Contemporary chemical lean validation – national standard for measurement – FINAL REPORT

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1.0 EXECUTIVE SUMMARY

Chemical Lean (CL) is defined as the amount of lean red meat compared to the amount of fat in a meat sample. CL determination by an approved method of sampling and testing is a mandatory, AUS-MEAT Ltd prescribed requirement for any bulk packed meat product destined for export. Presently, there are fifteen AUS-MEAT approved methods for CL analysis, ranging from classical wet chemical techniques to moisture determination using microwave ovens as well as specific instrumentally based techniques. One important aspect of the approved CL methods is that they are 'fit for purpose'. This can be tested by method validation where the method's performance is evaluated and tested to see if it is consistent with its intended outcomes. Inter-laboratory comparison (IC) is a suitable way to do this. In an IC, a method's performance can be assessed using a consensus value approach where the measured results of reference test materials from each particular method is compared to an assigned value (whether the mean or median of test results, or reference result) using statistical analysis (e.g. z-test). To date, no comprehensive study has been undertaken in Australia that compares a range of contemporary methods used for CL determination. The objectives of this project were:

- Engage with Australian meat processing industry to identify currently used methods for CL determination, and associated providers,
- Perform a comparison of methods used for CL analysis by Australian and overseas providers, and
- Develop a proposal for national standard for CL determination.

Key outcomes

- Engagement with Australian meat processors indicated a number of methods were deployed for CL measurement. These included chemical analyses (e.g. Soxhlet fat extraction and microwave moisture) to instrumental based techniques (e.g. near infrared reflectance (NIR) and transmittance (NIT), nuclear magnetic resonance (NMR), X-Ray).
- A range of meat homogenates consisting of beef (spanning CL65 to CL95), lamb and pork were used in a method comparison, which included Soxhlet fat extraction, microwave moisture analysis, along with NIR, NIT, NMR and X-Ray representative of instrumental techniques.
- The method comparison showed that each of the methods performed satisfactorily, and thus can be deemed as 'fit-for-purpose'. The methods were diverse and used different sample sizes. Given this breadth and diversity, we expected to observe differences in the result comparison. However, this was not the case.
- Engagement with the Australian meat industry is required to identify if a need exists for the development of a national standard relating to contemporary CL determination. At present, accreditation of industry standards is completed through AUS-MEAT Ltd with approvals made by the Australian Meat Industry Language and Standards Committee.

Recommendations

- The methods used in this study performed satisfactorily and can be regarded as 'fit-for-purpose' as well as suitable for contemporary CL determination.
- Suitable reference materials with a certified value of CL content are not available for Australian industry, and thus it is recommended that such materials are developed for industry use.
- This study forms the benchmark for contemporary CL determination in the Australian meat industry. It is recommended that future studies (such as this) are conducted to build on this project's outcomes and monitor the on-going method performance against a set of industry agreed performance standards.
- The development of a national standard requires industry involvement and acceptance. There would be benefit with consulting the Australian Technical Infrastructure Alliance (ATIA) in relation to such a standard, particularly in relation to global engagement.

2.0 INTRODUCTION

Chemical Lean (CL) is defined as the amount of lean red meat compared to the amount of fat in a meat sample. CL determination by an approved method of sampling and testing is a mandatory, AUS-MEAT Ltd prescribed requirement for any bulk packed meat (beef, sheep and pork) products destined for export markets. It is optional in other cases, e.g. bulk packed goat meat or bulk packed primals. There are currently fifteen AUSMEAT approved methods for chemical lean analysis, ranging from classical wet chemical techniques to moisture determination using microwave ovens as well as specific instrumentally based techniques. Anecdotal evidence suggested that some Australian processors use techniques such as Soxhlet extraction or microwave oven moisture determination as reference methods for quantitative CL determination, with instrumental techniques (e.g. near infrared (NIR) spectrometry) used as an "in-house" means to "monitor" CL tolerance within a processing facility rather than for quantitation. There also appears to be little information available which documents the methods used by Australia's overseas trading partners for CL determination.

One important aspect of the AUS-MEAT approved CL methods is that they are 'fit for purpose'; that is, they are suitable for providing quantitative values of fat in meat. This can be tested by method validation where the method's performance is evaluated and tested to see if it is consistent with its intended outcomes. Inter-laboratory comparison (IC) is a suitable way to do this; i.e., an IC can be used to assess the consistency of the CL measurements made in Australia as well as those performed overseas. A method's performance can be assessed using a consensus value approach where the measured results of the test materials of each particular method is compared to an assigned value (whether the mean or median of test results, or reference result) using statistical analysis (eg z-test). Inter-laboratory comparisons, and related proficiency testing, are recognised ways within the laboratory comparison and accreditation sector to develop an understanding of, as well as validating, a method's performance. To date, no comprehensive study has been undertaken in Australia that compares a range of contemporary methods used for CL determination. The outcomes of the comparison would be useful for possible validation of the methods used for CL determination in Australia and overseas trading partners.

The project objectives were to

- Engage with Australian meat processing industry to identify currently used methods for CL determination, and associated providers,
- Perform a comparison of methods used for CL analysis by Australian and overseas providers, and
- Develop a proposal for a national standard for CL determination.

A survey was undertaken with the Australian meat processing industry which provided information on the methods were being used for CL determination. This assisted with the selection of appropriate methods which were relevant and reflected contemporary practice for CL determination. These methods included classical wet chemical (Soxhlet fat extraction and microwave moisture) and instrumentally based. Additionally, the survey assisted with the selection of appropriate service providers. These methods were involved in a comparison that utilised beef of varying CL content, along with a pork and lamb sample. The emphasis on the beef samples reflected its economic importance in the export market. Homogenised lamb, beef and pork samples were forwarded to participating

facilities using industry standard transportation protocols for analysis using the nominated methods for CL determination. The evaluation of CL method performance was completed using two different metrics; the first was the normalised error while the second approach involved comparing the measured results with an assigned value (in this case, the aggregate median) using statistical analysis (robust *z*-scores). The outcomes from the comparison were presented to representatives of the Australian meat processing industry, with an anticipation that this could lead to a proposal for the development of an Australian national standard for CL determination. This information will also assist an on-going discussion with Australia's overseas trading partners relating to CL analysis, and create an awareness of Australia's measurement and quality standards, and the associated quality systems. One limitation for this project was the lack of a suitable reference material that could be used for all methods, using different sample sizes and homogeneity.

3.0 PROJECT OBJECTIVES

The project objectives were to

- Engage with Australian meat processing industry to identify currently used methods for CL determination, and associated providers,
- Perform a comparison of methods used for CL analysis by Australian and overseas providers, and
- Develop a proposal for national standard for CL determination

4.0 METHODOLOGY

4.1 Survey of methods for chemical lean (CL) determination in Australian meat processing industry

At present, there are 15 AUS-MEAT approved methods which can be used by the Australian meat processing industry to determine chemical lean (CL) in beef, lamb and pork products intended for export. The methods range from classical wet chemical techniques to moisture determination using microwave ovens as well as specific instrumentally based techniques. The AUS-MEAT approved methods for CL analysis are shown in Table 1. Where known, the details of the methods are provided in Appendix 1.

Table 1. AUS-MEAT approved methods for chemical lean (CL) determination

	Method name	Type of analysis
1	Soxhlet	Solvent extraction method
2	Babcock	Acid digestion method
3	Foss-let	Specific gravity of extract method
4	Anyl-Ray	X-Ray absorption method
5	Microwave	Moisture determination
6	MQ27	X-Ray CL analyser
7	Foss Meatmaster™ I and II	X-Ray CL system
8	Foss Foodscan™	NIT[A] technology
9	CEM: SMART Trac Fat and Moisture Analyser	NMR[B] technology
10	Smiths Detection Eagle™ Carton FA	X-Ray CL system
11	Foss Meatscan™	NIT CL system
12	Multiscan Series 3000 Food Analyser	NIT CL system
13	NDC Infralab™ e-Series Meat Analyser	NIR[C] CL system
14	Marel Trim Management System (Sensor-X)	X-Ray CL analyser
15	Perten Instruments (DA7250)	NIR CL system

[A]NIT = near infrared transmittance [B]NMR = nuclear magnetic resonance [C]NIR = near infrared spectroscopy

As part of this project, a survey was performed with the Australian meat processing industry to determine which methods were presently used for CL determination, and to develop an understanding whether these were performed either at the facility or with an external provider. Suitable facilities were identified from AUS-MEAT Accreditation Listing[1] with focus given to facilities noted as “Export Abattoir”. Contact was made with twenty five processing facilities and a series of questions was used to determine whether CL determinations were performed at the facility and, if so, which method/s were used at these sites. If an external provider was used then contact was attempted to determine which method was deployed.

[1] https://www.ausmeat.com.au/docs/AUS-MEAT%20Accreditation%20Listing.pdf

4.2 Preparation and distribution of meat homogenate samples.

Beef, sheep and pork are the most common meats used for bulk export, with beef the most significant in terms of export. Beef is shipped in higher volume and of higher economic importance compared to either pork or sheep meat. Based on feedback from the meat processing industry, a broad range of CL content for beef was chosen for the inter method comparison. Representative samples were chosen for beef spanning the range of CL65 to CL95, with single samples for lamb and pork for use in this study.

Meat was acquired from two different sources; beef of different CL content (3 X 27.2 kg cartons of CL65, CL70, CL75, CL80, CL85 and CL90) was purchased from one commercial vendor, while 3 cartons (27.2 kg) of beef (CL95), pork and lamb were purchased from another vendor. The set of beef samples from the first vendor had assigned values for CL content on the cartons. The samples were stored at 4 °C.

The meat samples were homogenised at the Food Processing Centre (FPC) located at the CSIRO, Werribee site, by combining the contents of two cartons and passing them through a Thompson 42 mincer with a 6 mm plate (Thompson Meat Machinery, Crestmead, Qld). The aggregate was collected and re-passed through the mincer, and then mixed using a commercial food/meat mixer (RC-100, Mainca USA Inc., St. Louis, Mo, USA). Part of the homogenate was re-packaged as a carton (27.2 kg) while the remainder was weighed into separate 1 kg packages and vacuum sealed (Cryovac® Barrier Shrink). All of the meat samples were stored at -20 °C until distribution for analysis. This was done to maintain the integrity of the samples. While it is possible that this may have some impact on the analysis, it was assumed to be negligible. The unused carton was set aside for X-Ray analysis.

The usual practice for sampling bulk-packaged cartons recommends the use of at least 24 cartons of product, and the use of a mincer of 2 to 4 mm for 1 to 2 kg of meat, for the purpose of calibration and verification of instruments for CL estimation (Eustace & McPhail, 2006). The present approach was different to this, aimed at producing a larger mass (54 kg) of homogenised meat product.

A package (1 kg) of each meat was used to form a set of homogenates in preparation for analysis. The sets were distributed to the different facilities performing the Soxhlet analysis, with only beef samples eligible for shipment to the US. All of the samples were sent to different vendors for measurement using NIR and NMR analyses as well as by the microwave moisture method. Measurements using NIT were performed at CSIRO, Werribee. A set of cartons (as received) were sent for X-Ray analysis.

No instruction was given to the vendors on how to perform the measurements, as it was assumed that best operational practice would be employed, and be reflective of usual industry practice. Not all homogenates were analysed by each method.

4.3 Selection of CL analysis methods for method comparison.

The outcomes from the survey were used to select appropriate CL analytical techniques for inclusion as part of the method comparison. As noted above, the microwave moisture method remains an important method for contemporary CL determination, and was included as part of this study.

