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Acknowledgements

Robyn Warner, Xiying Li, Cameron Steele, Peter McGilchrist, Darryl D'Souza, Robert Hewitt, David Pethick, Maddison Corlett and Sarah Stewart

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Abstract

Intramuscular fat (IMF) is related to eating quality in both beef and sheep, and probably in pork. However, the amount of IMF in pork is much lower than in the red meat species which presents problems in its' measurement. Near infra-red (NIR) and nuclear magnetic resonance (NMR) technologies have been shown to be able to predict IMF in lamb and in beef. However, the relationship between IMF and these technologies in pork is unknown. The objectives of this project was to determine the chemical IMF content of two pork muscles (*Longissimus thoracis et lumborum* (LTL) and *Semimembranosus* (SM)) and relate these values of measures obtained using NIR (with a SOMA device) and NMR in 60 pork carcasses.

The chemical IMF % of the LTL (1.04±0.051%) and SM (1.38±0.065%) were very low compared to other red meat but within the expected range. The IMF content of the SM was higher ($p<0.001$) than the LTL. All measures of SOMA LTL IMF were highly correlated ($p<0.001$) with chemical IMF%. Mean SOMA LTL IMF, geometric mean SOMA LTL IMF and the highest SOMA IMF accounted for 36.4%, 31.9% and 36.9% of the variation in chemical IMF %. There was a significant correlation ($p=0.008$) between chemical IMF % and NMR average p2f, although only 9% of the variation was accounted for. Interestingly, measures of SOMA IMF% were correlated with NMR p2f, indicating that they were measuring something similar.

All measures of SOMA SM IMF were correlated ($p<0.05$) with chemical IMF% but not to as great an extent as the LTL, although the correlations were much lower than for the LTL. Mean SOMA, geometric mean SOMA and highest SOMA IMF accounted for 6.7%, 6.4% and 6.4%, respectively, of the variation in chemical SM IMF %.

When muscles were combined, there were highly significant correlations ($p<0.001$) between SOMA measures of IMF and chemical measures of IMF, and these relationships were slightly improved by including muscle in the model. For example, the inclusion of muscle in the model relating mean SOMA to IMF described 27.7% of the variation compared to 25.5% in the simple model. Similarly, for the other relationships relating SOMA measures of IMF to chemical IMF in the pooled data set.

While these values are unlikely to be good enough to provide confidence in predicting IMF within the low ranges of IMF encountered in Australian pork LTL and SM, they do provide encouragement that with some finessing of the instrumentation and algorithms, which were specifically developed for lamb, an online tool can be developed.

The major conclusion from this project is that both the SOMA and NMR technology appear to be related to pork IMF, particularly in the LTL. These relationships exist despite the very low levels of observed IMF %. It is recommended that pork carcasses be manipulated nutritionally and genetically to increase the range in IMF to further test both SOMA and NMR over a greater range in IMF %.

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Executive Summary

The objectives of this project was to determine the chemical IMF content of two pork muscles (*Longissimus thoracis et lumborum* (LTL) and *Semimembranosus* (SM)) and relate these values of measures obtained using NIR (with a SOMA device) and NMR in 60 pork carcasses.

The chemical IMF % of the LTL (1.04±0.051%) and SM (1.38±0.065%) were very low compared to other red meat but within the expected range. The IMF content of the SM was higher ($p<0.001$) than the LTL.

All measures of SOMA IMF were highly correlated ($p<0.001$) with chemical IMF% in the LTL and to a lesser extent ($p<0.05$) in the SM. While these values are unlikely to be good enough to provide confidence in predicting IMF within the low ranges of IMF encountered in Australian pork LTL and SM, they do provide encouragement that with some finessing of the instrumentation and algorithms, which were specifically developed for lamb, an online tool can be developed.

The major conclusion from this project is that both the SOMA and NMR technology appear to be related to pork IMF, particularly in the LTL, despite the very low levels of observed IMF %. It is recommended that pork carcasses be manipulated nutritionally and genetically to increase the range in IMF to further test both SOMA and NMR over a greater range in IMF %.

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Contents

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1 Background

It is well established that intramuscular fat (IMF) is a major driver of eating quality in beef and lamb. Still, less is known about pork, possibly because the contemporary values for pork IMF are low. Using a Monte-Carlo risk analysis approach, Channon et al. (2016) indicated that sensory flavour and juiciness were increased by 9 and 15% when IMF was above 1.6% compared to below 1.6%. Still, because of the variation, these differences weren't significant. More recent data suggests that the IMF content is related to the objective measure of texture (Li et al. 2023 and unpublished) and there are nutritional and genetic levers to manipulate IMF (eg. D'Souza et al. 2008). Still, unless we know what the baseline IMF contents are across the various supply chains in Australia, it is unknown if there is any scope to improve pork eating quality through manipulating IMF. Also, in order to do this, it is important to have online technology to measure IMF in the loin or other muscles. Technologies such as Near Infrared absorptiometry (NIR) and nuclear magnetic resonance (NMR) have been developed for lamb and beef, albeit these species have much higher IMF contents than pork. Therefore this project was conducted to determine whether NIR and NMR could measure IMF in pork.

2 Objectives

1/ To determine the chemical IMF content of two pork muscles (*Longissimus thoracis et lumborum* (LTL) and *Semimembranosus* (SM)).

2/ To determine whether NIR (using the SOMA device) could be used to predict IMF content and of the pork LTL and SM.

3/ To determine whether NMR technology could be sued to predict the IMF content of the pork SM.

3 Methods

Samples of the *Longissimus thoracis et lumborum* (LTL) and *Semimembranosus* (SM) were collected from 60 loins and 60 topsides from a Costco kill at Sunpork in Kingaroy on 27th April 2022. Muscle pH, temperature, colour (L*, a*, b*) and SOMA readings were made within the boning room. SOMA was measured 3 times across a single cut surface of each of the muscles. Primals were transported to The University of Melbourne where samples were analysed for IMF%. The 60 portions of the loins were also thawed and scanned using the portable NMR device. These samples were collected under a University of Melbourne Animal Ethics Committee tissue scavenging approval.

