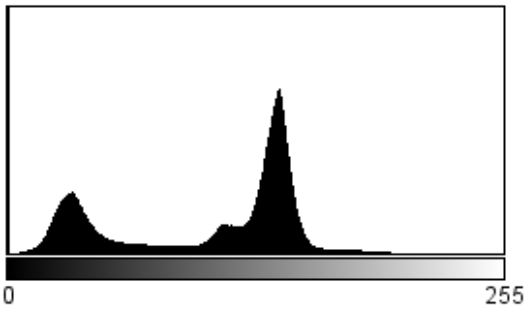


Primal #11A

2.5 ± 0.3 °C	35.3 ± 0.2 °C



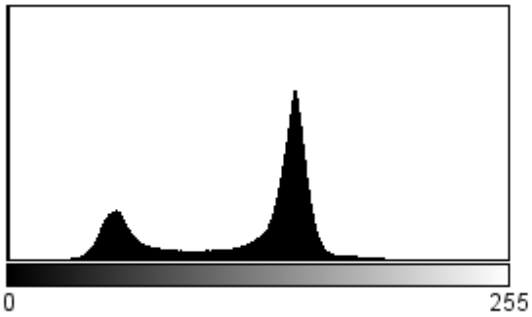
Count: 13369344
Mean: 8.235
StdDev: 32.037
Min: 0
Max: 255
Mode: 0 (12460533)



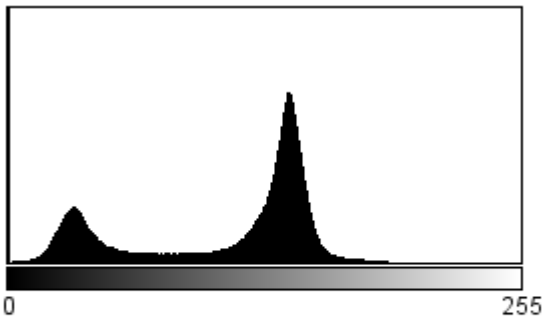
Count: 18350080
Mean: 5.645
StdDev: 26.074
Min: 0
Max: 255
Mode: 0 (17351893)

Primal #11B

2.5 ± 0.3 °C	35.3 ± 0.2 °C



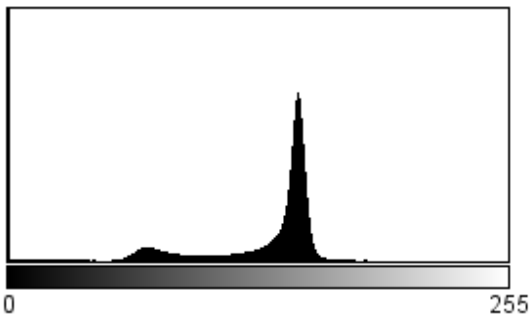
Count: 15728640
Mean: 8.234
StdDev: 32.043
Min: 0
Max: 255
Mode: 0 (14640290)



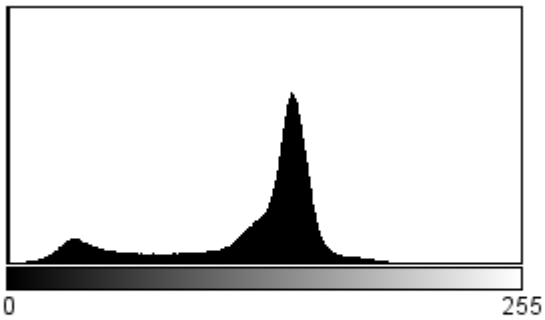
Count: 17825792
Mean: 6.686
StdDev: 28.500
Min: 0
Max: 238
Mode: 0 (16722573)

Primal #14A

2.5 ± 0.3 °C	35.3 ± 0.2 °C



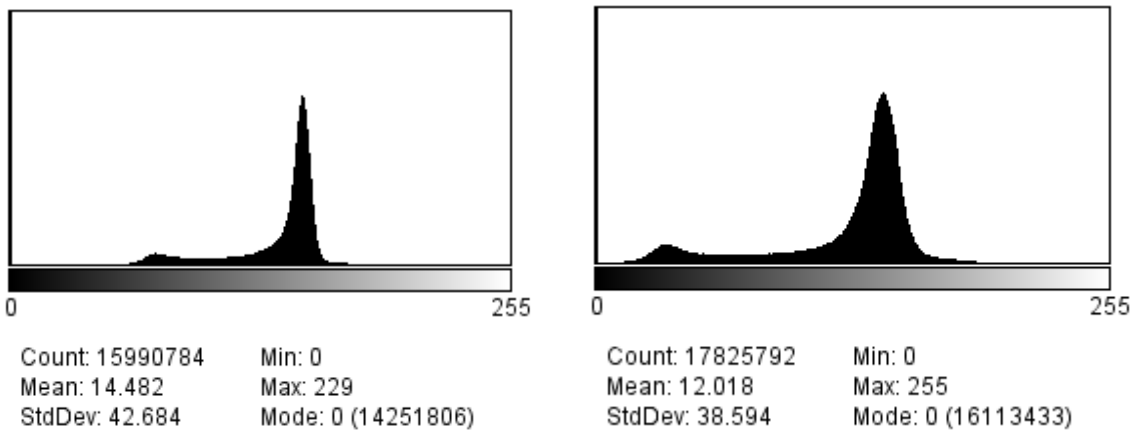
Count: 15204352
Mean: 11.126
StdDev: 37.529
Min: 0
Max: 199
Mode: 0 (13887195)



Count: 17563648
Mean: 9.133
StdDev: 33.684
Min: 0
Max: 255
Mode: 0 (16225975)