The Soxhlet method is used for the analysis of fat in meat, since the CL content of meat is related by CL = 100 - % fat, where the latter is the meat's fat content. The method is deemed by AOAC International as the Final Action method for the analysis of fat in meat. This method was included in this study, given its status for fat analysis, and it is also the method of choice for external providers performing CL measurements. For this analysis, four commercial providers (three based in Australia

and one in the USA) were used as part of the inter laboratory comparison. The US facility was accredited with the Meat Import Council of America (MICA) as an approved laboratory, which was part of the second objective for this project; that is, include overseas facilities as part of the study. This analysis was also performed at two universities involved with meat science research.

Other popular techniques used in meat processing facilities include those based on near infrared transmittance (NIT), and X-Ray analysis, with other approaches using near infrared (NIR) and nuclear magnetic resonance (NMR). These instrumental techniques were also included for the inter method comparison.

For this study, the authors have chosen to maintain anonymity of the participants.

4.3.1 Method description

Microwave moisture method

A mixture (500 g) of sand and silicon carbide (90:10 w/w) was prepared prior to the analysis. Glass jars, with a rubber band and 15 cm^2 Chux cloth material were pre-weighed (A). The sand/silicon carbide mixture was placed into a microwave oven, which was set to the maximum power. The mixture was heated at this temperature for 5 min. This pre-heated the oven before the analysis. The sample (20 g) was placed into a glass jar with weighing (B), and heated in the microwave oven. Measurements of the total mass were made until a constant value was obtained (C). The moisture content (M %) was determined using $M\ \% = 100\ \mathrm{X}\ \frac{B-C}{B-A}$, which was then used to determine the sample's chemical lean content (CL %), according to:

Beef with CL ≥ 80%	$CL\ \% = 1.21\ \mathrm{X}\ M\% + 5.44$
Beef with CL < 80%	$CL\ \% = 1.35\ \mathrm{X}\ M\% - 3.2$
Mutton	$CL\ \% = 1.25\ \mathrm{X}\ M\% + 2.7$
Pork	$CL\ \% = 1.27\ \mathrm{X}\ M\% + 1.1$

Near infrared reflectance (NIR)

Approximately 800 g sample was used for the analysis. The sample was packed into a plastic sample cup to the level of the cup, ensuring an even surface. The sample was placed into the instrument, and analysed. A representative view of meat and the instrument is shown in Figure 1.

Figure 1. Representative figure of meat homogenate being analysed by near infrared reflectance.

Near infrared transmittance (NIT) A

Approximately 500 g of the sample was placed into a food processor and homogenised for 30 to 60 s. The remaining portion was retained for repeat testing if necessary. Each meat sample was scanned in the NIR instrument four times. After homogenisation, the meat was weighed (85 g) and packed into the sample dish. The sample was scanned and the results recorded. The same sample was repacked into the dish and re-analysed. This step was repeated using a second sample aliquot.

Near infrared transmittance (NIT) B

Approximately 200 g sample was placed into a sample cup, with care taken to avoid air pockets in the samples. The samples were packed in a consistent manner and packed to the level of the sample cup. The sample temperature was in the range of 10 to 20 °C. After the instrument had started and performed appropriate diagnostics, the sample in the cup was placed into the holder in the instrument. The analysis was started after the door had been locked into place. When complete, the sample was removed from the instrument and the result was stored for later processing.

Near infrared transmittance (NIT) C

Approximately 180 g sample was placed into a sample cup. Care was taken to avoid air pockets in the samples, and that they were packed to the level of the sample cup, and in a consistent manner. The sample temperature was in the range of 10 to 20 °C. After the instrument had started and performed appropriate diagnostics, the cup plus sample was placed into the holder in the instrument. The analysis was started after the door had been locked into place. When complete, the sample was removed from the instrument and the result was stored for later processing.

Nuclear magnetic resonance (NMR)

The meat samples were thawed and analysed as received with no further grinding. Two glass fibre pads were tared with the sample (5 to 6 g) placed in the centre of one pad and covered with the second one. Sample was pressed flat between the pads using a sample press. The pad containing sample was then dried before being transferred to the NMR spectrometer, which had been conditioned using the internal heater and analysed to give a fat measure for the sample.

Soxhlet extraction

The Soxhlet method involves the removal of fat from the meat with an organic solvent by continuous extraction. Commercial facilities use this method as the benchmark for fat analysis. For the analysis, a meat sample (5 g) is weighed into a Soxhlet thimble and heated at 102 °C for 5 hr. After cooling, the thimble and contents are extracted with an organic solvent for 6 hr; typically, this is done with petroleum spirit (b.p. 60 to 80 °C) but other solvents can also be used, along with different (longer) periods for extraction. After cooling, the flask containing the solvent is removed and the solvent is evaporated. The flask and contents are dried at 102 °C until constant weight is reached. The fat content of the sample (% fat) = 100 x $\frac{W_2 - W_1}{S}$ where S, W_1 and W_2 are the weights of the sample, empty flask and flask with extracted fat, respectively. The chemical lean (CL) content is calculated as CL = 100 - (% fat).

X-Ray

The cartons were passed through the X-Ray analyser, and the results were recorded.

4.4 Comparison of results

4.4.1 Analysis of variance (ANOVA)

An ANOVA relating the CL results with the methods was performed using R (R Core Team, 2018).

4.4.2 Normalised error (E_n)

The normalised error (E_n) is one metric which can be used to assess whether results can be regarded as satisfactory or otherwise. It was calculated using:

$$E_n = \left| \frac{x_{lab} - X_{reference}}{\sqrt{U_{lab}^2 + U_{reference}^2}} \right|$$

Where || represents the absolute value, x_{lab} is the participant's measured result, $X_{reference}$ is the assigned value, U_{lab} is the uncertainty of the participant's measurement and $U_{reference}$ is the uncertainty relating to the assigned value (Softić, Zaimović-Uzunović, & Bašić, 2012). The participants in the study were not requested to provide estimates for measurement uncertainty relating to the results. Thus, assumptions were made to calculate the normalised error. Firstly, the value for the participant's result was taken as the central tendency estimator which, in the case of duplicate measurements (x_1 and x_2) of a homogenate, was taken as $\frac{x_1+x_2}{2}$ while, when $n \geq 3$ measurements were made for a homogenate, the mean was used in the case. The median of the results relating to the homogenate was used for $X_{reference}$ (Table 2). In order to estimate U_{lab}, the standard deviation (σ) of the results was used for $n \geq 3$ measurements with U_{lab} = 2 *x* σ while, for duplicate measurements, the standardised difference was used, meaning that U_{lab} = 2 *x* $\frac{|x_1-x_2|}{\sqrt{2}}$, and for the reference value, $U_{reference}$ = 2 *x* normalised interquartile range (IQR) of the homogenate (Table 2). The normalised IQR is calculated using $\frac{Q(3)-Q(1)}{1.349}$ where $Q(3)$ and $Q(1)$ are the values at the 75th and 25th percentiles respectively (Proficiency Testing Australia, 2016), which provides a good estimate of the standard deviation. The calculation was performed using R (R Core Team, 2018). When $E_n \leq 1$, the results would be regarded as "satisfactory" while, for $E_n > 1$, the results would be regarded as "not satisfactory". The X-Ray result was excluded from this analysis as there was only one result.

4.4.3 z-Scores

The *z*-score is the most widely used approach employed for interlaboratory studies, and is calculated using $z = \frac{x - x_{pt}}{\sigma_{pt}}$ where x is the measured result, x_{pt} is the assigned value and σ_{pt} is the 'standard deviation for proficiency testing' (Hibbert, 2018). The assigned value, x_{pt}, can either be a reference value (such as from a certified material) or a consensus value obtained by central tendency estimators such as the mean or median. The latter was used for this study, and the median of all results from the appropriate group was employed for each respective group. For σ_{pt}, the normalised interquartile range (IQR) was used as a robust estimate for the standard deviation. Another approach to estimate the value for σ_{pt}, was to use a modified form of the Horwitz curve, which is intended to provide a reasonable value that is regarded as "fit for purpose" for proficiency testing studies. It was calculated using

$$\sigma_{pt} = \begin{cases} 0.22c, if\ c < 1.2\ X\ 10^{-7} \\ 0.02c^{0.8495}, if\ 1.2\ X\ 10^{-7} \leq c \leq 0.138 \\ 0.01c^{0.5}, if\ c > 0.138 \end{cases}$$

where c is the concentration on a mass per mass basis (Thompson, 2000). This meant that the CL measurements were transformed to the equivalent fat concentration by calculating $c = \frac{100-\text{CL}}{100}$, and the z-score calculated using the median and related σ_{pt} for each group of samples based on the transformed data. The calculations were performed using R (R Core Team, 2018)

In usual practice, normalised errors and z-scores are used in proficiency testing (PT) studies to assess the performance of testing laboratories, and not methods. However, for the Australian meat industry, approvals for determinations (such as CL content) is usually sought by, and awarded to, the related equipment vendor, with final decisions made by the Australian Meat Industry Language and Standards Committee (AMILSC) (Eustace & McPhail, 2006). Given this vendor based approach used by industry, it was reasoned that the use of normalised errors and z-scores would be the most suitable for this study.

5.0 PROJECT OUTCOMES AND DISCUSSION.

5.1 Survey results of methods for chemical lean determination in Australian meat processing industry

Figure 2 summarises the survey results and shows that the microwave moisture method was the most commonly used technique among participating facilities. Instrumental methods including X-ray, NIR and NMR, were used by fifteen facilities, while two processors contracted external providers who used Soxhlet extraction for determining CL content (Figure 1). It should be noted that some facilities used more than one method.

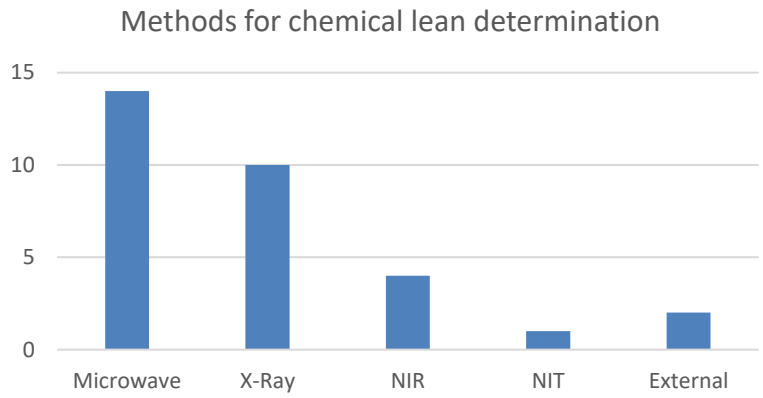


Figure 2. Tally of methods used for chemical lean determination by Australian meat processors.

Note: NIT = near-infrared transmittance NIR = near-infrared reflectance

No facilities used other wet chemical techniques such as Babcock or Foss-let techniques, presumably either due to the age of the techniques, their relative degree of complexity in application or the need for specialised chemicals. For example, the Babcock method requires concentrated sulphuric acid which is highly corrosive while the Foss-let method utilises perchloroethylene (also known as tetrachloroethylene) which is classified as a Group 2A carcinogen and regarded as probably carcinogenic to humans. Given the nature of these chemicals, it is easy to understand why these methods are no longer deployed in Australian meat processing facilities since chemicals would represent an occupational health and safety issue. With the older instrumental techniques (e.g. Anyl-Ray and MQ27), the availability of the newer techniques would replace the use of such equipment. Some of the instrumental methods have only been recently accredited since 2012 which could create some delay in the industry adoption of these methods.

Anecdotal comments from the survey indicated that the microwave moisture method was used due to its simplicity and the minimal equipment required for its deployment. One operator stated that the method was particularly suitable for smaller facilities where only one technician is available to do CL determinations, along with other required duties and responsibilities. Another commented that instrumental methods (such as X-Ray or NIR) tend to be favoured by larger meat processors. Determinations based on X-ray analysis are suitable for large volumes of meat (using cartons) and readily deployed in larger meat processing facilities with the economies of scale to afford the infrastructure expense. Additionally, the instrumental techniques would be easier to use, and have capacity to process large volumes of meat.