Intramuscular fat (IMF) content was determined using the AOAC method 991.36 (AOAC, 1995) with some modifications. Briefly, triplicate of 3.5 g freeze-dried samples were powdered and wrapped in a folded Watman no.1 filter paper. Each sample was placed in a Soxhlet apparatus using diethyl ether as extraction solvent. Intramuscular fat content was expressed as percent fat of fresh meat.

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Thawed samples of LTL were scanned for NMR estimates of IMF. NMR Data was collected on the Oscar 2.0 NMR system running at 12.3 MHz with the magnet temperature set to a few degrees above room temperature of 20° C and controlled by a heat pump. The outputs were sent to HTS-110 for data analysis and the University of Melbourne were provided with the NMR parameter p2f.

Data were analysed for summary statistics and by correlation and regression and analysis using Genstat Version 21.1.

4 Results

The chemical IMF % of the LTL (1.04±0.051%) and SM (1.38±0.065%) were very low compared to other red meat but within the expected range. The IMF content of the SM was higher ($p<0.001$) than the LTL.

Table 1. Summary statistics for chemical intramuscular fat content (%) of 60 LTL and SM muscles.

	LTL	SM
Mean	1.04	1.38
Median	0.915	1.29
Minimum	0.443	0.579
Maximum	2.25	2.84
Lower quartile	0.757	0.968
Upper quartile	1.26	1.71
Standard error of the mean	0.0511	0.0649
Number of observations	60	60

SOMA was measured 3 times across each of the muscles and the results were highly variable. Therefore, chemical IMF% was compared to either the geometric mean SOMA IMF, the arithmetic mean SOMA IMA or the highest of the 3 recorded values. All measures of SOMA LTL IMF were highly correlated ($p<0.001$) with chemical IMF% (Table 2). Mean SOMA LTL IMF accounted for 36.4% of the variation in chemical IMF % (Figure 1). Similarly, geometric mean SOMA LTL IMF and the highest SOMA IMF reading accounted for 31.9% (Figure 2) and 36.9% (Figure 2) of the variation in chemical IMF %. There were some significant correlations between SOMA LTL IMF values and measures of colour and pH in the pork loin (Table 2). However, the inclusion of LTL pork colour and pH in multiple regressions didn't result in any further improvement in the estimation of chemical IMF % from SOMA alone (data not shown). There was a significant correlation ($p=0.008$) between chemical IMF % and NMR average p2f (Table 2, Figure 4), although only 9% of the variation was accounted for. Interestingly, measures of SOMA IMF% were correlated with NMR p2f, indicating that they were measuring something similar (Table 2). For example, NMR p2f was correlated with the geometric mean SOMA IMF, accounting for 17.7% of the variation (Figure 5).

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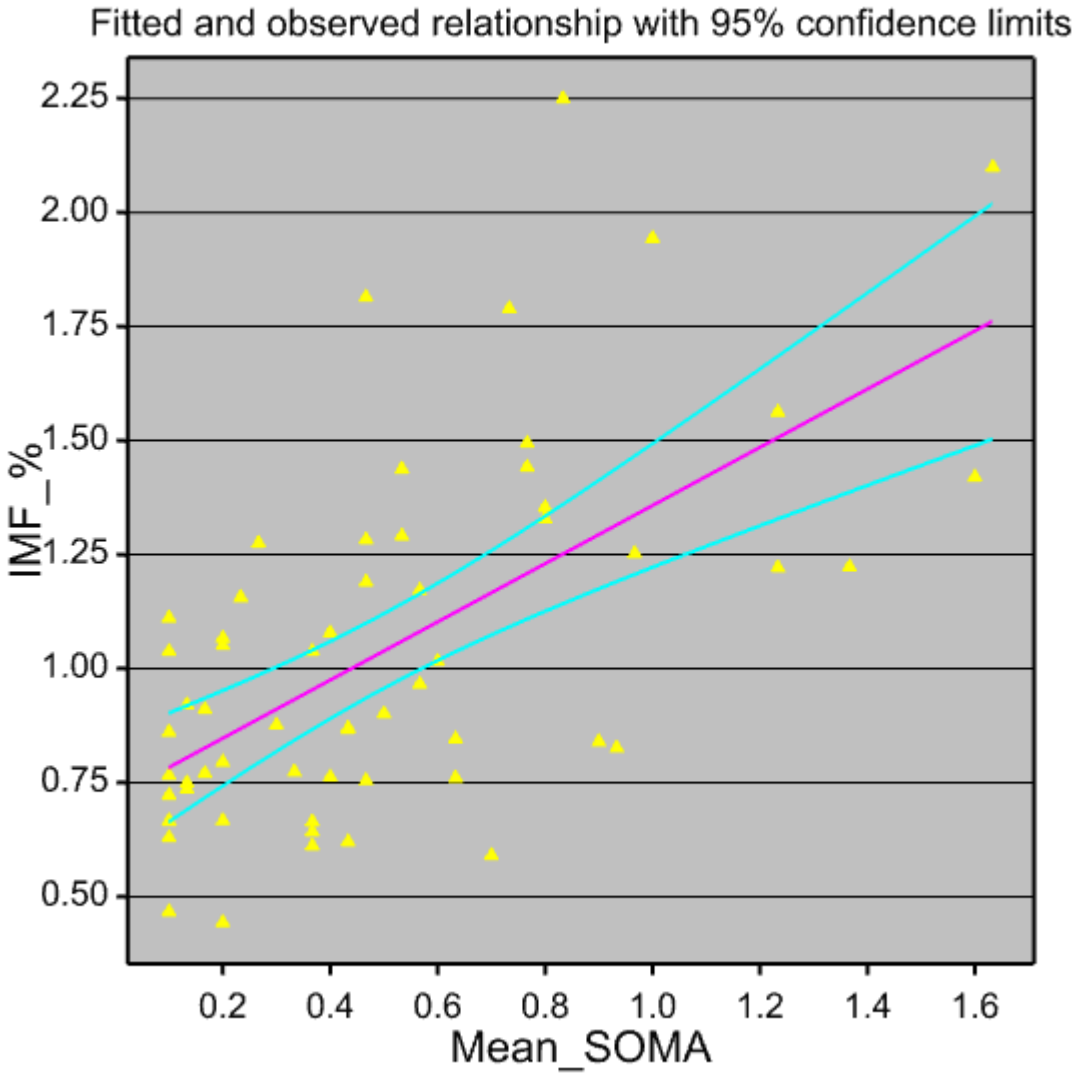

Figure 1. Chemical analysed IMF versus the mean of three SOMA IMF outputs across the loin muscle (IMF % = 0.72 (±0.068) + 0.638 (±0.108) * Mean SOMA, R^2 = 0.364, p<0.001).