Primal #15A

2.5 ± 0.3 °C	35.3 ± 0.2 °C



Primal #15B

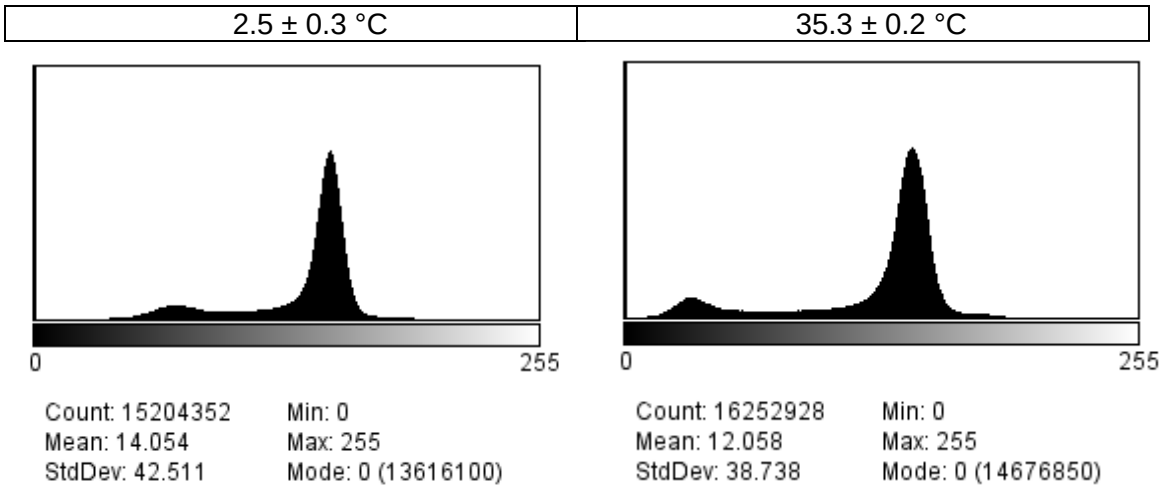


Figure 6. – Histogram of changes in cut off pixel ranges (grey scales) for fat (far left hump) and lean (far right hump) of each of the six primals evaluated as core temperature rises from 2.5 (far left histogram) to 35.3 °C (far right histogram)

The threshold level for each primal was then determined based on the steps reported in Table 3. The average and weighed average grey scale values are reported in Tables 4 and 5 for upper threshold levels for fat and lean, respectively. Figure 7 illustrates the non-linear nature of greyscale threshold values as the temperature changes.

Table 4. Average, weighted averages and standard error (s.e.) of upper grey scale threshold for fat/lower grey scale value for lean across 6 primals (n=339) at specific temperatures

Temperature	n	Average	Weighted average	s.e.
2	339	99.30	107.34	1.15
5	356	85.60	86.94	0.99
15	356	88.99	87.53	0.91
25	362	89.58	88.73	0.59
35	398	88.94	92.32	0.63

Table 5. Average, weighted averages and standard error (s.e.) of upper grey scale threshold for lean across 6 primals (n=339) at specific temperatures

Temperature	n	Average	Weighted average	s.e.
2	339	206.74	219.23	2.66
5	356	187.87	187.90	1.55
15	356	185.70	181.46	1.44
25	362	185.15	183.62	1.89
35	398	226.92	242.87	1.89

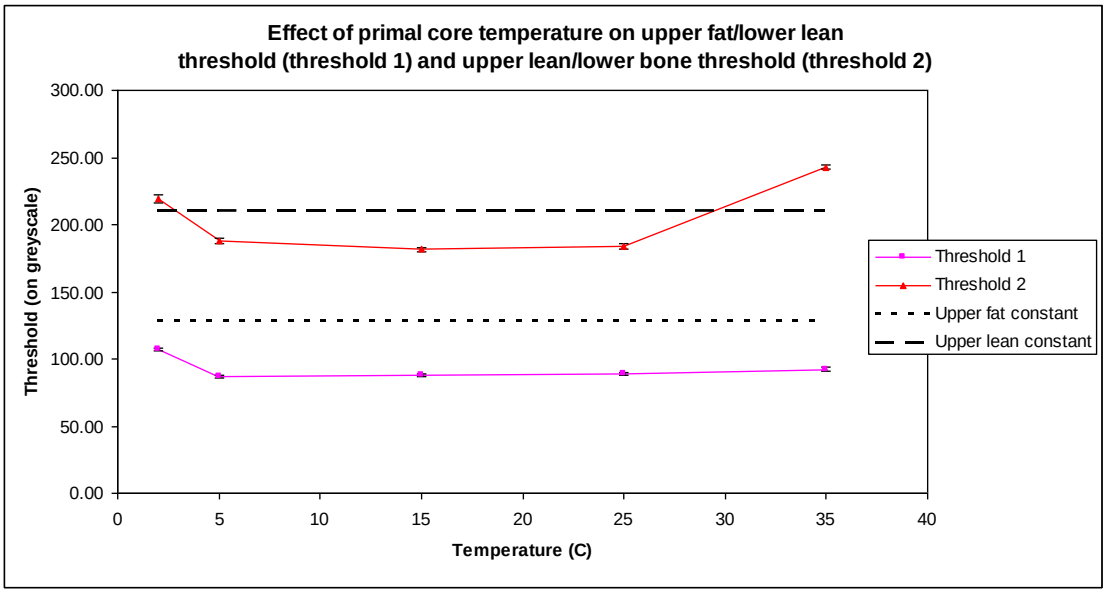


Figure 7. Weighted average across 6 primals at a range of temperatures for upper fat and lean greyscale thresholds with the current default greyscale constant for upper fat and lean thresholds

Conclusions

This study has illustrated (Figure 7) the non-linear nature of greyscale threshold values as the temperature changes and justifies the significance of changing the grey scale settings. Differences in greyscale thresholds will exist (e.g. between primals) and therefore determining the thresholds is an important step in getting accurate information.

The steps outlined in Table 3 could be coded up into a procedure that could be implemented into a fully automated system for estimating total fat and lean in a trimmed primal and provide an estimate of retail beef yield in the primal cut. However, upon further evaluation of using the auto threshold techniques (Table 3) the auto threshold technique did not prove to be highly accurate when compared with 2 and 35 degC differences in temperature. Results on this are reported on in the following study.