5.2 Chemical Lean Analysis

Appendix 2 presents the complete set of measurements for each set of meat homogenates, based on the analytical measurement technique and related vendor. A preliminary analysis was made of the data set to ensure that the data was normal (i.e. Gaussian). Figure 3(a) shows the histogram of the CL measurements made by the various analytical techniques for each homogenate and it can be seen that approximately normal curves were apparent for each sample while Figure 3(b) shows the quantile-quantile (QQ) plots of the CL content for each meat homogenate. QQ plots are intended as a visual check for normality of the data, where approximately linear plots are observed with Gaussian data, which is the case for each homogenate. It is apparent that one result could be identified as an outlier, which was the X-Ray measurement (Appendix 1).

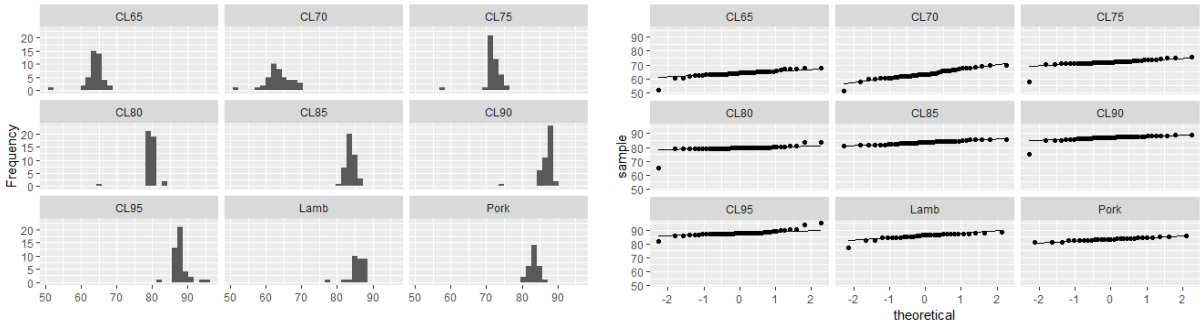


Figure 3 (a) Histograms and (b) quantile-quantile plots (QQ-plots) of the aggregate CL content for each meat homogenate using all analytical techniques.

Table 2 summarises the results for each homogenate. In spite of the presence of the outlier, the span of the measurements for each sample group was very good with the largest span associated with the beef sample, CL70, with a median of 63.0 $\pm$ 2.7 (normalised IQR). Most of the beef samples were close to the stated CL values, except for CL70 and CL95.

Table 2. Summary statistics of the aggregate chemical lean (CL, %) measurements for beef of differing CL content (CL65, CL70, CL75, CL80, CL85, CL90 and CL95), lamb and pork using all analytical techniques.

Sample	Median[A]	Norm. IQR[B]	Min[C]	Max[D]	Range[E]
CL65	64.2	1.3	52.0	67.7	15.7
CL70	63.3	3.2	51.0	69.4	18.4
CL75	71.8	1.4	58.0	75.5	17.5
CL80	79.6	0.6	65.0	83.7	18.7
CL85	83.8	1.1	81.0	85.9	4.9
CL90	87.3	0.8	75.0	89.0	14.0
CL95	87.7	1.1	82.0	95.0	13.0
Lamb	86.5	1.7	77.0	88.2	11.2
Pork	83.3	1.2	80.8	86.0	5.2

Note[A]n = 43, except for Lamb and Pork where n = 31 and 29, respectively [B]Norm. IQR = normalised interquartile range between the 75th and 25th percentiles (IQR = $[Q_3 - Q_1]/1.349$), used as an approximation for standard deviation [C]Min = minimum [D]Max = maximum [E]Range is the difference between minimum and maximum.

Figure 4 shows the distribution of the CL content, according to meat homogenate and measurement technique. For some homogenates, the results are similar across each technique (eg CL80 in Fig. 4(a)), but for other samples there were marked differences across the techniques for beef homogenates (eg CL75, Fig. 4(a), and lamb and pork homogenates (Fig. 4(b)). An ANOVA indicated that there were differences between the CL results and the different methods ($P < 0.001$). This could be related to sample inhomogeneity. All meat samples though were extensively homogenised prior to distribution to the participating laboratories, thus we assume (although cannot completely discount) that this variation was not associated with a lack of sample homogeneity. Even though the ANOVA results]are statistically significant, it is important to identify whether such differences are practically significant. Inevitably, there will be some variation of CL content across samples (Vander Heyden & Smeyers-Verbeke, 2007), and it would be useful to differentiate between the sample variation and the variance that arises from the measurement process.

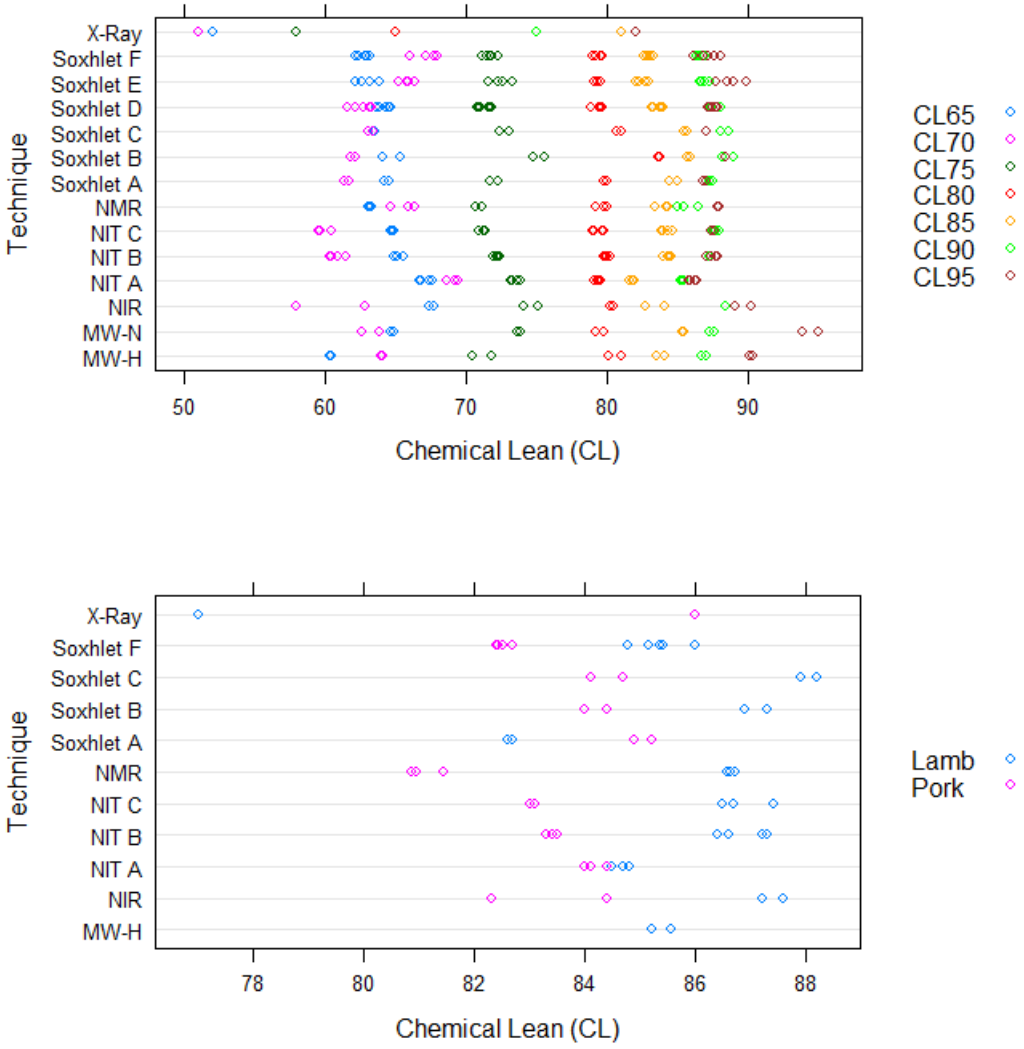


Figure 4. Plot of the CL content distribution for (a) beef of different CL content and (b) lamb and pork, based on measurement technique.

Note: MW-H = microwave moisture method for homogenised samples MW-N = microwave moisture method for non-homogenised samples NIT = near-infrared transmittance NIR = near-infrared reflectance NMR = nuclear magnetic resonance

5.3 Comparison of CL results

5.3.1 Normalised error (E_n)

The normalised errors (E_n) were calculated for each homogenate and measurement technique (Table 3). The result shown in bold represents when $E_n > 1$. Overall, nearly all of the values are less than 1, which meant the results would be regarded as satisfactory. This was not the case for the Soxhlet B results for beef homogenate CL80 (E_n = 2.77) while the microwave moisture results for non-homogenised beef CL95 were close to 1 (E_n = 0.91). In future work, it would be useful to have estimations for the measurement uncertainty for this metric.

Table 3. The normalised error (E_n) for chemical lean measurements for beef of differing CL content (CL65, CL70, CL75, CL80, CL85, CL90 and CL95), lamb and pork.

Technique	CL65	CL70	CL75	CL80	CL85	CL90	CL95	Lamb	Pork
MW-H	-0.56	0.02	-0.06	0.27	0.00	-0.18	0.49	-0.10	
MW-N	0.08	0.00	0.24	-0.07	0.32	0.03	0.91		
NIR	0.48	-0.03	0.28	0.46	-0.05	0.43	0.25	0.08	0.00
NIT A	0.39	0.14	0.21	-0.18	-0.41	-0.76	-0.33	-0.16	0.14
NIT B	0.13	-0.06	0.05	0.24	0.10	0.04	-0.04	0.03	0.01
NIT C	0.08	-0.08	-0.08	-0.13	0.06	0.14	-0.06	0.04	-0.04
NMR	-0.15	0.05	-0.10	0.01	0.02	-0.35	0.02	0.01	-0.36
Soxhlet A	0.02	-0.04	0.02	0.20	0.16	0.02	-0.16	-0.33	0.29
Soxhlet B	0.05	-0.03	0.36	**2.77**	0.41	0.31	0.14	0.05	0.15
Soxhlet C	-0.13	0.00	0.11	0.77	0.35	0.30	-0.14	0.13	0.17
Soxhlet D	-0.02	-0.01	-0.06	-0.18	-0.04	0.12	-0.04		
Soxhlet E	-0.14	0.06	0.07	-0.22	-0.24	-0.15	0.13		
Soxhlet F	-0.20	0.09	-0.02	-0.13	-0.17	-0.19	-0.08	-0.10	-0.14