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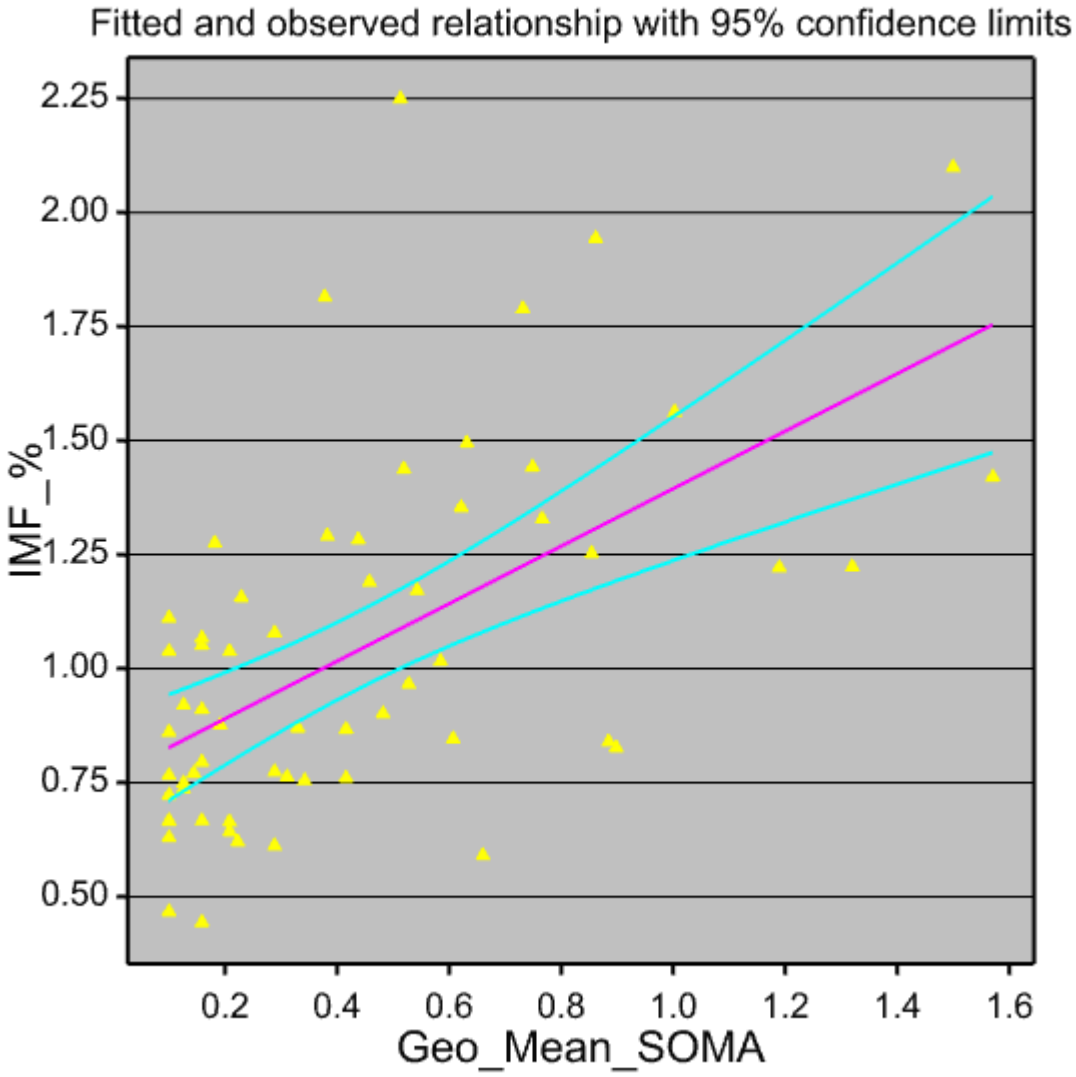

Figure 2. Chemical analysed IMF versus the geometric mean of three SOMA IMF outputs across the loin muscle (IMF % = 0.76 (±0.067) + 0.630 (±0.118) * Gometric Mean SOMA, R^2 = 0.319, p<0.001).