Study 4

Introduction

Red meat contains various amounts of intramuscular fat, giving it an appearance similar to a marble pattern. Currently the quantity of intramuscular fat is visually assessed to provide a marbling score. The marbling score has an impact on the financial price which can be demanded for retail beef. However, marbling score is determined from a single point, a subjective measurement, and thus may not give an accurate assessment of marbling throughout a primal cut. Further, because marbling is visually assessed it is prone to subjective variation (sampling error). X-ray computed tomography has previously been used to analyse the body composition of individual animals and primal cuts, and may therefore provide a more accurate way to assess marbling. However, the method by which CT scan images are separated into muscle, fat and, bone, first needs to be assessed to determine accuracy. There are two main areas in which accuracy could be improved, the first is during the CT scanning stage, and the second is the method of image analysis employed. During the CT scanning stage, decreasing the cross-sectional thickness and spacing will improve the clarity of images allowing for more accurate analysis. However, this would also increase the scanning time and increase the number of images that require analysis. The method of image analysis may also impact upon accurate composition determination. CT scan images are usually assessed using a frequency plot of the grey scale values.

The boundary method (current standard used in AutoCAT; version 1998) uses thresholds (i.e., boundaries) to determine fat, muscle and bone. However, the distributions of fat, muscle and bone do not provide clearly separate and discrete boundaries. As a result, setting a specific threshold would serve to underestimate either the proportion of fat or muscle present in a scan. Further, the temperature at which CT scanning is carried out may be expected to impact the position of threshold values due to the impact of temperature on muscle and fat density. This therefore requires adjustment of threshold values (i.e. thresholds can not be set as a constant) which further complicates image analysis. Bayesian mixture modelling may provide an alternative method to analysing frequency plot data (Alston *et al.*, 2004). This method assumes that the frequency data is comprised of numerous normal distributions, calculated such that the sum of normal distributions provides the best-fit to the frequency data. Whilst this method is more complicated, it removes the necessity for thresholds which reduces the number of assumptions made during analysis, and accounts for overlapping fat, muscle and bone distributions thereby providing more accurate estimations. Figure 8 is provided to give a clearer explanation of the differences between the Boundary method and the Mixture model method. As such the aim of this study is to compare the accuracy of image analysis carried out using the boundary method and the mixture model method.

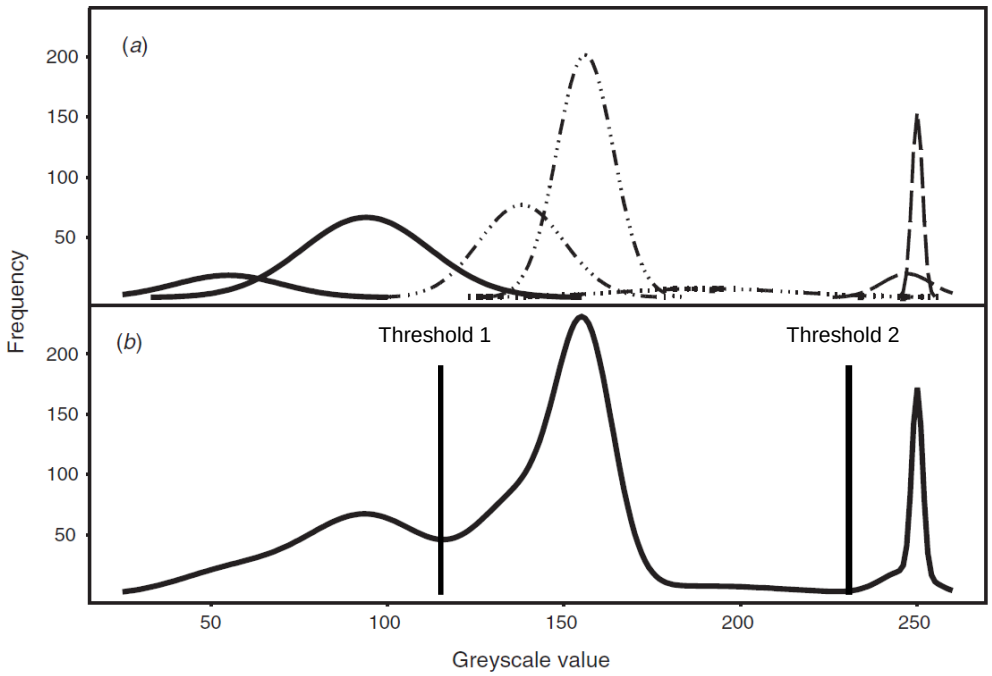


Figure 8. (a) Mixture model method: Solid lines represent fat components. Dotted and dashed lines represent carcass muscle components. Dashed lines represent components of cartilage and bone. (b) Boundary Method: Fat component is given below threshold 1. Muscle component is given between thresholds 1 and 2. Bone component is given above threshold 2. Note: the sum of normal distributions shown in (a) result in the frequency plot provided for the Boundary method (b). Adapted from Alston *et al*. (2004).

As mentioned in Study 3, after further examination of the auto threshold technique and the correlation with areas of ImageJ images between 2 and 35 degC (R^2 = 0.71) it became clear that if intramuscular percent were to be calculated from CT images then a more accurate method was required. Therefore, the objective of study 4 was to compare the accuracy of image analysis carried out using the boundary method (as mentioned in Study 3) and the mixture model method (Alston *et al.*, 2004).

Material and methods

Primal cuts

20 Angus MSA Grade 3 striploins were purchased from a local Armidale Butcher, 10 of which were grass fed and the other 10 were grain fed. All 20 primal cuts were vacuum packed and transported (at 1-2°C) to the University of New England Meat Sciences CT unit for X-ray computed tomography (CT) scanning.

CT scanning

Primal cuts were scanned at 2°C using a Picker Ultra Z Spiral CT scanner (Philips Medical Imaging Australia, Sydney NSW). The X-ray tube operated at 130kV and 100mAs. A pitch of 1.5, field of view of 300 mm, couch height of 275mm, and cross-sectional thickness and spacing of 10 mm were used. This resulted in 30 cross-sectional images per primal (each primal was 300mm in length). The primals were then denuded of their subcutaneous fat and run through the CT scanner again at 2°C with the same scanner setup. All primals were then placed in a water path to heat the primals to 35°C. Once the primals had reached this temperature, they were CT

scanned again using the same setup (the only difference being the primal temperature).