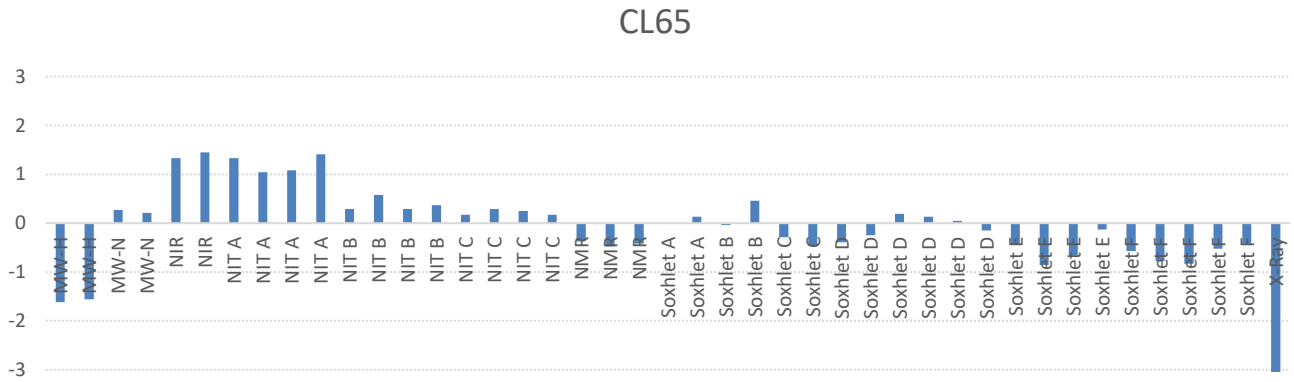
5.3.2 z-Scores

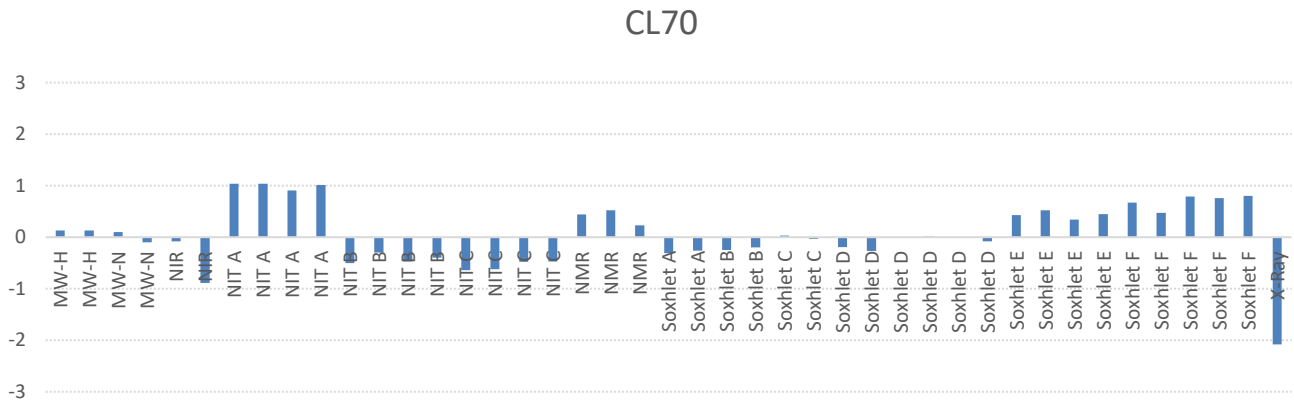
The robust z-scores for the CL results were calculated for each set of homogenate with $z = \frac{\text{CL} - \text{median(CL)}}{\text{Normalised IQR(CL)}}$ using the median and normalised IQR for each homogenate (Table 2). Appendix 3 provides a summary of the z-scores using this approach. The numbers highlighted in bold indicate where $z > |3|$. Figure 5 shows the plot of the z-scores for each homogenate, based on each measurement. It should be noted the minimum and maximum values for the abscissa of Figure 5 are -3 and 3, respectively, and that there are some points beyond these values (Appendix 3). The use of z-scores is aimed at providing a transparently but widely applicable scoring system for participants in proficiency testing, and provide an appropriate scaling of the difference between the measured results and the 'assigned value' (Analytical Methods Committee, 2016). This approach provides a value which is deemed to be satisfactory or otherwise. This assessment is made using (Vander Heyden & Smeyers-Verbeke, 2007):

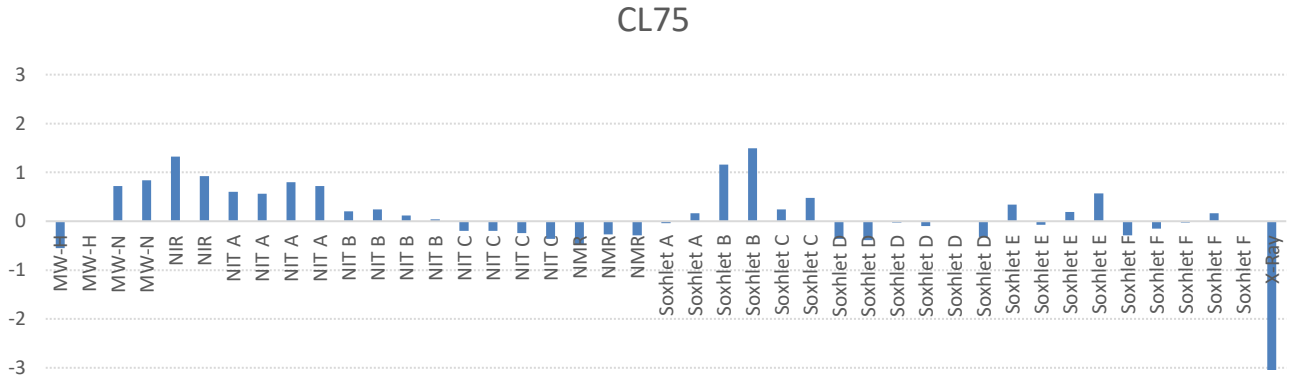
$$\begin{cases} |z| < 2, \ satisfactory\ performance \\ 2 \leq |z| \leq 3, questionable\ \ performance \\ |z| > 3, unsatisfactory\ performance. \end{cases}$$

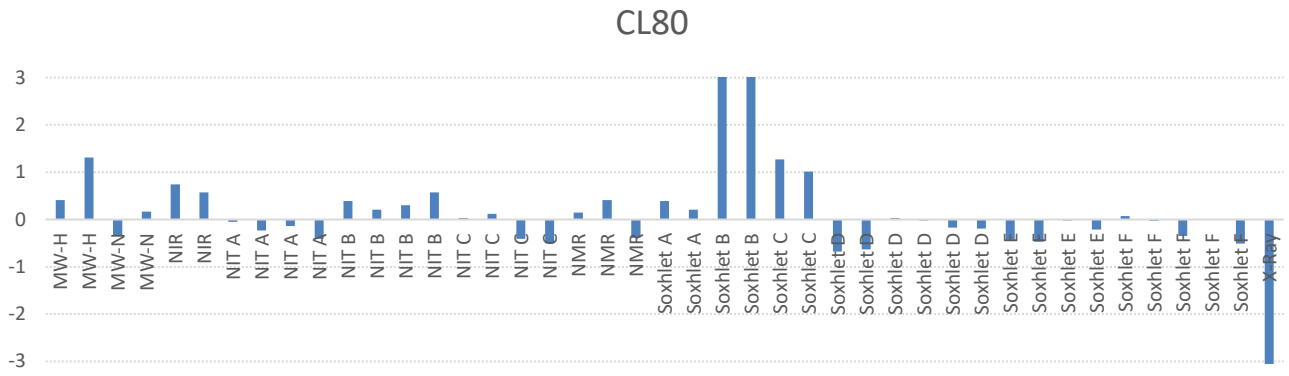
Overall, most of the results could be regarded satisfactory since most of the z-scores were below 2, meaning that they lie within 95% of the median value (Appendix 2). Those results that would be

deemed as not satisfactory were the X-Ray results for the beef homogenates CL65, CL75, CL80, CL90 and CL95 as well as lamb. The results for the microwave moisture method of the non-homogenised beef homogenate CL95 and Soxhlet results for beef CL80 from Laboratory B, would also be regarded as not satisfactory. These were not surprising, given the E_n results (Table 3). The X-Ray result for beef CL80 would be regarded as questionable but this was just marginal (z = 2.03). The results from the X-Ray analysis were not unexpected, given the observed difference of the original result from the other results from the other techniques. It should be noted though that the number of unsatisfactory results was relatively quite small, representing < 3% of the total data set. The corollary to this, of course, is that nearly all of the results were satisfactory.








CL80
3
2
1
0
-1
-2
-3
MW-H
MW-H
MW-N
MW-N
NIR
NIR
NIT A
NIT A
NIT A
NIT A
NIT B
NIT B
NIT B
NIT B
NIT C
NIT C
NIT C
NIT C
NMR
NMR
NMR
Soxhlet A
Soxhlet A
Soxhlet B
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Soxhlet C
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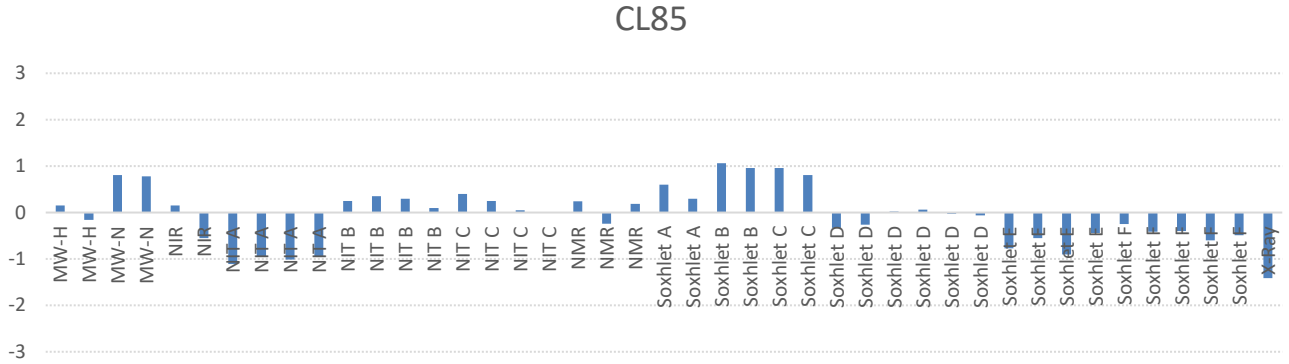
Soxhlet D
Soxhlet D
Soxhlet E
Soxhlet E
Soxhlet E
Soxhlet E
Soxhlet F
Soxhlet F
Soxhlet F
Soxhlet F
X-Ray


CL85
3
2
1
0
-1
-2
-3
MW-H
MW-H
MW-N
MW-N
NIR
NIR
NIT A
NIT A
NIT A
NIT A
NIT B
NIT B
NIT B
NIT B
NIT C
NIT C
NIT C
NIT C
NMR
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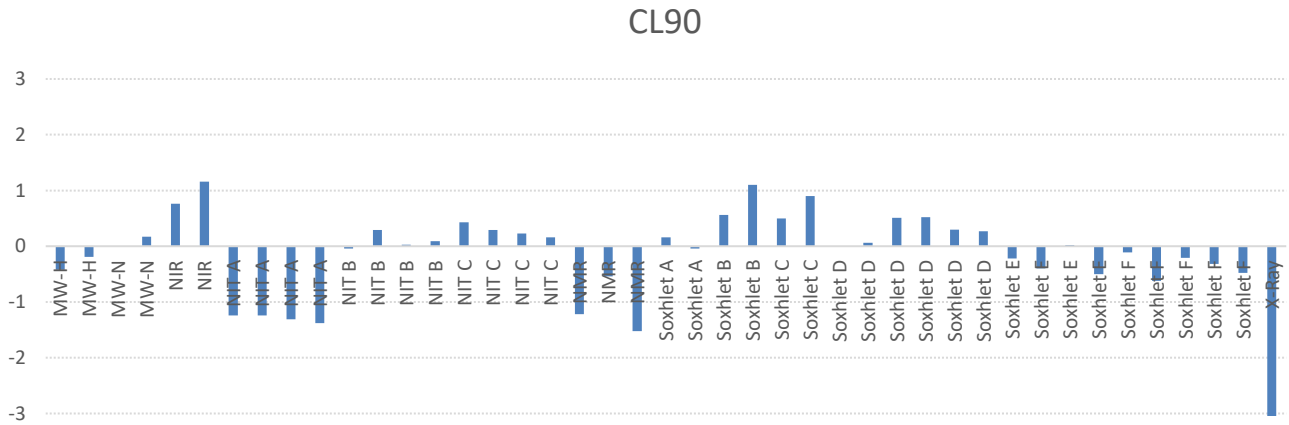
X-Ray