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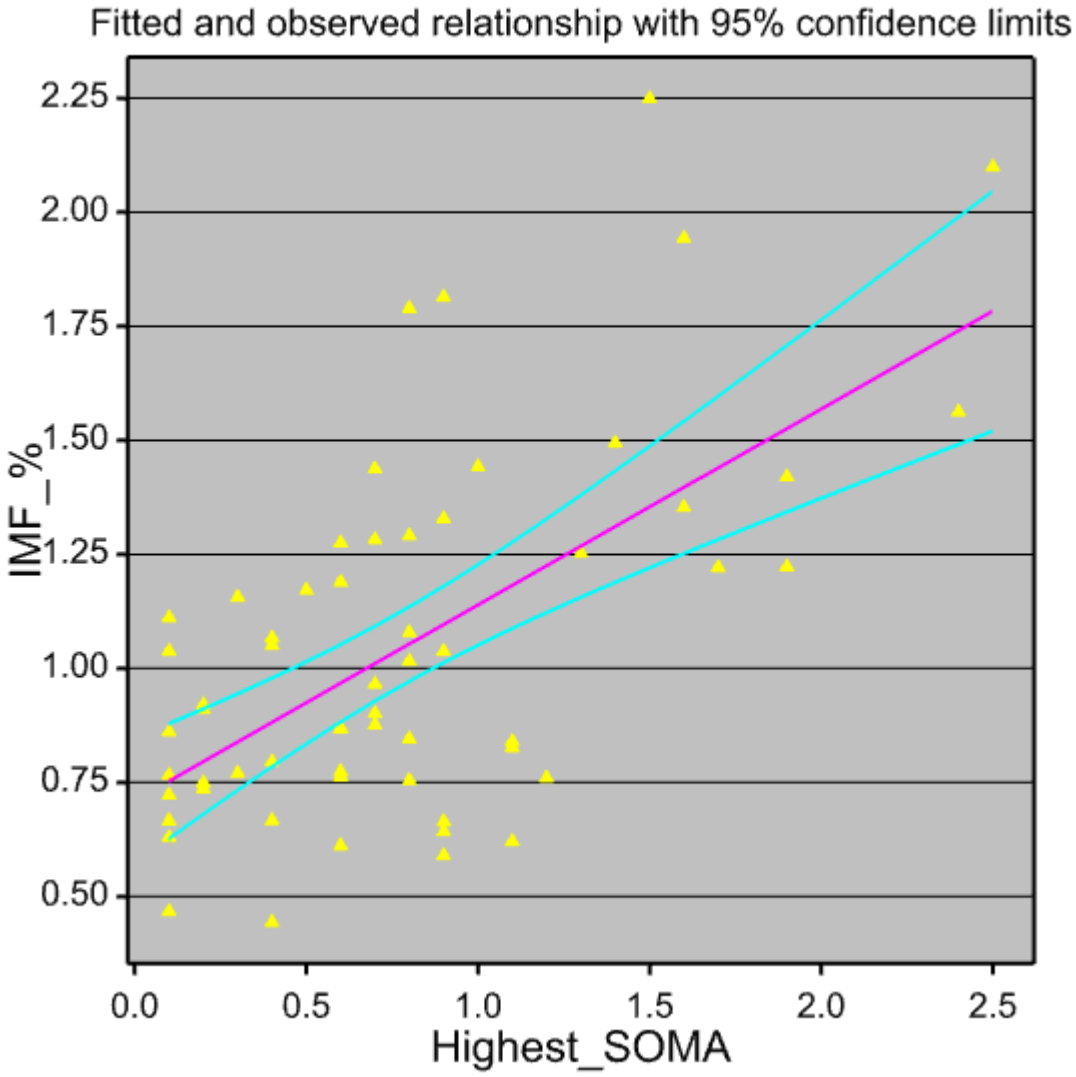

Figure 3. Chemical analysed IMF versus the highest of three SOMA IMF outputs across the loin muscle (IMF % = 0.71 (±0.069) + 0.429 (±0.072) * Highest SOMA, R^2 = 0.369, p<0.001).

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Table 2 Correlation matrix between chemical IMF%, SOMA, colour, pH and temperature of chilled LTL primals and NMR measures on thawed LTL samples (n=60).

IMF%	1	-								
SOMA Geometric Mean	2	0.57***	-							
SOMA Arithmetic Mean	3	0.61***	0.98***	-						
Highest SOMA	4	0.62***	0.87***	0.94***	-					
NMR p2f	5	0.32**	0.47***	0.40**	0.31**	-				
L	6	0.17	0.53***	0.51***	0.43***	0.40**				
a	7	0.24	0.41**	0.46***	0.45***	0.34**	0.19		-	
b	8	0.28*	0.46***	0.48***	0.45***	0.41**	0.62***	0.63***	-	
pH	9	-0.12	-0.50***	-0.47***	-0.37**	-0.53	-0.67***	-0.30*	-0.43***	-
Temp	10	0.03	0.08	0.07	0.07	0.09	0.04	0.10	-0.02	-0.08
		1	2	3	4	5	6	7	8	9

Table 3 Correlation matrix between chemical IMF%, SOMA, colour, pH and temperature of chilled SM primals (n=60).

IMF%	1	-							
SOMA Geometric Mean	2	0.28*	-						
SOMA Arithmetic Mean	3	0.29*	0.99***	-					
Highest SOMA	4	0.28*	0.87***	0.92***	-				
L	6	-0.20	0.35**	0.34**	0.25	-			
a	7	0.20	0.37**	0.35**	0.26*	0.11	-		
b	8	0.03	0.29*	0.28*	0.23	0.55***	0.65***	-	
pH	9	0.18	-0.32*	-0.33*	-0.27*	-0.71***	-0.30**	-0.53***	-
Temp	10	0.31*	-0.02	-0.03	-0.02	-0.25	0.14	-0.07	0.08
		1	2	3	4	5	6	7	8

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Table 4 Correlation matrix between chemical IMF%, SOMA, colour, pH and temperature of combined chilled LTL and SM primals (n-120).

IMF%	1	-							
SOMA Geometric Mean	2	0.49***	-						
SOMA Arithmetic Mean	3	0.51***	0.99***	-					
Highest SOMA	4	0.49***	0.89***	0.94***	-				
L	6	-0.19*	0.14	0.13	0.10	-			
a	7	0.40***	0.58***	0.58***	0.50***	-0.28**	-		
b	8	0.23*	0.46***	0.46***	0.41***	0.32***	0.64***	-	
pH	9	0.17	-0.17	-0.16	-0.13	-0.73***	0.12	-0.31***	-
Temp	10	0.36***	0.29**	0.28**	0.25**	-0.34***	0.55***	0.18*	0.21*
		1	2	3	4	5	6	7	8