Image analysis & methodology
Frequency plots across all CT scan images for the 2°C with subcutaneous fat, 2°C denuded and 35°C denuded primals were generated using Image J software (National Institutes of Health, USA). These frequency plots were then analysed using either the boundary method or the mixture model method.

Boundary method
Upper threshold greyscale values for fat (threshold 1) and muscle (threshold 2) were calculated separately for each set of primals under the assumption that treatment and temperature would have an impact on frequency plots. Treatment (denuded or not) was expected to have an impact on threshold values because denuded primals contain less fat with which to determine threshold values. Temperature is also expected to impact threshold value estimation via temperature dependent shifts in density. The thresholds calculated for each set of primals is given in Table 6. Greyscale values between 1 to threshold 1 are designated as fat, those between threshold 1 and 2 are assigned to muscle, whilst values above threshold 2 are assumed to be cartilage or bone.

Table 6. Greyscale values used as thresholds for determining fat and muscle content for primals at 2°C with subcutaneous fat, 2°C denuded and 35°C denuded.

Threshold	2°C with subcutaneous fat	2°C denuded	35°C denuded
1	126	114	113
2	213	214	205

The total pixel count and the pixel count falling into each range (i.e. being identified as fat or muscle) count was subsequently calculated from the frequency plots.

Mixture model method
The mixture model methodology was implemented using the XSTAT add-in for Microsoft® excel. Alternatively there are numerous packages within the R statistical package that can carry out this type of analysis. The total pixel count, fat pixel count and muscle pixel count were recorded.

Comparison of analysis methodology
Due to the same primals being used for each of the sets (2°C with subcutaneous fat, 2°C denuded and 35°C denuded), the pixel count for muscle should be very similar for the 2°C with subcutaneous fat and the 2°C denuded frequency plots, and fat and muscle proportions (fat or muscle pixel count divided by the total pixel count) should be very similar for the 2°C denuded and the 35°C denuded frequency plots. Therefore, comparison of these values provides an estimation of the accuracy of analysis methodology.

Results
The R^2 between the muscle pixel count for the 2°C with subcutaneous fat and the 2°C denuded frequency plots was 0.71 using the Boundary method, and 0.96 using the Mixture model method. The R^2 between the muscle and fat proportions (when comparing the 2°C denuded and the 35°C denuded frequency plots) was 0.81 and 0.47, respectively using the Boundary method; and 0.97 and 0.99, respectively when using the Mixture model method.

Conclusions

The Mixture model method of analysing frequency plots obtained from CT scans was identified as providing a much greater accuracy when determining fat, muscle and bone components. This methodology could be automated into a software package with the advantage that the user does not need to provide threshold inputs and results can be obtained at the time of CT scanning rather than requiring someone to carry out the analysis. Further, to obtain accurate weight estimates from CT scans, a series of standards of known density should be run for a range of temperatures. Greyscale pixel values for these density standards can then be used to estimate the effect of temperature on density (and thus our frequency plot estimations), and will also remove the requirement to take constant density values for fat, muscle and bone (i.e. density becomes a range over the 256 grey scale with each pixel value having a known density).

References

Alston C.L., K.L. Mengersen, J.M. Thompson, P.J. Littlefield, D. Perry and A.J. Ball (2004). Statistical analysis of sheep CAT scan images using a Bayesian mixture model. *Aust. J. Agric. Res.,* **55:** 57-68.

Phase 1.2 Determine the relationship between the MSA marbling score, chemical intramuscular fat (IMF) (%), and the CT scanning image of IMF (%) of the 10 mm slices.

Introduction

This phase of the project was to determine the relationship between MSA marbling score, chemical intramuscular fat (IMF, %), and the CT scanning image of IMF. As reported in Study 4 of Phase 1.1 the accuracy of estimating fat is greatly improved if the mixture model is used to analyse how much lean and fat is in a primal cut at a specific temperature. The mixture model has not yet been implemented to analyse fat and lean for the work in this phase and hence no CT scanning image of IMF is reported.

Material and methods

Primal cuts

20 Angus MSA Grade 3 striploins were purchased from a local Armidale Butcher, 10 of which were grass fed and the other 10 were grain fed (Study 4 Phase 1.1).

CT scanning

Striploins were used in Study 4 Phase 1.1 to compare different methods to estimate fat. All striploins (Figure 9) were denuded and sliced into steaks of 20mm thickness (Figure 10). All steaks (n = 299) were photographed and sent off to a certified MSA grader and graded.

Samples to estimate chemical IMF% were determined as described by Perry et al. (2001). In brief, steaks were diced into 1cm cubes. Cubes were blended to that of a mince consistency for approximately 30 seconds. They were loosely placed into 50ml sample tubes that had all been weighed beforehand (without lids). Samples were then weighed to determine the 'wet' weight of the samples (again without lids on). These samples were then placed in the freeze drier for approximately one week. Once the samples were dry, they were again weighed (without lids) to determine the dry sample weight, as well as a dry percentage to test that the samples were dried properly (<35%). The critical value was raised to approximately 30%, as the high IMF% was taken into consideration. To prepare samples for the NIR, all samples were further ground using a coffee grinder to form a fine powder. In between grinding, all equipment was cleaned to prevent contamination. In between drying, grinding and the NIR measurements, samples were stored in the cool room at approximately 2°C. Before samples were measured using the NIR machine, they were dried again in a 50°C oven overnight prior to scanning.

In order to prepare samples for the NIR scans, 5 grams of each sample were placed into a cup and then run through the NIR machine. Each cup was cleaned between samples to ensure that there was minimal contamination between samples.