CL90
3
2
1
0
-1
-2
-3
MW-H
MW-H
MW-N
MW-N
NIR
NIR
NIT A
NIT A
NIT A
NIT A
NIT B
NIT B
NIT B
NIT B
NIT C
NIT C
NIT C
NIT C
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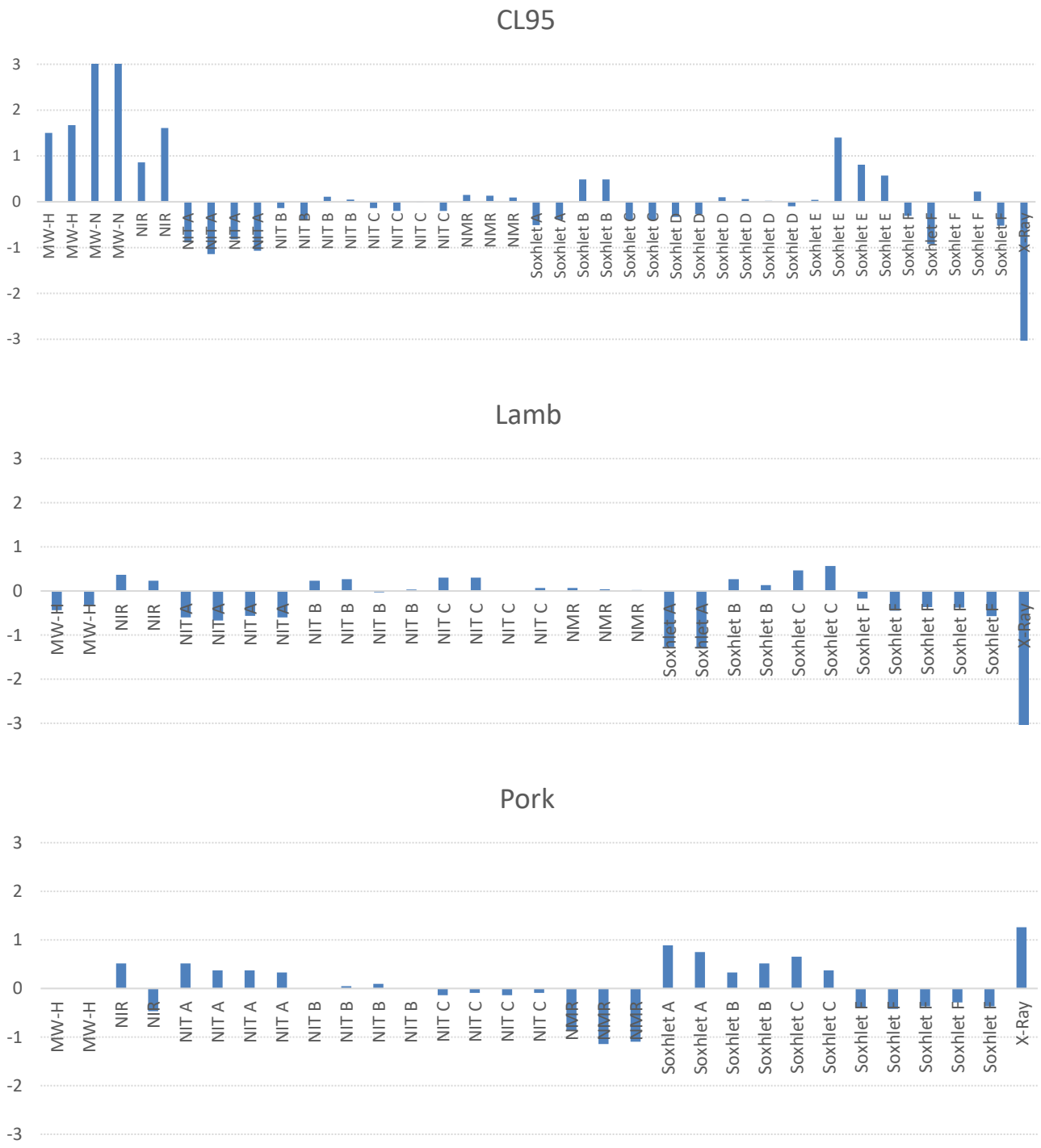

Figure 6. Plot of *z*-scores for beef of differing chemical lean content (CL65, CL70, CL75, CL80, CL85, CL90 and CL95), lamb and pork based on analytical method.

Note: MW-H = microwave moisture method for homogenised samples MW-N = microwave moisture method for non-homogenised samples NIT = near-infrared transmittance NIR = near-infrared reflectance NMR = nuclear magnetic resonance

The comparison of the CL results using z-scores (based on the median and the normalised IQR) has demonstrated that nearly all of the results were satisfactory and thus, using this approach, the analytical techniques used for this study would be regarded as suitable for the determination of CL content in Australian meat, whether it would be beef, lamb or pork. It needs to be noted though that some caution is needed in interpreting z-scores since this approach is used to identify whether the intended method is fit for its intended purpose. This was done by scaling the data in order to allow for statistical evaluation. It is neither intended nor designed to be used as a metric that rates one method as 'better' compared to another. A z-score which falls between $\pm$ 2 would indicate that there was no reason to suspect that that the analytical procedure would need call for revision (Analytical Methods Committee, 2016). In this study, for example, there was only one sample for the Soxhlet method which would be regarded as unsatisfactory from a total of forty-nine samples (across homogenates and providers). Given that most of the results were satisfactory, it was reasonable to assume that this was more related to that specific sample than the technique. This would also be the case for the result for the non-homogenised beef CL95 sample which was analysed by the microwave moisture method. Again, it was more likely that this was sample specific, given that the remainder of the samples analysed by this technique were satisfactory. With regards to the X-Ray results, it's feasible that the analyser may have needed calibration since these results were consistently below the others.

An alternative approach for calculating the z-scores was also investigated, based on a modified form of the Horwitz equation. This approach is recommended to provide values that are 'fit-for-purpose' and is consistently used for proficiency testing studies. Given the CL content relates to the fat content of the meat, the CL results were converted to the equivalent fat content (on mass per mass basis) using $\frac{100-\mathrm{CL}}{100}$, and the median and related σ_{pt} (section 4.4.2) for the results based on homogenate type were determined (Table 4). For comparison, this was also done for the CL content as well (Table 4). The median and σ_{pt} of the fat content were used to calculate the associated z-scores, shown in Appendix 4. In comparison to the first approach, the z-scores were quite different in this case and much larger in their magnitude. If these had have been used to assess the results and related methods then this which would have led to the different conclusion in which the results would have been regarded as not satisfactory. In fact, the number of data points where $|z| \geq 3$ was 155 while, for $2 \leq |z| < 3$, the number of points was 110. The reason for this lies in the differences between σ_{pt} used for each approach. The value for σ_{pt} is larger for the normalized IQR compared to the approach detailed in section 4.4.2. For example, σ_{pt} = 1.1 and 1.7 for beef CL95 and lamb, respectively, based on the normalised IQR (Table 2) while the equivalent values were 0.094 and 0.093 (Table 4) using the modified Horwitz equation. This represents over an order of magnitude in difference between the values, and thus explains the difference between the two sets of z-scores. The use of the modified Horwitz equation has not been recommended for proficiency testing of methods relating to fat determination, along with other empirical analytes which are method dependent (Horwitz & Albert, 2006; Rivera & Rodríguez, n.d.). Yet, the ANOVA results (§ 5.2) and these z-scores suggest that further work is required to identify what may be the cause of this difference between these approaches, and whether there is of any practical significance.

Table 4. The median and σ_{pt}[A] of fat (g/g) and chemical lean content (%) for beef of differing CL content (CL65, CL70, CL75, CL80, CL85, CL90 and CL95), lamb and pork.

Sample	Fat content (g/g)		CL content (%)	
	Median	σ_{pt}	Median	σ_{pt}
CL65	0.358	0.0060	64.2	0.080
CL70	0.367	0.0061	63.3	0.080
CL75	0.282	0.0053	71.8	0.085
CL80	0.204	0.0045	79.6	0.089
CL85	0.162	0.0040	83.8	0.092
CL90	0.127	0.0035	87.3	0.093
CL95	0.124	0.0034	87.6	0.094
Lamb	0.135	0.0037	86.5	0.093
Pork	0.167	0.0041	83.3	0.091

[A]see § 4.4.2.

It should be noted that some techniques needed samples of known CL content to be used as calibration standards for measurement. For this purpose, the beef homogenates which had been analysed by Soxhlet extraction (from providers A, B and C), and close to the stated CL content, were used for this purpose. The homogenates were CL65, CL75, CL80, CL85 and CL95. The remaining samples i.e. beef CL70 and CL90, pork and lamb) were treated as unknowns. It was useful to identify whether this may not have introduced some error for these measurements if there were significant differences between the central measures of the smaller data set compared to the larger one. Table 5 shows the mean and standard deviation for the smaller set of results, which were provided as 'known' values. Only a relatively small difference (approx. 2 %) was found between the medians for the smaller sample set (Table 5) in comparison to the medians of the larger set (Table 2), indicating that the impact would be relatively small (if any) and very unlikely to impact on the measured values for the 'unknown' samples.

Table 5. The mean and standard deviation (SD) of chemical lean content (%) for selected beef homogenates (CL65, CL75, CL80, CL85 and CL90) analysed by Soxhlet extraction (providers A, B and C).

Beef	Mean ± SD[A]
CL65	64.1 ± 0.8
CL75	73.2 ± 1.5
CL80	81.5 ± 1.7
CL85	85.4 ± 0.6
CL90	88.0 ± 0.6

[A]n = 6

Overall, the comparison using normalised error and robust z-scores has shown the methods have performed satisfactorily, across a wide range of CL content for three different species. The normalised error was calculated using the measurement uncertainty for each set of measurements, while the z-scores indicated how far the results were dispersed from the central tendency indicator (in this case, the median). The ANOVA results and use of the modified Horwitz curve provides a contrast, which does warrant further investigation. In the study, the methods were diverse, including instrumental based techniques (NIR, NIT, NMR and X-Ray) and chemical analysis (Soxhlet fat extraction and microwave moisture), which used different sample sizes (e.g. ranging from 5 g to 500 g to 27.2 kg). Given this

breadth and diversity, it would not been surprising to see if there were differences in the result comparison. However, this was not the case. This would indicate that this approach was useful in demonstrating the performance of the methods for the meats of different CL content.

5.4 Discussion

As noted in Section 4.1, there are presently fifteen methods approved by AUS-MEAT Ltd which can be used by the Australian meat processing industry for chemical lean (CL) determination of export meat products (Table 1). AUS-MEAT Ltd is the Australian meat industry's non-for-profit organisation which, in part of its function, has oversight of the quality and standards processes within the industry[2]. Approvals for determinations (such as CL content) are made by the Australian Meat Industry Language and Standards Committee (AMILSC), who ensure that the accuracy, repeatability and reproducibility of measurements are satisfactory, along with a number of other factors (Eustace & McPhail, 2006). Equipment approval is usually sought, and awarded to, the related equipment vendor. At its simplest, approval of a method is provided if equivalency of results is demonstrated between the proposed method and that of established methods, such as Soxhlet extraction (Eustace & McPhail, 2006). In context, this represents an industry set approach to standardisation that is 'fit for purpose' for the needs of Australian meat processing industry.