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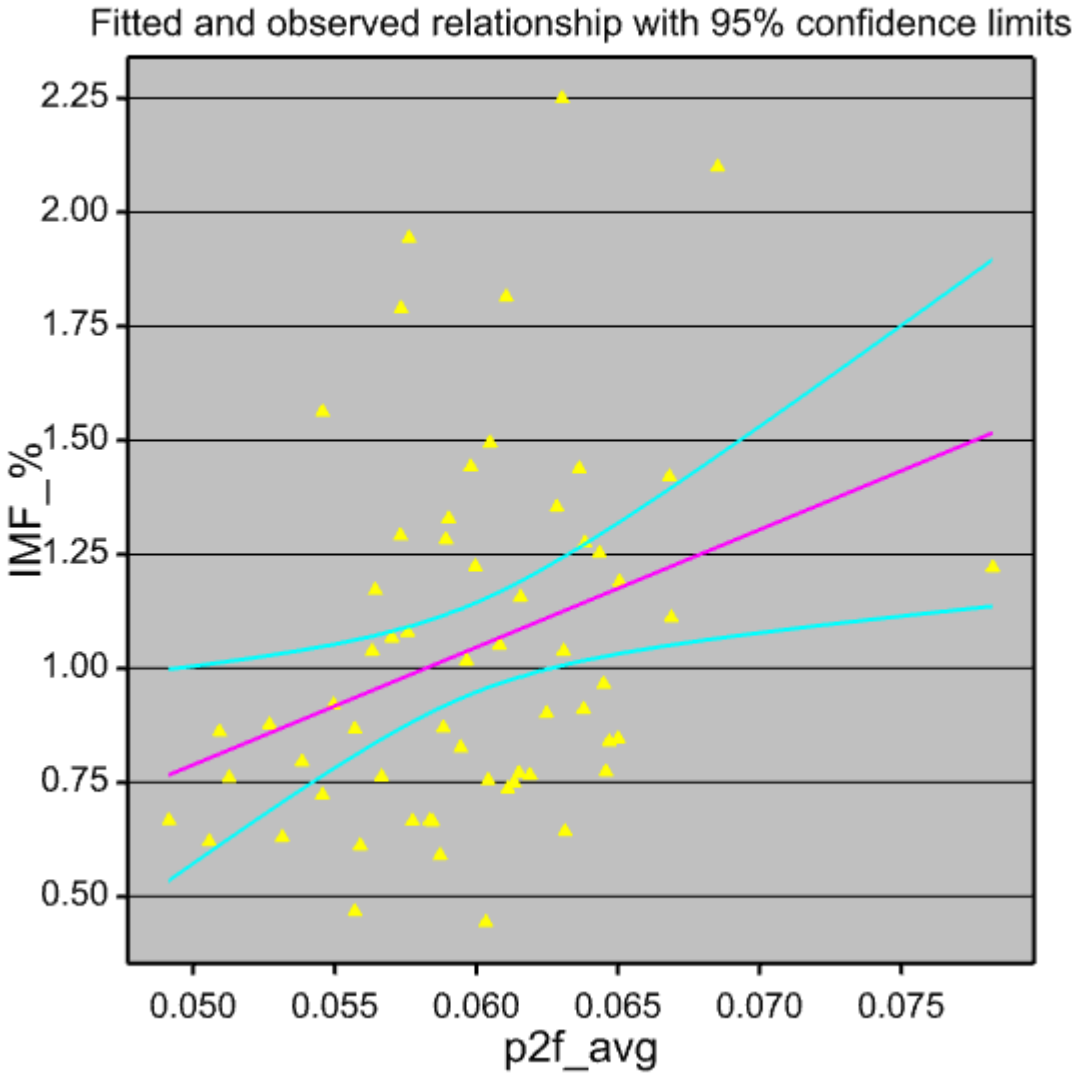

Figure 4. Chemical analysed IMF versus NMR determined IMF on 60 thawed loin muscles (IMF % = -0.50 (±0.594) + 25.8 (±9.92) * NMR p2f, R^2 = 0.089, p=0.012).

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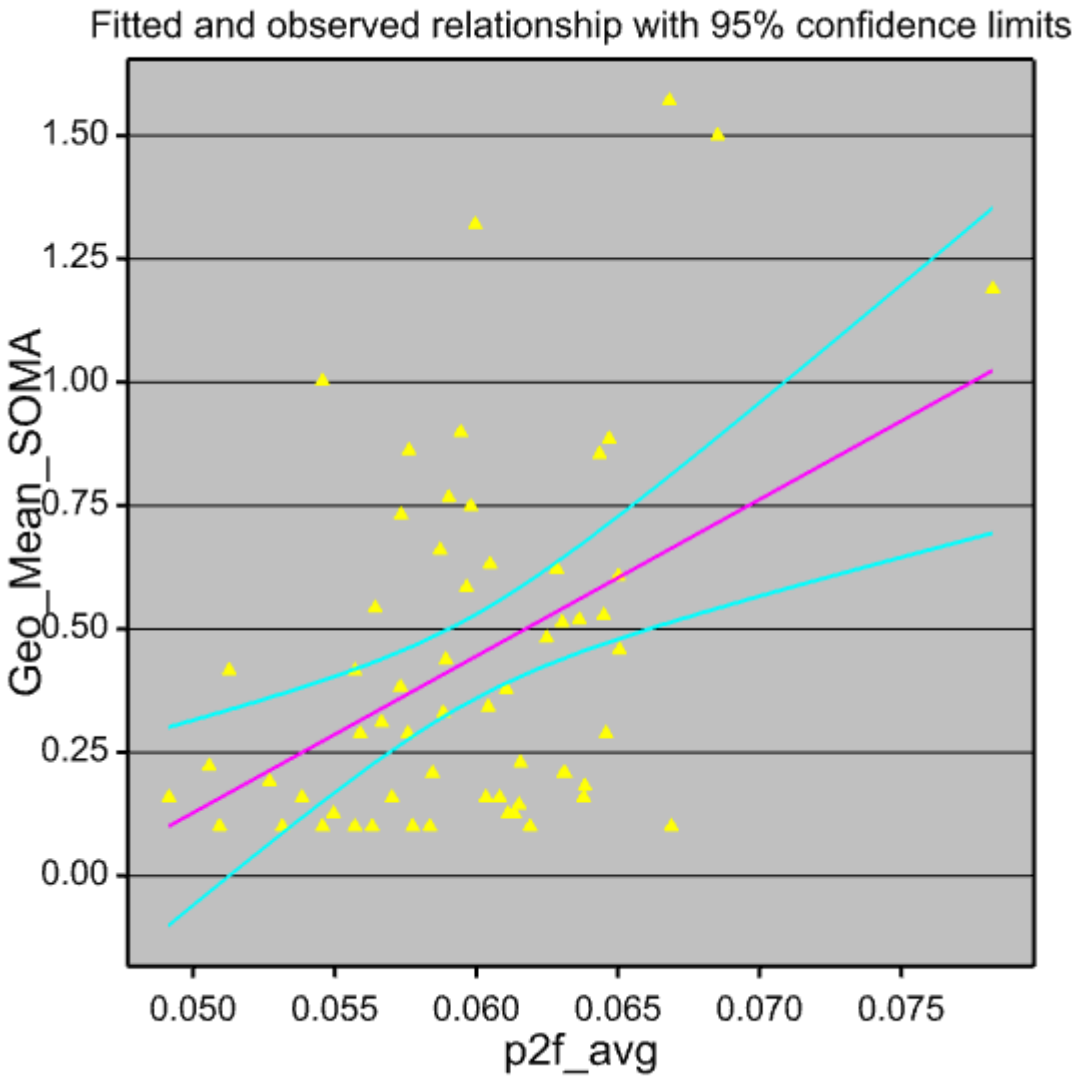