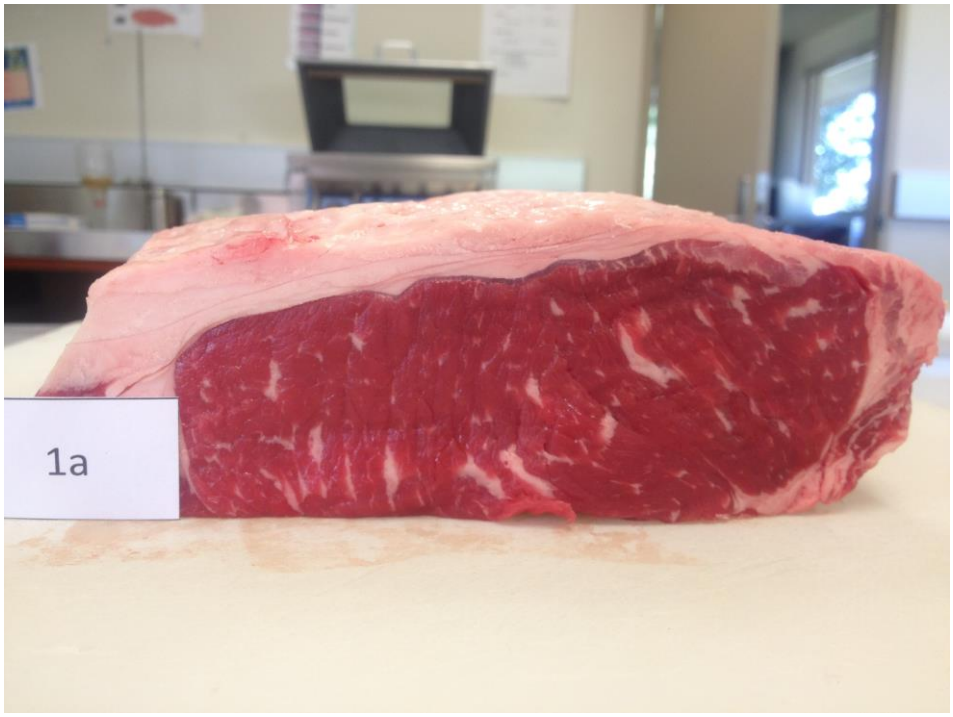


Figure 9. Striploin primal used in the study

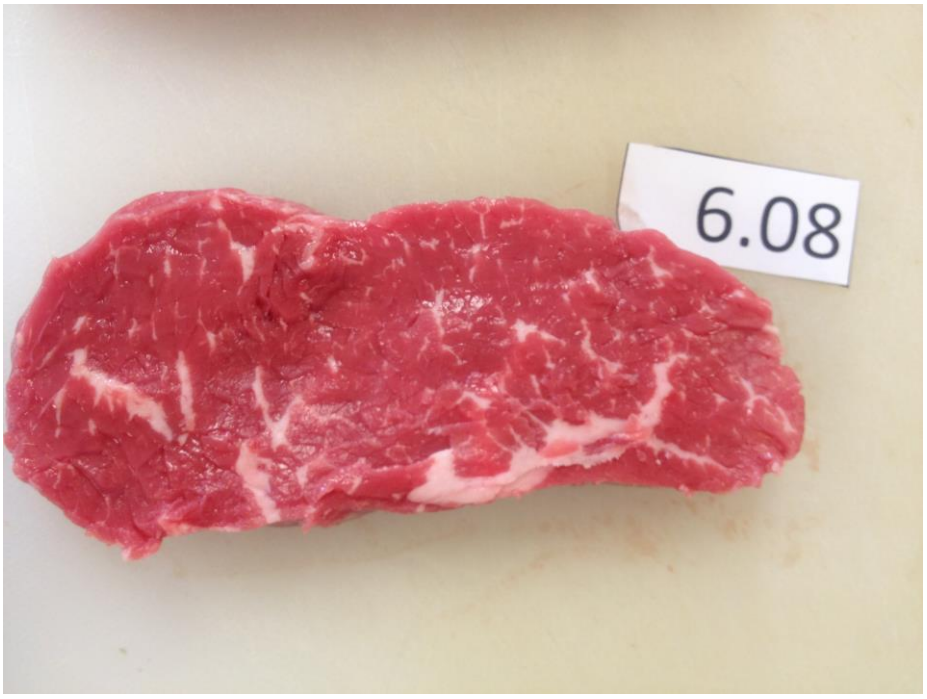


Figure 10. A 20 cm thick slice of a primal

Results

The results of the MSA grade for each slice across 20 primals are reported in Figure 11.