The industry survey revealed that not all of the approved methods were being used by industry. Notably, these included the older wet chemical techniques (e.g. Babcock and Foss-let), along some instrumental methods (e.g. Anyl-Ray and MQ27). The wet chemical techniques use hazardous chemicals (e.g. concentrated sulphuric acid and tetrachloroethylene) which would represent a potential health and safety issue for meat processing facilities. Based on the industry survey, there is also no evidence that these methods are currently used in meat processing industries. Thus, it is suggested that a review is made of these approved methods and an assessment made on the suitability of these methods for contemporary practice.

The method comparison demonstrated that the methods performed satisfactorily for different meats with a range of varying CL content. The normalised error (E_n) and robust z-scores were used as metrics to test the suitability of methods for CL determination. For the former metric, all but one result was deemed to be satisfactory while, for the latter, there was no consistent evidence to suggest that any method's performance was unsatisfactory. In fact, the number of points that could be regarded as such was quite low (< 2 % of the overall data set). Thus, it can be concluded that these methods are suitable for determining CL content of these species, and of varying CL content.

A wide range of different methods, including instrumental based techniques (NIR, NIT, NMR and X-Ray) and chemical analysis (Soxhlet fat extraction and microwave moisture), were included for the method comparison. This was done to ensure coverage of the techniques indicated in Table 1 as well as ensure that the techniques reflected contemporary industry practice (Figure 1). Even though first described in 1997, the microwave moisture method still remains in use in industry largely due to its simplicity and the minimal equipment required for its deployment. This method's performance was also satisfactory in the comparison. At present, alternative ways for measuring moisture could be available and thus it would be useful to consider whether these could be used for CL determination. The Soxhlet fat extraction technique remains as the AOAC Final Action Method for the analysis of fat

[2] https://www.ausmeat.com.au/about-us/history/

in meat and it is also recognised by industry as a reference chemical analytical method (Anonymous, 1998). In this study, the Soxhlet results were less than 1 σ from the aggregate median and thus were regarded as satisfactory for CL determination. The instrumental methods also performed well for the different homogenates with the results close to the aggregate median. Overall, the comparison would indicate that these methods would be regarded as 'fit for purpose' for CL determination.

One important factor for CL determination relates to sample size. At one extreme, cartons (27.2 kg) are deployed for X-Ray analysis while, at the other, 5 g of sample is needed in the case of Soxhlet fat extraction. With a carton of high CL content, it is very likely that the content will be heterogeneous, and so appropriate sampling strategies are needed to ensure that the sample is sufficiently homogenous for techniques requiring smaller sample sizes. This was regarded as important for this study and effort was made to ensure that sufficient sample (54 kg, see § 4.2) was produced for the method comparison. Other strategies have been described elsewhere (Eustace & McPhail, 2006). It was anticipated that the preparation of the meat homogenates would allow the meat to be well-mixed (uniformly distributed) for the method comparison. In other areas where interlaboratory, and method, comparative studies are performed, there usually is a reference material which is available. Sometimes, these materials are certified, meaning that there is a prescribed value assigned to the material. Such materials are, or have been, available for the measurement of fat in meat; e.g.(Kolar, Faure, Torelm, & Finglas, 1993; Welch et al., 2001). Kolar *et al* (1993) describe the analysis of a "fresh" meat product with a stated fat content of 21.11 $\pm$ 0.11 g per 100 g while Welch *et al* (2001) describe the development of National Institute of Science and Technology (NIST) Standard Reference Material (SRM) 1546a, which has a certified value of 18.96 ± 0.40 g per 100 g. In the case of the former, the sample size was 200 g while, for NIST SRM 1546a, the sample consists of 4 x 85 g with a high cost ($1,000). Materials such as these would be useful for this study but the associated cost would be prohibitive due to amount of sample needed to cover the project's scope. Future studies may need to consider the development of suitable reference materials to which certified values can be assigned, and thus be used to cover the scope of the different measurement techniques. One approach to develop such a material could be to take lean meat and add sufficient fat to reach a particular CL level, ensuring that the combination is sufficiently mixed and homogenous. It's suitability as a reference material could be confirmed using a similar range of techniques deployed in this study.

To the best of the authors' knowledge, this represents the first time that a comprehensive study, both in terms of methods and meats, has been undertaken to review and investigate CL determination in the Australian meat industry. For future studies, this study's outcomes are a useful benchmark for comparative purposes. In fact, it is recommended that further comparative studies are conducted in the future to build on these outcomes, and thus obtain further information and knowledge on the on-going performance of these methods in industry.

The third objective of this project related to the development of a proposal for a national standard for CL determination. The intent of developing such a standard was to assist in any on-going discussion with Australia's overseas trading partners relating to CL analysis, and to also create an awareness of Australia's measurement and quality standards, and the associated quality systems. As previously noted, oversight of the quality and standards processes within the Australian meat industry is performed by AUS-MEAT Ltd with approvals made by the AMILSC, which is comprised of meat industry representatives. The development of such standards provides confidence to the industry that there are a particular level of quality (Wilson-Wilde, 2018). Before developing such a proposal, engagement

with the Australian meat industry would be required to ascertain whether there was a need for such a standard. If there is no industry interest then the existing industry infrastructure would thus be regarded as fit for its intended purpose. However, if there was sufficient interest then a national standard could be proposed and developed. It is useful to note that, at the national level, the Australian Technical Infrastructure Alliance (ATIA)[3] exists to ensure that the nation gets the best value of its standards and conformance infrastructure, supporting the Australian government and industry, both nationally as well as globally. The ATIA consists of the Joint Accreditation System of Australia and New Zealand (JASANZ), the National Association of Testing Authorities (NATA), the National Measurement Institute (NMI) and Standards Australia. In developing a national standard for CL determination, there would benefit to the Australian meat industry by consulting with the ATIA, particularly in relation to global engagement.

[3] http://www.atia.org.au/Default.aspx

6.0 CONCLUSIONS/RECOMMENDATIONS

Conclusions

- Engagement with Australian meat processors indicated a number of methods are currently deployed for CL measurement. These included chemical analyses (e.g. Soxhlet fat extraction and microwave moisture) to instrumental based techniques (e.g. NIR, NIT, X-Ray). The microwave moisture method was used due to its simplicity and the minimal equipment required for its deployment, while the instrumental techniques would also be popular due to their ease of use, and capacity to process large volumes of meat.
- A set of standardised reference meat homogenates was prepared, consisting of beef, lamb and pork. A larger number of beef samples were prepared, spanning the range of CL65 to CL95, reflecting the economic importance of this meat product as an export commodity. These homogenates were used in a method comparison, which included Soxhlet fat extraction and microwave moisture analysis, along with NIR, NIT, NMR and X-Ray representative of instrumental techniques.
- The method comparison demonstrated that each method performed satisfactorily, and thus be deemed as 'fit-for-purpose'. The comparison was made using the normalised error and z-scores, and spanned across a wide range of CL content for three different species. The normalised error was calculated using the measurement uncertainty for each set of measurements, while the z-scores indicated how far the results were dispersed from the central tendency indicator (in this case, the median). The methods were diverse and used different sample sizes. Given this breadth and diversity, it would not have been surprising to see differences in the result comparison. However, this was not the case.
- Industry engagement is required with the Australian meat industry to identify if a need exists for the development of a national standard relating to contemporary CL determination. At present, accreditation of industry standards is completed through AUS-MEAT Ltd with approvals made by the Australian Meat Industry Language and Standards Committee.

Recommendations

- The methods used in this study were found to perform satisfactorily and thus can be regarded as 'fit-for-purpose', and suitable for contemporary CL determination.
- Suitable reference materials with a certified value of CL content would be of industry benefit, and it is suggested that such materials be developed for use by the Australian meat industry.
- This study forms the benchmark for contemporary CL determination. It is recommended that further studies be continued to monitor the on-going performance of CL analysis in the Australian meat industry.
- The development of a national standard requires industry involvement and acceptance. If needed, there would be benefit with the Australian meat industry consulting the Australian Technical Infrastructure Alliance (ATIA), particularly in relation to global engagement.

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9.0 APPENDICES

9.1 AUS-MEAT methods for chemical lean determination

This appendix provides detail of the AUS-MEAT approved methods for chemical lean determination, where available.

Soxhlet – Ether extraction method (Anonymous, 1998)

The Soxhlet method involves removal of fat from the meat with ether by continuous extraction. The sample is weighed (5 g) into a Soxhlet thimble and heated at 102 °C for 5 hr. After cooling, the thimble and contents are extracted with petroleum spirit (b.p. 60 to 80 °C) for 6 hr. After cooling, the flask containing petroleum spirit is removed and the solvent evaporated. The flask and contents are dried at 102 °C until constant weight is reached. The fat content in sample (as %) = 100 x $\frac{W_2 - W_1}{S}$ where S, W_1 and W_2 are the weights of the sample, empty flask and flask with extracted fat, respectively.

Babcock – Acid digestion method (Muhl & Eustace, 1977)

The Babcock method uses sulphuric acid to dissolve the meat's non-fat solid component to facilitate the release of fat. Minced meat (9 g) is added to 50% Babcock bottle. After the addition of H_2O (10 mL), the bottle is shaken to suspend the meat in the water. Sulphuric acid (25 mL, S.G. 1.825) is carefully added to the bottle with continuous shaking. After the digestion is complete, hot water is added to the bottle's neck and allowed in a hot water bath (70 to 72 °C) until the fat is collected in a clear layer at the top. The length of the fat column is then measured.

Foss-let – Specific gravity of extract method (Usher, Green, & Smith, 1973)

Foss-let method involves rapid extraction of fat in meat using perchloroethylene using a mechanical shaker and semi-automated measurement of the specific gravity of the extract (Kropf, 1984). The sample is weighed into an extraction chamber and, after the addition of a known volume of perchloroethylene, the chamber is vibrated for 2 min. After filtration, the specific gravity of the extracted solvent is measured and directly converted to fat content (as %) by reference to a table. It has been adopted by the AOAC as First Action method for fat/crude fat in meat and meat products (AOAC 976.21).

Microwave – Moisture determination (Eustace, McPhail, & Small, 2006)

A homogenised sample (20g) is weighed into *ca* 200 to 250 mL beaker (glass or propylene) as duplicates. The duplicate samples are placed into pre-heated microwave oven and the samples are dried for a predetermined time. After cooling (under desiccation), the samples are weighed. The moisture content is determined using $100 \text{ x } \frac{C - A}{B - A}$ where A, B and C respectively represent the weight of beaker, beaker plus sample and beaker plus sample after drying (g). The chemical lean content for the meat sample is related to the moisture content (% H_2O) using the following formulae:

<u>*Beef*</u>

CL% = (1.21 x % H_2O) + 5.44 for CL > 80%

CL% = (1.35 x % H_2O) – 3.2 for CL < 79%

<u>*Mutton*</u>

CL% = (1.25 x % H2O) + 2.7

Pork

CL% = (1.27 x % H2O) + 1.1

Anyl-Ray – X-Ray absorption method (Anonymous, 2013)

"The Anyl-Ray is one of the most widely-used laboratory or offline fat analysis instruments and has been used by the meat industry for more than three decades. It's based on the difference in x-ray absorption between fat and lean meat, in a sample of precise thickness and weight. The process involves a technician filling a cup with a 5.9 kg sample, before placing it in the Anyl-Ray. X-rays are then passed through the cup and, as lean meat absorbs more x-rays than fat, the Anyl-Ray measures the x-rays that pass through the cup to determine the lean/fat ratio. This method is capable of providing accurate fat analysis for meat at any temperature, provided it can be thoroughly compacted into the sample cup, which is a problem for frozen meat. However, in practice, its use is limited to boneless ground meat as bones are known to cause inaccurate readings. Despite the large sample size reducing sampling error, the Anyl-Ray remains a sample-based instrument that requires skilled labour."