Figure 5. The geometric mean SOMA IMF versus NMR determined IMF on 60 thawed loin muscles (Geometric mean SOMA = -1.46 (±0.515) + 31.7 (±8.59) * NMR p2f, R^2 = 0.177, p<0.001).

All measures of SOMA SM IMF were correlated (p<0.05) with chemical IMF% but not to as great an extent as the LTL (Table 3). Mean SOMA, geometric mean SOMA and highest SOMA IMF accounted for 6.7% (Figure 6), 6.4% and 6.4%, respectively, of the variation in chemical SM IMF % (Table 3). There were some even more significant correlations between SOMA IMF values and measures of colour and pH in the pork SM (Table 2). However, the inclusion of SM pork colour and pH in multiple regressions didn't result in any further improvement in the estimation of chemical IMF % from SOMA alone (data not shown).

When muscles were combined, there were highly significant correlations (p<0.001) between SOMA measures of IMF and chemical measures of IMF (Table 3), and these relationships were slightly improved by including muscle in the model. For example, the inclusion of muscle

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in the model relating mean SOMA to IMF described 27.7% of the variation (Figure 7) compared to 25.5% in the simple model (Table 3). Similarly, for the other relationships relating SOMA measures of IMF to chemical IMF in the pooled data set (data not shown).

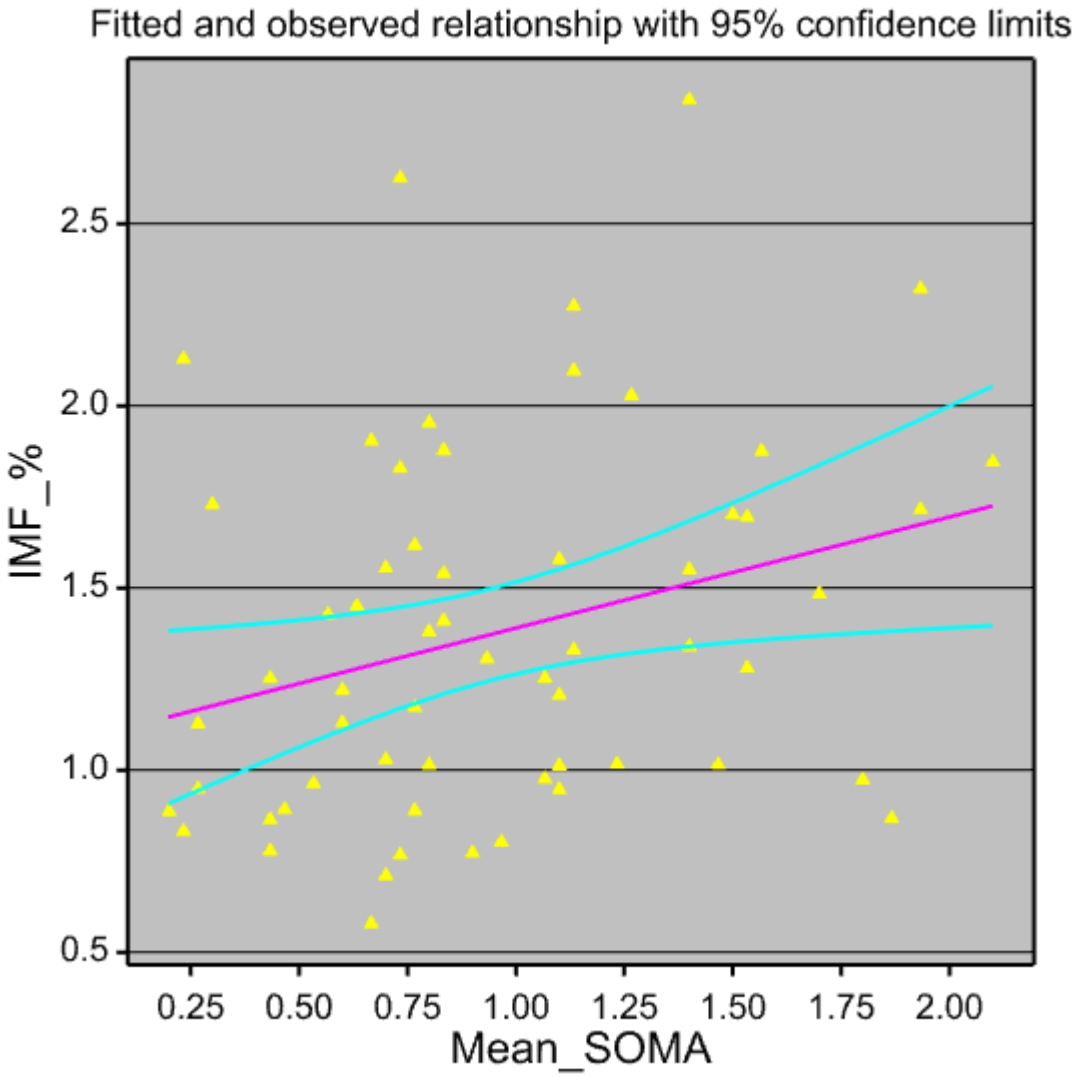

Figure 6. Chemical analysed IMF versus the mean of three SOMA IMF outputs across the SM muscle (IMF % = 1.09 (±0.142) + 0.305 (±0.133) * Mean SOMA, R^2 = 0.067, p=0.025).