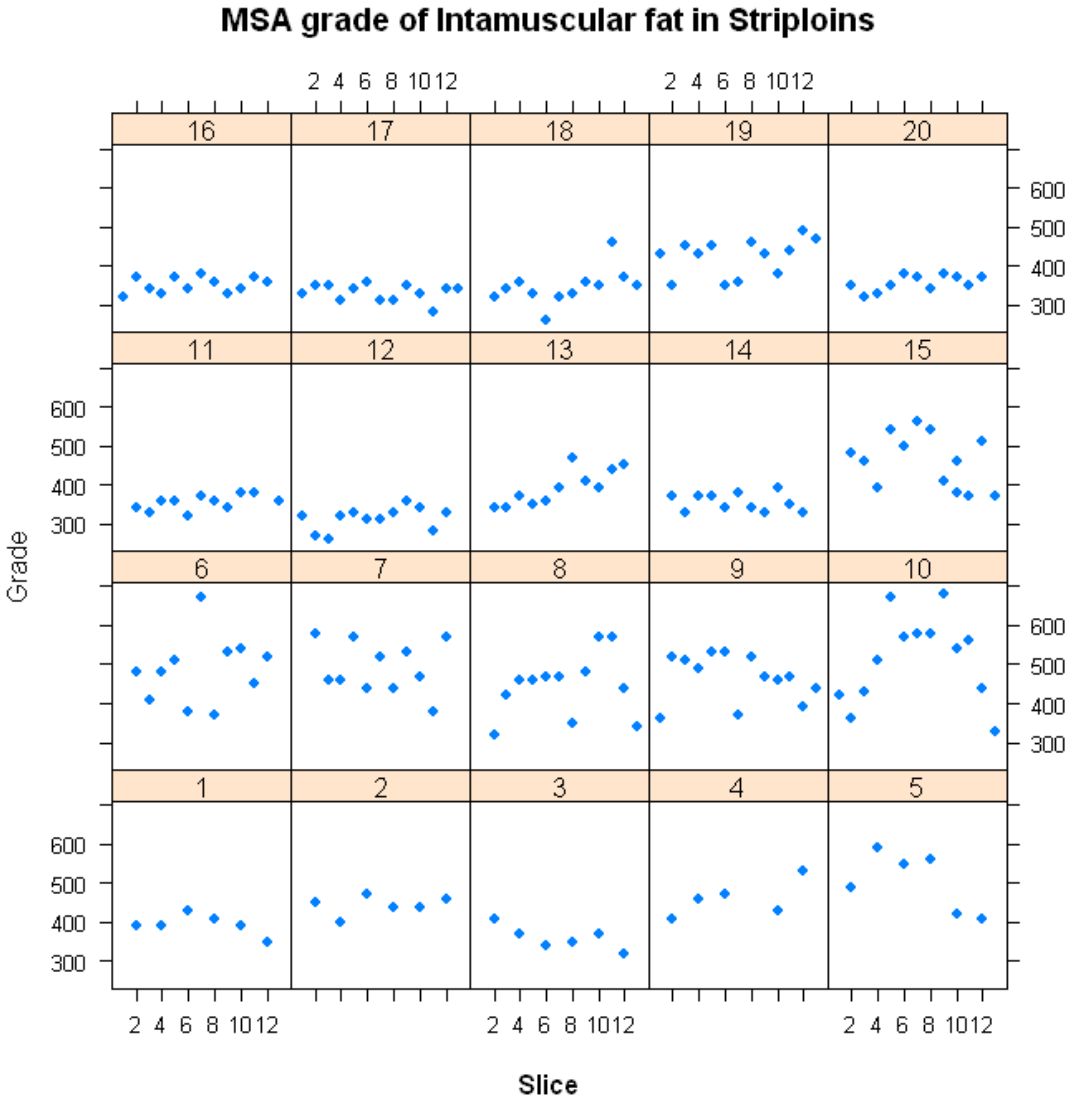


Figure 11. MSA grade of steaks (20mm thick) across 20 striploin primals.

The results of the NIR chemical IMF analysis for each slice across 20 primals is reported in Figure 12.

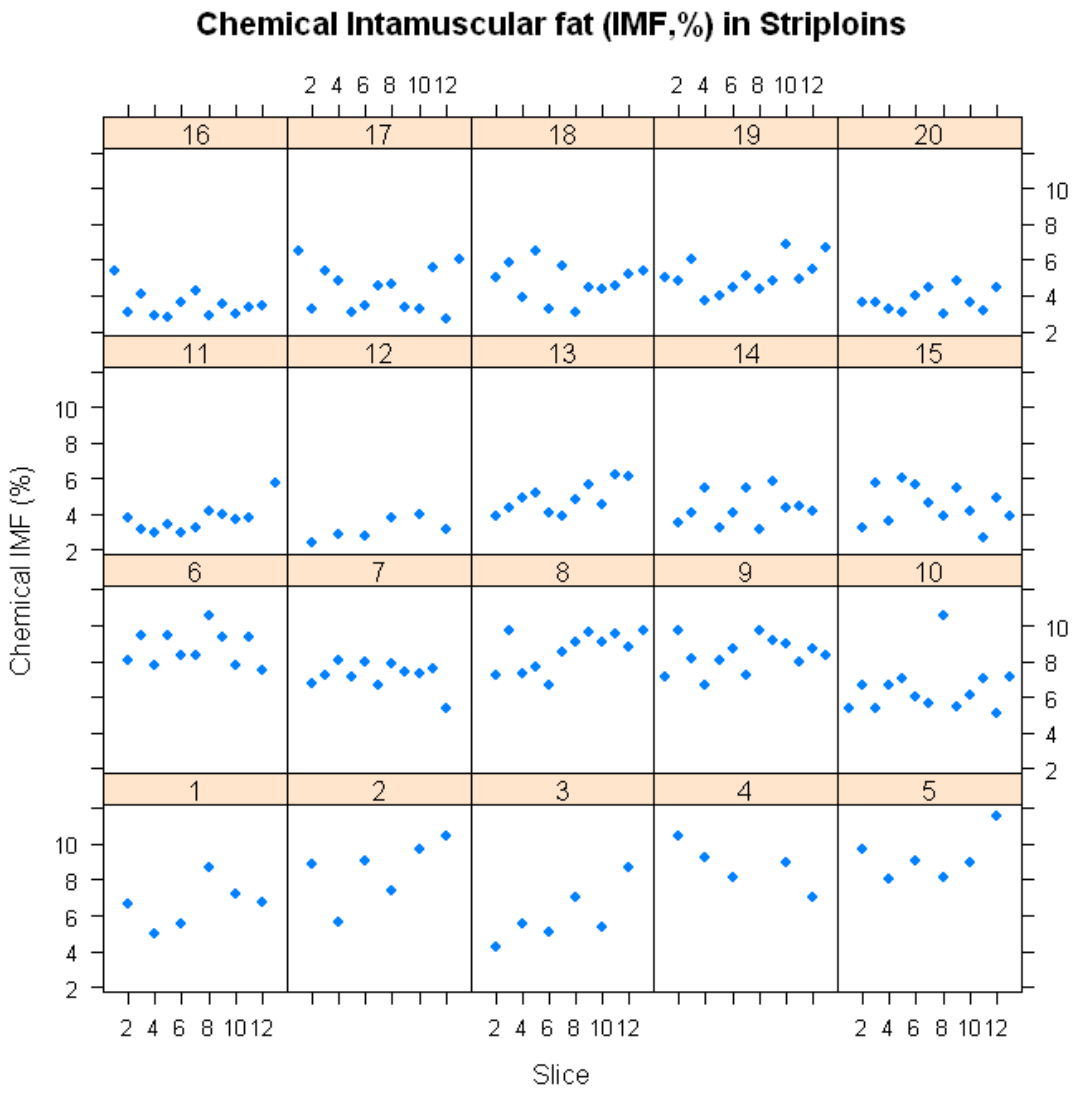


Figure 12. Chemical IMF (%) of steaks (20mm thick) across 20 striploin primals.

The relationship between MSA grade and chemical NIR IMF is reported in Figure 13 with the trend of the relationship shown.