MQ27 – Chemical Lean Meat Analyser (Anonymous, 1997)

"A number of TOBEC (Total Body Electromagnetic Conductivity) machines, specifically targeting the measurement of CL in boxed manufacturing meat, have been commercialized. Much of the published market assessment of these devices has been undertaken by the Meat Research Laboratory of the CSIRO (Cannon Hill, Australia), reflecting Australia's position as the world's largest exporter of manufacturing meat (predominantly beef). One of the first systems developed was the EMME Model M60. In an industrial trial in 1977, the device demonstrated an accuracy of ±7.68% against the manufacturer's claimed accuracy of ±2% of the value determined chemically 'with appropriate temperature control' due to the technology being very sensitive to the operating environment."

"In 1991, the EMME-M60 was compared with a second generation TOBEC machine, the MQ-25 (Meat Quality Incorporated (formerly Agmed Incorporated), Springfield, IL, USA), and was assessed for measuring cold-boned beef. The MQ-25 operated at throughput rates in excess of 16 cartons per minute and demonstrated standard error of estimate (SEE) for single cartons ranging from 1.34 to 1.91 dependent on meat piece size. A further evaluation in 1994 of a more recent model (MQ-27) for monitoring lean meat content of hot-boned beef concluded that results (SEE of 1.71) were comparable with results previously obtained for cold-boned beef. Chemical evaluation of CL was implemented by core sampling frozen cartons, taking two 20 g subsamples from each core and testing for fat content using the microwave drying procedure described by CSIRO. The MQ-27 is marketed on the basis of an achievable accuracy (SEE) per carton of between 1.5% and 2%.

An important tenet of TOBEC is the direct relationship between the temperature of the meat product and its electroconductivity. The vast majority of published prediction equations include product temperature as an independent variable. In the 1991 trial, the temperature of the meat near the geometric centre and at four other points was measured using a digital probe thermometer for each carton. The arithmetic mean of these values was used to correct the mean electrical conductivity measurement for the temperature of the meat according to a formula provided by the manufacturer of the equipment.

The hot-boning trial in 1994 found that temperature did not play a significant role in the performance of the machine. Note, however, that this trial was on hot-boned meat, which would have been a

temperature somewhere between 10 and 20 degrees warmer than the equivalent cold-boning operation. Ongoing anecdotal evidence from numerous installations within Australasian meat plants (predominantly cold-boning plants) indicates that the temperatures both of the product and the operating environment are critical to maintaining accurate and consistent operation of TOBEC equipment." (Clarke, 2014)

FOSS MeatMaster™ X-Ray CL Analyser

"In the meat industry, X-ray systems are being used extensively for detecting the presence of foreign bodies. Usually, X-ray images are acquired using only a single X-ray tube operating at a fixed electron acceleration voltage. In recent years, dual-energy X-ray systems have become available. Here, images are acquired with either two X-ray tubes operating at different voltages or with two detector systems, one sensitive to high-energy photons and the other to low-energy photons. The technology is comparable to the dual-energy X-ray analysis systems used for many years at hospitals for measuring bone density and quantifying obesity in patients. A number of companies that have been active in marketing foreign body detectors have switched to using X-rays at two energies. The advantage of using dual energies is that it makes it easier to find certain low-density foreign bodies in the products. At the same time, the systems can be used to find the fat content in boxes of meat or in bulk products on a conveyor. Some examples of these systems are the MeatMaster™, from the company Foss, and the Eagle FA. These instruments can analyse 100 – 150 t hr^{-1} of fat content and are able to measure through boxes of meat with thicknesses up to 20 cm. During routine operation, accuracies of 0.8 – 1% fat are achievable. A few of the Japanese suppliers of X-ray inspection systems will be presenting similar dual X-ray energy machines in the very near future." (Borggaard, 2014)

"A more sophisticated approach to rapid methods for fat is the use of X-rays such as the Anyl-Ray instrument (KatridgPak, Tegetec, Frederikssune, Denmark), the MeatMaster™ (FOSS, Hilleroed, Denmark), and the EAGLE™ FA (EAGLE Product Inspection, Tampa, Florida, USA). This approach utilises an X-ray machine that measures the differential in X-ray absorption between fat and lean. The X-ray method is non-destructive so the sample is not damaged or lost. Calibration of the machine with a standard allows determination of the fat content in unknown samples. A unique aspect to the Anyl-Ray instrument is that it requires a 5.9 kg sample. The relatively large sample size is an advantage because it is easily representative of the larger amount being sampled. The MeatMaster™, on the contrary, is designed to scan and measure meat materials in-line at up to 20 t hr^{-1}. The measurement requires less than 1 min and standard deviations have been reported to be 0.60–0.80%. The EAGLE™ FA is designed to scan as many as 30 cartons (up to 28 kg) per minute or bulk products at up to 120 t per hour with results that are within 1.0% of actual fat content. This system includes a 45 s automatic calibration, which is effective for fresh or frozen products, and will also detect contaminants such as metal, glass, and bone. All of these methods utilising X-ray technology have been well accepted in the industry for formulation estimates but have not shown the performance required of official methods." (Sebranek, 2014)

Foss FoodScan™ NIR CL System (Anderson, 2007)

"A collaborative study was conducted to evaluate the repeatability and reproducibility of the FOSS FoodScan™ near-infrared spectrophotometer with artificial neural network calibration model and database for the determination of fat, moisture, and protein in meat and meat products. Representative samples were homogenised by grinding according to AOAC Official Method 983.18.

Approximately 180 g of ground sample was placed in a 140 mm round sample dish, and the dish was placed in the FoodScan. The operator ID was entered, the meat product profile within the software was selected, and the scanning process was initiated by pressing the "start" button. Results were displayed for percent (g/100 g) fat, moisture, and protein. Ten blind duplicate samples were sent to 15 collaborators in the United States. The within-laboratory (repeatability) relative standard deviation (RSD_r) ranged from 0.22 to 2.67% for fat, 0.23 to 0.92% for moisture, and 0.35 to 2.13% for protein. The between-laboratories (reproducibility) relative standard deviation (RSD_R) ranged from 0.52 to 6.89% for fat, 0.39 to 1.55% for moisture, and 0.54 to 5.23% for protein. The method is recommended for Official First Action."

CEM Smart Trac - Fat & Moisture Analyser (Leffler et al., 2008)

"A peer-verified method is presented for the determination of percent moisture and fat in meat products by microwave drying and nuclear magnetic resonance (NMR) analysis. The method involves determining the moisture content of meat samples by microwave drying and using the dried sample to determine the fat content by NMR analysis. Both the submitting and peer laboratories analysed 5 meat products by using the CEM SMART system (moisture) and the SMART Trac (fat). The samples, which represented a range of products that meat processors deal with daily in plant operations, included the following: (1) fresh ground beef, high-fat; (2) deboned chicken with skin; (3) fresh pork, low-fat; (4) all-beef hot dogs; and (5) National Institute of Standards and Technology Standard Reference Material. The results were compared with moisture and fat values derived from AOAC-approved methods, 950.46 (Forced Air Oven Drying) and 960.39 (Soxhlet Ether Extraction)."

"Based on the results of this study, it is recommended that the method for determination of moisture and fat in meats by microwave and NMR analysis should be adopted as First Action" Official AOAC Method 2008.06

Smiths Detection Eagle™ Carton FA (minimum lot size: 10 cartons)

• Material Discrimination X-ray operates on the same principle as fat analysis, DEXA (Dual Energy X-ray Analysis) to discriminate materials by their chemical composition (atomic number).

• MDX is valuable in difficult or "busy" images that contain high variations in image density.

• In the meat industry today, the most common use of MDX is to detect bone. (Hincksman, 2015)

"The accuracy with which the Eagle™ FA DEXA (dual-energy X-ray absorptiometry) scanner measured the chemical lean percentage (CL) of boneless beef in 27.2 kg cartons was evaluated under actual production operating conditions in a commercial meat plant. Forty cartons of boneless beef (CL range 60 to 98) were scanned in triplicate (Scan CL), and the results obtained were compared with chemically determined estimates of CL from three laboratories (Lab CL) performed in triplicate on each of three samples taken from the minced and blended contents of each carton. Differences between CL estimates from the different laboratories and from the different sub-samples were small and not statistically significant. The relationship between Scan CL and the Lab CL had an R^2 value of 99.6% and a residual standard deviation (RSD) of 0.79. When estimates of errors associated with sampling and laboratory analysis (RSDs of 0.4 to 0.6) were taken into account, the Scan CL RSD was estimated to be between 0.6 and 0.7. The mean difference between Scan CL and Lab CL was 0.28. Thus the measures of accuracy were within the specifications for the scanner, which are stated as being an RSD of less than 1 and an average difference between Scan CL and actual CL of less than 1 percentage point.

Estimates of CL obtained by analysing cores (about 1.5% of the total carton) taken from the cartons, were not closely related to Scan CL with an RSD of 2.00 for this relationship. Small but statistically significant differences were found between the CL groups (65, 80, 90, & 95%) in the deviations between Lab CL and Scan CL. It is concluded that the Eagle™ FA on-line DEXA scanner provides a rapid on-line means of accurately estimating the CL content of boneless beef in standard cartons." (Purchas, Archibald, West, & Bartle, 2006)

9.2 Chemical lean (CL) results

Technique	Replicate	CL65	CL70	CL75	CL80	CL85	CL90	CL95	Lamb	Pork
MW-H	1	60.3	64.0	70.4	80.0	84.1	86.6	90.0	85.2	
MW-H	2	60.5	64.0	71.8	81.0	83.5	87.0	90.3	85.6	
MW-N	1	64.8	63.9	73.6	79.2	85.4	87.3	95.0		
MW-N	2	64.7	62.7	73.9	79.8	85.3	87.5	93.8		
NIR	1	67.4	62.8	75.1	80.4	84.1	88.4	89.0	87.6	84.4
NIR	2	67.7	58.0	74.1	80.2	82.7	89.0	90.2	87.2	82.3
NIT A	1	67.4	69.4	73.3	79.5	81.6	85.4	86.2	84.7	84.4
NIT A	2	66.7	69.4	73.2	79.3	81.9	85.4	85.8	84.5	84.1
NIT A	3	66.8	68.6	73.8	79.4	81.8	85.3	86.3	84.8	84.1
NIT A	4	67.6	69.2	73.6	79.1	81.9	85.2	85.9	84.7	84.0
NIT B	1	64.9	60.3	72.3	80.0	84.3	87.2	87.4	87.2	83.3
NIT B	2	65.6	61.5	72.4	79.8	84.5	87.7	87.0	87.3	83.4
NIT B	3	64.9	60.5	72.1	79.9	84.4	87.3	87.8	86.4	83.5
NIT B	4	65.1	60.9	71.9	80.2	84.0	87.4	87.7	86.6	83.3
NIT C	1	64.6	59.5	71.3	79.6	84.6	87.9	87.4	87.4	83.0
NIT C	2	64.9	59.6	71.3	79.7	84.3	87.7	87.3	87.4	83.1
NIT C	3	64.8	60.4	71.2	79.1	83.9	87.6	87.6	86.5	83.0
NIT C	4	64.6	60.5	70.9	79.0	83.8	87.5	87.3	86.7	83.1
NMR	1	63.3	65.9	70.6	79.7	84.3	85.4	87.9	86.7	81.4
NMR	2	63.1	66.3	71.1	80.0	83.3	86.5	87.8	86.6	80.9
NMR	3	63.2	64.6	71.1	79.1	84.2	85.0	87.8	86.6	81.0
Soxhlet A	1	64.2	61.4	71.7	80.0	85.0	87.5	86.8	82.7	85.2
Soxhlet A	2	64.5	61.7	72.2	79.8	84.4	87.2	87.0	82.6	84.9
Soxhlet B	1	64.1	61.8	74.7	83.7	85.9	88.1	88.4	87.3	84.0
Soxhlet B	2	65.3	62.1	75.5	83.6	85.7	88.9	88.4	86.9	84.4
Soxhlet C	1	63.5	63.4	72.4	81.0	85.7	88.0	87.0	87.9	84.7
Soxhlet C	2	63.1	63.1	73.0	80.7	85.4	88.6	87.0	88.2	84.1
Soxhlet D	1	63.3	62.1	70.9	78.8	83.1	87.2	87.1		
Soxhlet D	2	63.6	61.6	70.8	78.8	83.3	87.4	87.2		
Soxhlet D	3	64.7	63.3	71.7	79.6	83.8	88.0	87.8		
Soxhlet D	4	64.5	63.3	71.5	79.5	83.9	88.0	87.7		
Soxhlet D	5	64.3	63.2	71.8	79.4	83.7	87.7	87.6		
Soxhlet D	6	63.8	62.8	71.0	79.4	83.7	87.7	87.5		
Soxhlet E	1	63.1	65.8	72.6	79.1	82.3	86.9	87.7		
Soxhlet E	2	62.1	66.3	71.6	79.0	82.7	86.7	89.9		
Soxhlet E	3	62.6	65.2	72.3	79.5	82.0	87.3	88.9		
Soxhlet E	4	63.9	65.9	73.2	79.3	82.9	86.5	88.5		
Soxhlet F	1	62.8	67.2	71.1	79.6	83.3	87.1	87.1	86.0	82.4
Soxhlet F	2	62.3	66.0	71.4	79.5	83.0	86.3	86.1	85.2	82.4
Soxhlet F	3	62.2	67.9	71.7	79.2	83.0	86.9	87.6	85.4	82.5
Soxhlet F	4	63.0	67.7	72.2	79.6	82.6	86.8	88.0	85.4	82.7
Soxhlet F	5	63.2	68.0	71.8	79.0	82.8	86.5	86.8	84.8	82.5
X-Ray	1	52.0	51.0	58.0	65.0	81.0	75.0	82.0	77.0	86.0