There was no significant correlation between IMF% determined in the LTL and the SM (r=0.21, p=0.11).

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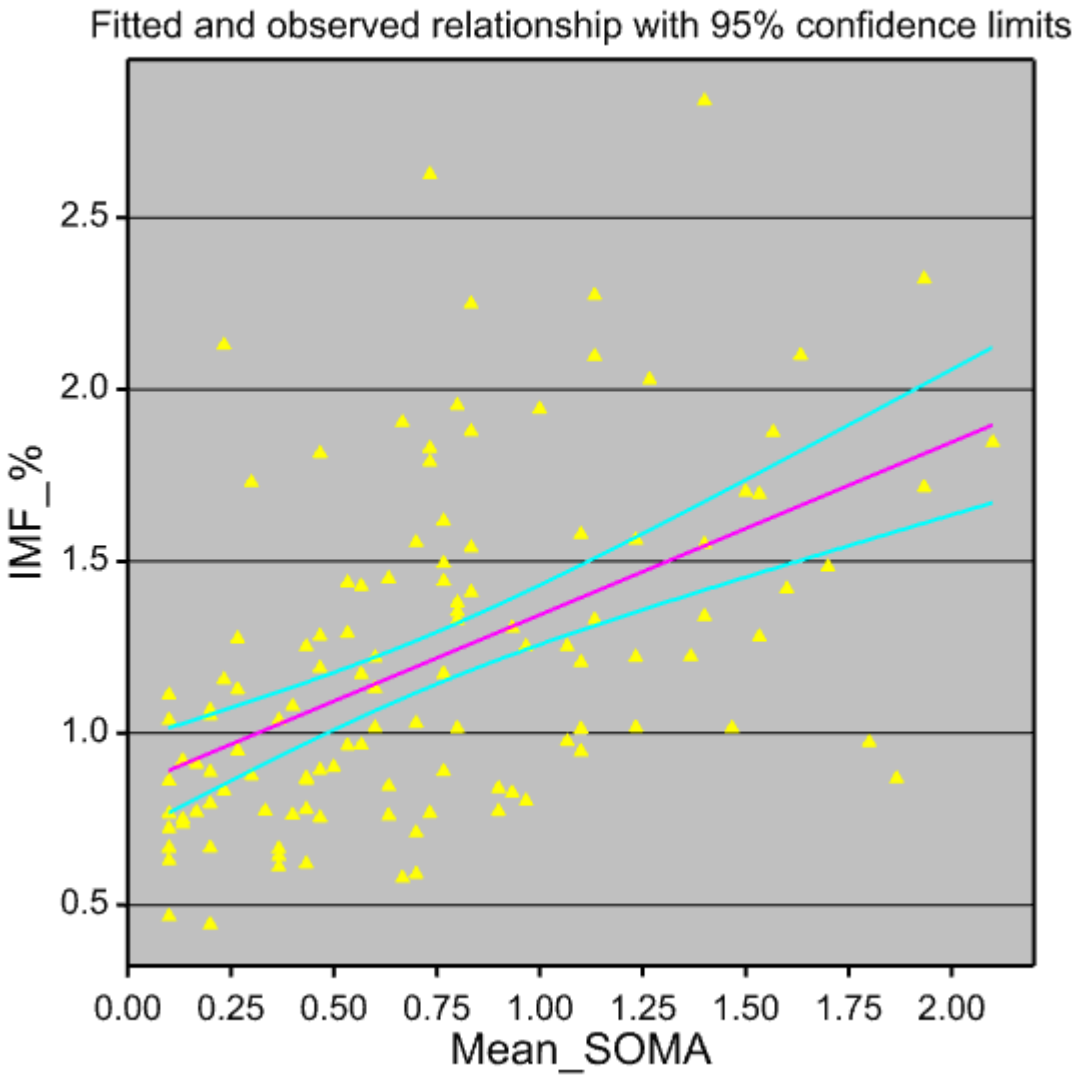

Figure 7. Chemical analysed IMF versus the mean of three SOMA IMF outputs across both the LTL and the SM muscles (IMF % = 0.842 (±0.069) + 0.503 (±0.079) * Mean SOMA, R^2 = 0.251, p<0.001).