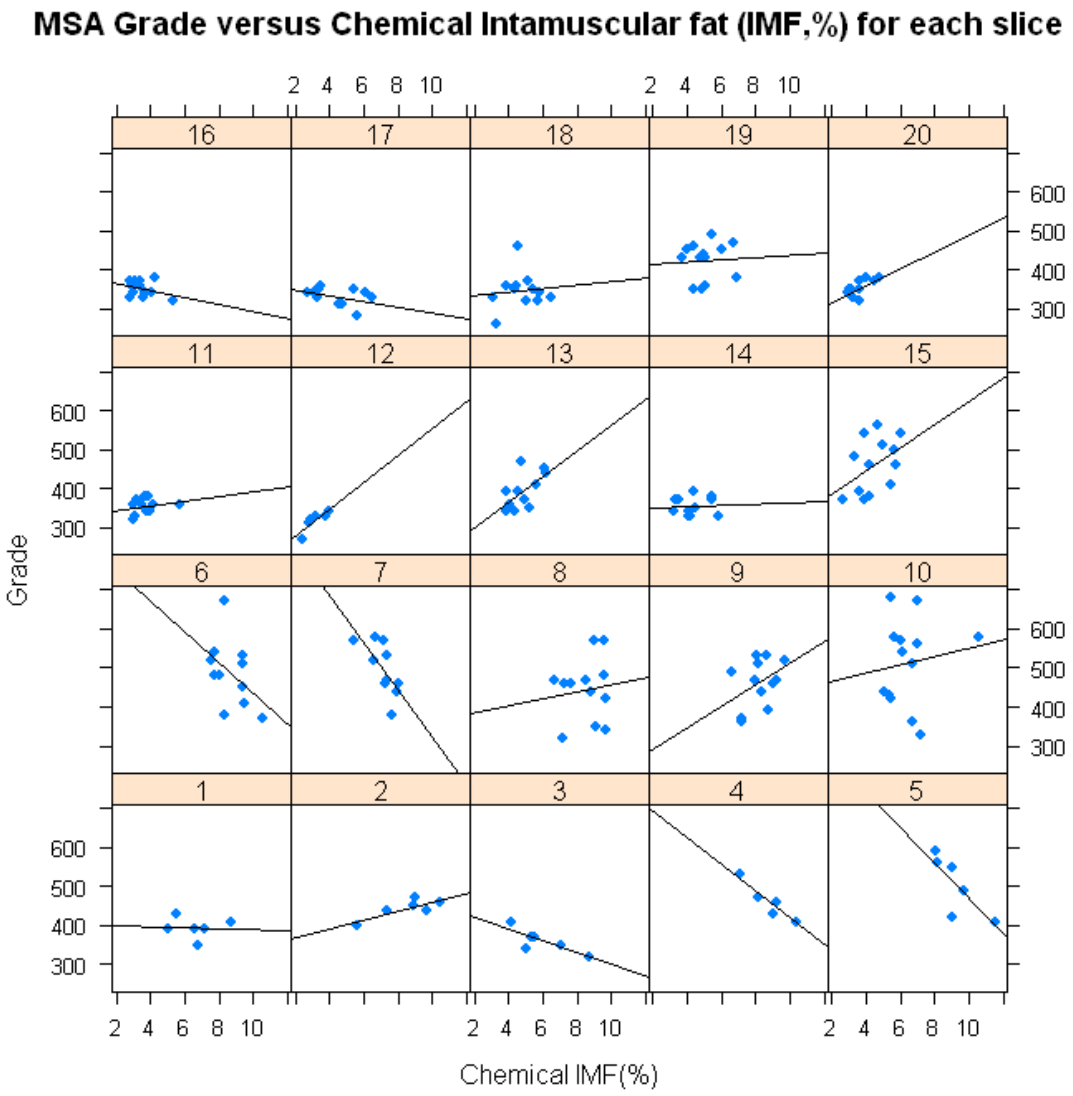


Figure 13. Relationship between chemical IMF(%) and MSA grade of steaks (20mm thick) across 20 striploin primals; linear lines for each primal represent the trend.

Conclusions

The relationship of MSA grade across primals is variable as illustrated in Figure 11. In some of the primals a sinusoidal pattern is present [Figure 11. (primals 6,7,11-20)] (demonstrated in a previous study personal communication John Thompson). Variation of NIR chemical intramuscular fat(%) within a primal was also illustrated in Figure 12. The relationship between MSA grade and NIR chemical intramuscular fat (%) as shown in Figure 13 demonstrates that agreement between MSA grade and chemical intramuscular fat (%) does not always match.

This study has demonstrated the variation of intramuscular fat in primal cuts and further work is required to develop a relationship between MSA grade and chemical intramuscular fat (%) and intramuscular fat (%) from CT scanned images.

References

Perry D, Shorthose WR, Ferguson DM, Thompson JM (2001) Methods used in the CRC program for the determination of carcass yield and beef quality. Aust. J. of Exp. Agric. 41: 953–957.

Phase 1.3 Develop equations to estimate IMF (%) in primal cuts

Introduction

Equations to estimate not only total fat and lean in a primal but also intramuscular fat in a primal cut under hot-boning conditions would provide carcass attributes that would assist the beef industry move forward in a value based system. Equations have been developed based on the following objective: develop an equation to estimate the total amount of IMF in 1 primal cut.

Data from the striploin has been used to develop the equation. To estimate IMF (%) in individual slices portions of fat (subcutaneous and intermuscular) need to be removed from images so that IMF can be calculated. Techniques using ImageJ can be implemented to determine intramuscular fat but more time is required to develop these routines. Manually removing the unwanted fat (i.e., subcutaneous and intermuscular fat) would take approximately 12 to 15 minutes. Hence, an automated process would speed up the assessment of a primal.