9.3 CL *z*-scores using median and normalised IQR.

Technique	CL65	CL70	CL75	CL80	CL85	CL90	CL95	Lamb	Pork
MW-H	-1.62	0.13	-0.55	0.41	0.15	-0.42	1.50	-0.43	
MW-H	-1.56	0.13	-0.01	1.31	-0.16	-0.19	1.67	-0.32	
MW-N	0.27	0.10	0.72	-0.35	0.81	0.00	**4.58**	0.37	0.51
MW-N	0.21	-0.10	0.84	0.17	0.78	0.17	**3.87**	0.23	-0.47
NIR	1.33	-0.08	1.32	0.74	0.15	0.76	0.86	-0.60	0.51
NIR	1.45	-0.89	0.92	0.57	-0.55	1.16	1.61	-0.67	0.37
NIT A	1.33	1.04	0.60	-0.05	-1.11	-1.24	-0.89	-0.57	0.37
NIT A	1.04	1.04	0.56	-0.23	-0.96	-1.24	-1.14	-0.60	0.33
NIT A	1.08	0.91	0.80	-0.14	-1.01	-1.31	-0.82	0.23	0.00
NIT A	1.41	1.01	0.72	-0.41	-0.96	-1.38	-1.07	0.27	0.05
NIT B	0.29	-0.50	0.20	0.39	0.25	-0.04	-0.14	-0.03	0.09
NIT B	0.58	-0.30	0.24	0.21	0.35	0.29	-0.39	0.03	0.00
NIT B	0.29	-0.47	0.12	0.30	0.30	0.03	0.11	0.30	-0.14
NIT B	0.37	-0.40	0.04	0.57	0.10	0.09	0.05	0.30	-0.09
NIT C	0.17	-0.64	-0.20	0.03	0.40	0.43	-0.14	0.00	-0.14
NIT C	0.29	-0.62	-0.20	0.12	0.25	0.29	-0.20	0.07	-0.09
NIT C	0.25	-0.48	-0.24	-0.41	0.05	0.23	-0.01	0.07	-0.87
NIT C	0.17	-0.47	-0.36	-0.50	0.00	0.16	-0.20	0.04	-1.14
NMR	-0.37	0.44	-0.47	0.15	0.24	-1.22	0.15	0.02	-1.10
NMR	-0.48	0.52	-0.27	0.41	-0.24	-0.54	0.13	-1.27	0.89
NMR	-0.42	0.23	-0.29	-0.39	0.19	-1.52	0.09	-1.30	0.75
Soxhlet A	0.00	-0.31	-0.04	0.39	0.60	0.16	-0.51	0.27	0.33
Soxhlet A	0.13	-0.26	0.16	0.21	0.30	-0.04	-0.39	0.13	0.51
Soxhlet B	-0.04	-0.25	1.16	**3.66**	1.06	0.56	0.49	0.47	0.65
Soxhlet B	0.46	-0.20	1.49	**3.57**	0.96	1.10	0.49	0.57	0.37
Soxhlet C	-0.29	0.03	0.24	1.27	0.96	0.50	-0.39		
Soxhlet C	-0.46	-0.03	0.48	1.01	0.81	0.90	-0.39		
Soxhlet D	-0.39	-0.19	-0.36	-0.68	-0.33	-0.01	-0.33		
Soxhlet D	-0.25	-0.27	-0.39	-0.63	-0.26	0.06	-0.27		
Soxhlet D	0.19	0.00	-0.03	0.03	0.02	0.51	0.10		
Soxhlet D	0.13	0.01	-0.10	-0.02	0.06	0.52	0.06		
Soxhlet D	0.05	-0.01	0.00	-0.17	-0.03	0.30	0.02		
Soxhlet D	-0.15	-0.08	-0.32	-0.19	-0.06	0.27	-0.10		
Soxhlet E	-0.44	0.43	0.34	-0.43	-0.77	-0.22	0.04		
Soxhlet E	-0.86	0.52	-0.07	-0.47	-0.55	-0.40	1.40		
Soxhlet E	-0.66	0.34	0.19	-0.02	-0.90	0.02	0.81		
Soxhlet E	-0.13	0.45	0.57	-0.21	-0.44	-0.50	0.57		
Soxhlet F	-0.57	0.67	-0.29	0.07	-0.25	-0.11	-0.31	-0.17	-0.40
Soxhlet F	-0.78	0.47	-0.15	-0.03	-0.41	-0.62	-0.92	-0.45	-0.42
Soxhlet F	-0.83	0.79	-0.03	-0.35	-0.40	-0.21	0.00	-0.36	-0.37
Soxhlet F	-0.52	0.76	0.16	0.00	-0.60	-0.32	0.22	-0.38	-0.29
Soxhlet F	-0.42	0.80	-0.01	-0.51	-0.49	-0.48	-0.52	-0.57	-0.37
X-Ray	**-5.07**	*-2.08*	**-5.53**	**-12.87**	-1.41	**-8.20**	**-3.51**	**-3.17**	1.26

9.4 CL *z*-scores using median and modified Horwitz curve

Technique	CL65	CL70	CL75	CL80	CL85	CL90	CL95	Lamb	Pork
MW-H	6.53	-1.30	2.59	-1.04	-0.72	1.78	-7.11	3.56	
MW-H	6.27	-1.25	0.05	-3.27	0.80	0.81	-7.91	2.60	
MW-N	-1.07	-1.00	-3.38	0.89	-3.98	0.00	-21.65		
MW-N	-0.84	1.00	-3.95	-0.42	-3.83	-0.75	-18.32		
NIR	-5.35	0.75	-6.22	-1.86	-0.75	-3.28	-4.07	-3.01	-2.69
NIR	-5.85	8.67	-4.34	-1.41	2.73	-5.01	-7.61	-1.92	2.45
NIT A	-5.35	-10.14	-2.83	0.13	5.47	5.35	4.18	4.93	-2.69
NIT A	-4.18	-10.14	-2.65	0.58	4.72	5.35	5.36	5.48	-1.96
NIT A	-4.35	-8.82	-3.78	0.36	4.97	5.64	3.89	4.66	-1.96
NIT A	-5.68	-9.81	-3.40	1.02	4.72	5.93	5.07	4.93	-1.71
NIT B	-1.17	4.87	-0.95	-0.97	-1.24	0.17	0.65	-1.92	0.00
NIT B	-2.34	2.89	-1.14	-0.53	-1.74	-1.27	1.83	-2.19	-0.24
NIT B	-1.17	4.54	-0.58	-0.75	-1.49	-0.12	-0.53	0.27	-0.49
NIT B	-1.50	3.88	-0.20	-1.41	-0.50	-0.40	-0.24	-0.27	0.00
NIT C	-0.67	6.19	0.93	-0.09	-1.99	-1.84	0.65	-2.47	0.73
NIT C	-1.17	6.03	0.93	-0.31	-1.24	-1.27	0.94	-2.47	0.49
NIT C	-1.00	4.71	1.12	1.02	-0.25	-0.98	0.06	0.00	0.73
NIT C	-0.67	4.54	1.68	1.24	0.00	-0.69	0.94	-0.55	0.49
NMR	1.47	-4.32	2.19	-0.37	-1.19	5.24	-0.71	-0.58	4.58
NMR	1.92	-5.09	1.25	-1.02	1.17	2.30	-0.59	-0.33	6.00
NMR	1.67	-2.27	1.35	0.98	-0.94	6.53	-0.42	-0.16	5.75
Soxhlet A	0.00	3.06	0.18	-0.97	-2.98	-0.69	2.42	10.41	-4.65
Soxhlet A	-0.50	2.56	-0.76	-0.53	-1.49	0.17	1.83	10.69	-3.92
Soxhlet B	0.17	2.40	-5.47	-9.16	-5.22	-2.42	-2.30	-2.19	-1.71
Soxhlet B	-1.84	1.90	-6.98	-8.93	-4.72	-4.72	-2.30	-1.10	-2.69
Soxhlet C	1.17	-0.24	-1.14	-3.18	-4.72	-2.13	1.83	-3.84	-3.43
Soxhlet C	1.84	0.25	-2.27	-2.52	-3.98	-3.86	1.83	-4.66	-1.96
Soxhlet D	1.56	1.82	1.70	1.71	1.62	0.05	1.57		
Soxhlet D	1.01	2.66	1.81	1.59	1.29	-0.26	1.27		
Soxhlet D	-0.78	0.00	0.15	-0.07	-0.11	-2.21	-0.47		
Soxhlet D	-0.50	-0.05	0.48	0.04	-0.27	-2.24	-0.26		
Soxhlet D	-0.19	0.10	0.00	0.43	0.16	-1.28	-0.09		
Soxhlet D	0.62	0.80	1.49	0.46	0.28	-1.15	0.45		
Soxhlet E	1.79	-4.20	-1.59	1.09	3.78	0.95	-0.18		
Soxhlet E	3.48	-5.06	0.35	1.17	2.71	1.73	-6.61		
Soxhlet E	2.66	-3.26	-0.88	0.05	4.45	-0.09	-3.81		
Soxhlet E	0.50	-4.35	-2.68	0.53	2.16	2.13	-2.69		
Soxhlet F	2.29	-6.49	1.36	-0.19	1.22	0.46	1.47	1.40	2.11
Soxhlet F	3.13	-4.53	0.70	0.07	2.01	2.68	4.34	3.68	2.19
Soxhlet F	3.34	-7.71	0.13	0.88	2.00	0.92	0.00	2.99	1.93
Soxhlet F	2.08	-7.38	-0.77	0.00	2.95	1.36	-1.06	3.11	1.50
Soxhlet F	1.67	-7.76	0.05	1.27	2.43	2.06	2.46	4.70	1.93
X-Ray	20.39	20.21	25.97	32.21	6.96	35.29	16.57	26.03	-6.61