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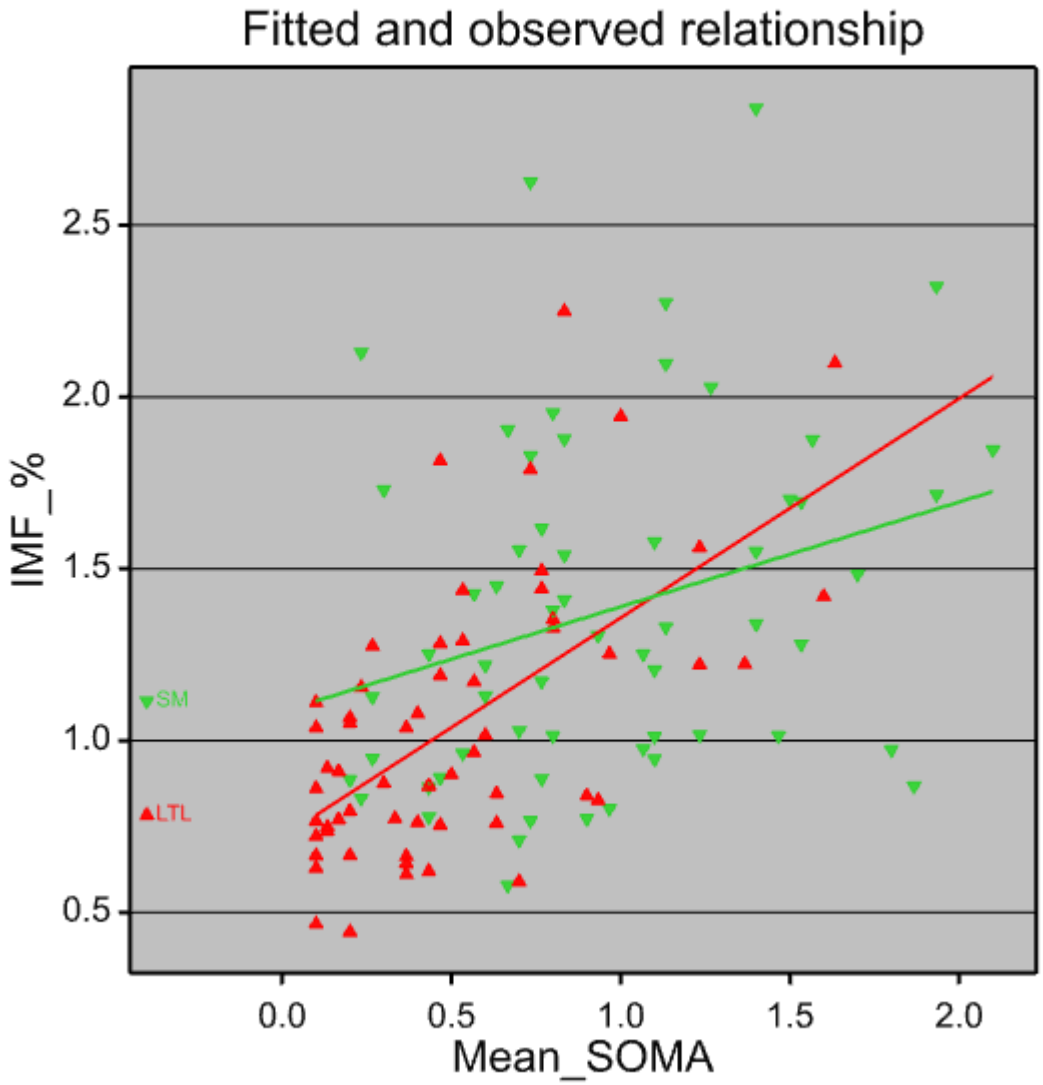

Figure 8. Chemical analysed IMF versus the mean of three SOMA IMF outputs across both the LTL and the SM muscles (IMF % = 0.720 (±0.088) + 0.638 (±0.140) * Mean SOMA for LTL, and IMF % = 1.09 (±0.148) + 0.305 (±0.179) * Mean SOMA for SM, R^2 = 0.277, p<0.001).

5 Discussion

The chemical IMF % of both the LTL (1.04%) and SM (1.38%) were very low compared to other red meat species, but were within the expected range. For example, For example, Li *et al.* (2022) reported a value of 1.10 % and 1.16 for Australian market purchased LTL and *Biceps femoris* (BF) IMF%, respectively.

The role of IMF in pork eating quality within the range of IMF% currently encountered in AOACXAustralia is still equivocal. Using a Monte-Carlo approach to assess published literature, Channon et al. (2016) partitioned LTL IMF % into two categories (< 1.6 and > 1.6%)

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to look at the effect on eating quality. Channon et al. (2016) reported that the relative sensory tenderness, flavour and juiciness of LTL with an IMF above 1.6% compared to that below 1.6% was 0.98, 1.09 and 1.15. However, the errors around these estimates meant that none of these ratios was significant. While Li et al (2022) found no significant correlations between IMF % and objective eating quality in the LTL and BF, more recent data found that there were significant negative correlations between IMF % and chewiness, hardness, and cooking loss in the LTL but not the BF (Li et al unpublished). Also, Li et al (2023) found that IMF% was negatively related to Warner Bratzler Shear Force (WBSF) LTL and BF. Therefore, there does appear to be some relationship between eating quality and IMF% in Australian pork, even within the range of IMF % encountered. The existence of these relationships may justify developing techniques to measure IMF % online to endure eating quality or to assist in breeding programs.

Importantly, despite the very low concentrations of IMF, there were significant relationships between both technologies developed to predict IMF and actual IMF. While these values are unlikely to be good enough to provide confidence in predicting IMF within the low ranges of IMF encountered in Australian pork LTL and SM, they do provide encouragement that with some finessing of the instrumentation and algorithms, which were specifically developed for lamb, an online tool can be developed. The best SOMA predictions described 37% of the variation in IMF in the LTL but only 7% in the SM. Interestingly, the relationship between IMF and the SOMA IMF output could be described by a single equation that described 26% of the variation (Figure 7, Table 4)and was only marginally improved to 28% by including muscle in the model (Figure 8). Therefore, it appears that the SOMA measures the same characteristic in each muscle. The greater variation in the SM is not unexpected because of the more heterogeneous nature and size of this muscle.

The NMR parameter was not as strongly related to IMF % as SOMA, but given the low range in IMF, it is perhaps not surprising. Interestingly, the NMR parameter was more highly correlated to the SOMA estimate of IMF %, which provides confidence that both technologies are measuring the same component(s). Feedback from the NMR scientists was that perhaps there were some high outliers in the chemical estimates of IMF %, and these values did appear to be outliers in a number of relationships. However, we have no reason to doubt these values and removing the 3 highest values didn't improve any of the relationships.

6 Conclusions and Recommendations

The major conclusion from this project is that both the SOMA and NMR technology appear to be related to pork IMF, particularly in the LTL. These relationships exist despite the very low levels of observed IMF %. It is recommended that pork carcasses be manipulated nutritionally and genetically to increase the range in IMF to further test both SOMA and NMR over a greater range in IMF %.

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