Materials and methods

Data from the Beef CRC experimental trial (unpublished data) on Angus, Hereford, Wagyu/Angus steers were used. All primals were weighed and scanned and total fat was estimated in the primals using the AutoCat software (Jopson version 1998) (unpublished data). [Note: the mixture model method would increase the accuracy of estimating intramuscular fat as mention above in Study 4 of Phase 1.1]. Subsequently the intramuscular and intermuscular fat was removed from the CT scanned images and subcutaneous fat (kg) was estimated using the AutoCat software and in addition subcutaneous and intermuscular fat were removed to determine the percentage of IMF in the primal cut. By subtraction intermuscular fat was determined; i.e., for each primal total, subcutaneous, intermuscular, and intramuscular fat (kg) was estimated. Software was then written in R to create a file that could then calculate fat (kg) in individual slices (unpublished). Intramuscular fat (%) was then calculated [IMF fat (kg/(IMF fat (kg) + lean (kg))x100]. The results of estimating IMF from the CRC experiment on the Striploin across different slices is shown in figure 15.

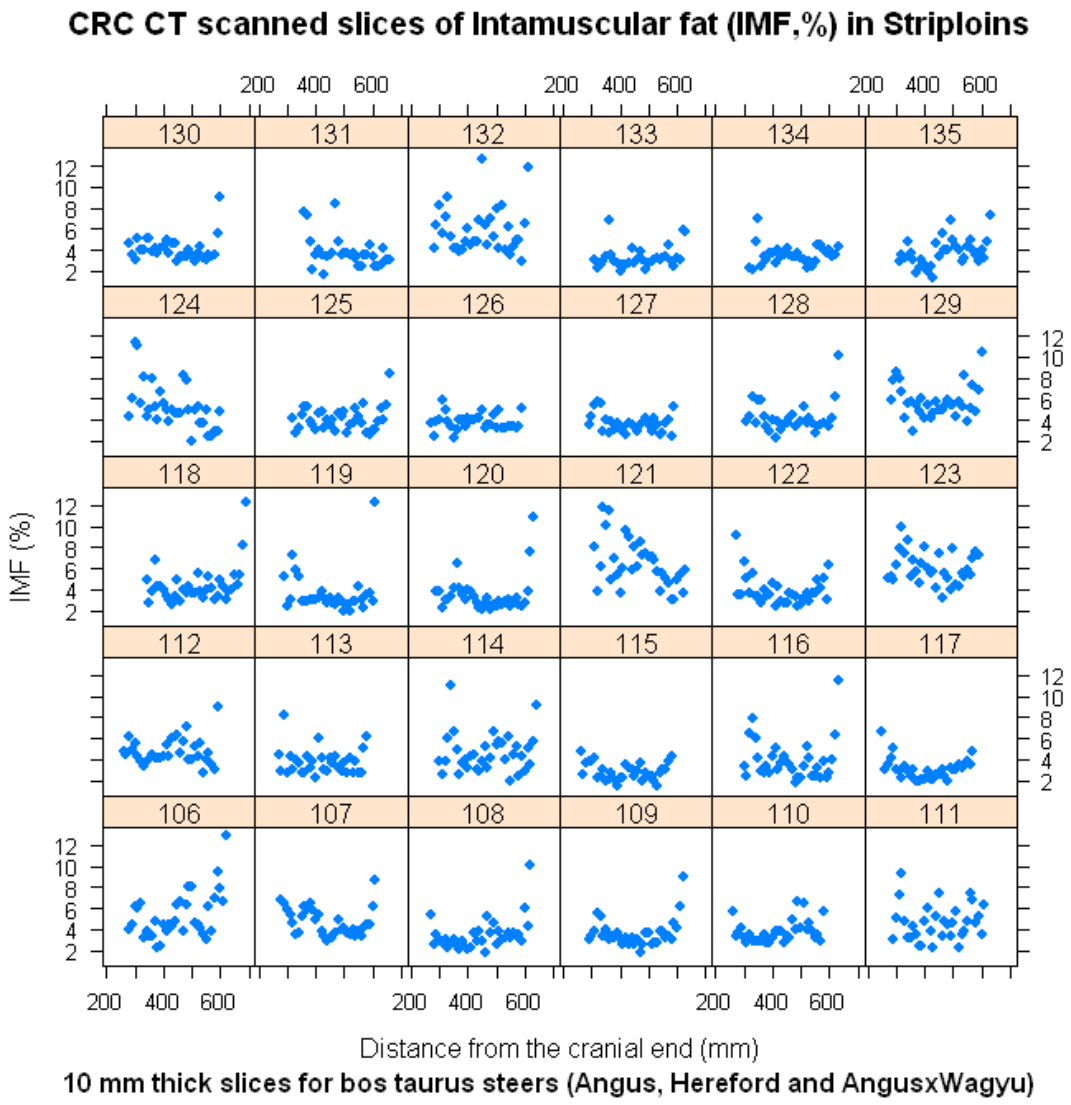


Figure 15. CT scanned intramuscular fat (%) of individual slices within 30 Bos Taurus steers (Angus, Hereford, and AngusxWagyu) striploins

A linear regression using the stepwise regression procedure in the R statistical package was then used to develop the following equation:

TotalIMF (%) = exp(0.58 + 0.0039xTotalWt + 0.027xSlice12 + 0.043xSlice14
+ 0.079xSlice16 + 0.031xSlice20) AdjR^2= 0.84

Note: Slice12 refers to the slice 10 mm thick, 120mm from the cranial end etc.

Conclusions

Further work will evaluate this equation with an independent data set after a mixture model technique has been established and can therefore be used to estimate intramuscular fat with higher accuracy. However, a technique that uses the 2 dimensional attributes of slices (e.g. n=3) that estimates the amount of IMF in a primal (i.e., 3 dimensional) may well prove to be a far better method.

Acknowledgements

Numerous staff from the Meat Science faculty at UNE were involved and their assistance is greatly appreciated! The work undertaken by Georgie Lawrence, an honours student who received a scholarship from the AMPC funds, was greatly appreciated. Georgie put in many long hours to see that experiments were conducted according to experimental protocol and were completed in a timely manner. Mark Barnett, a post doc, also contributed significantly to the experimental study with 6 primals. Lastly, I would like to acknowledge Yan Laurenson who has provided significant insight into the processing of CT images. Yan is also a post-doc with excellent skills in modelling and has therefore been able to evaluate different methods in how fat might be estimated.