



Australian Government

Department of Agriculture, Fisheries and Forestry

Technical Report

Program and KPI: Sub-program 2.2 KPI 3.24

Report Title: Report on the relationship between fatty acid profile and consumer sensory scores

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Date published: 31 January 2023



Citation

McGilchrist P., Saker, N. (2023). Report on the relationship between fatty acid profile and consumer sensory scores. *An Advanced measurement technologies for globally competitive Australian meat Project*. January.

Acknowledgements

Meat and Livestock Australia are thanked for funding the consumer testing for this project. John Dee and JBS Beef City are thanked for processing the cattle along with Janine Lau from MLA for grading the carcasses. Stockyard Beef and AACo are thanked for supplying the cattle and the UNE Chemistry department is appreciated for analysing the fatty acids for this project. The UNE Meat Science team are appreciated for conducting all the consumer testing.

Abstract

This study aimed to evaluate the potential effect of fatty acid content on eating quality when carcasses were selected in a case-control fashion based on marbling. Three cohorts of 36 carcasses were selected from Angus, Wagyu Angus F1 cross, purebred Wagyu, and Wagyu *Bos indicus* F1 cross, all of which had been long fed (≥ 200 DOF). The chuck roll, bolar blade, striploin, D-rump, and outside flat were consumer tested using the grill cook method. Sensory scores for CMQ4 were analysed within cohorts against cut and carcass traits for Australian consumers. The results showed that cut, IMF%, rib fat and all the fatty acids except linoleic acid have a significant effect on CMQ4. The inclusion of muscle explains 47% of variation in the model and IMF% explains a further 12% of variation. The inclusion of oleic and palmitic fatty acids explains a further 11% and 12% of variation in the model, whereas palmitoleic, myristic and stearic explain only a further 2%, 4% and 5% of the model and linolenic explains no significant variation in the model. Inclusion of monounsaturated (MUFA) and polyunsaturated (PUFA) had significant effect on CMQ4, whereas, saturated fatty acids (SFA) was not significant when included in the model.

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1 Introduction

The Australian Wagyu cattle breed has dominated premium international markets over recent decades. However, within the Meat Standards Australia (MSA) eating quality grading system there is currently no adjustment for fatty acids as differences in eating quality are explained through variables existing in the model. There is a perceived assumption of a benefit of fatty acids and which is not explained by the current MSA model predictions. Within the existing version of the MSA model, Wagyu influenced cattle represent around 2% of the data underpinning the predictions which include animals with MSA marbling scores up to the maximum 1190. The objectives of the project were to 1) determine if there is a fatty acid effect on eating quality that is over and above IMF and the current MSA model prediction utilising both long fed (≥ 200 DOF) purebred and F1 animals; 2) In the event of the identification of an effect, to quantify this based on clipped meat quality 4 score (CMQ4).

2 Methodology

2.1 Carcase selection

Carcases were selected from commercial slaughter animals from multiple sources. The selection criteria were that cattle were fed together in the same feedlot pen and included at least two breeds, one of which was Wagyu or Wagyu cross. Table 1 outlines the breeds collected across three cohorts.

Table 1. Cattle breeds and number of carcasses selected for cohorts 1, 2 and 3, including the number of days on a high energy feedlot ration (DOF)

Breed	Cohort 1 (200 DOF)	Cohort 2 (300 DOF)	Cohort 3 (300 DOF)
Angus	18	-	-
Wagyu x Angus	18	-	18
Wagyu x <i>Bos indicus</i>	-	18	-
Wagyu Purebreds	-	18	18

2.1.1 Cohort 1

The 36 animals for this kill were sourced from a single feedlot and had been fed together in the same pen for 200 days, prior to processing at a commercial abattoir on the same day. The cattle consisted of Angus (AAAA) and F1 Angus Wagyu cross cattle (WYAA). In total, 18 heads of Angus and 18 heads of F1 Angus Wagyu were selected to be part of the cohort out of a larger kill group. Animals were selected as case and control pairs with Angus and F1 Angus Wagyu carcasses matched as close on marbling and ossification as possible. From these 36 head, the striploin, bolar blade, outside flat, D-rump and chuck roll were collected and cut into grill samples.

2.1.2 Cohort 2

The 36 animals for this kill were sourced from a single feedlot and all cattle had been fed together in the same pen for 300 days prior to processing at a commercial abattoir on the

same day. The cattle consisted of F1 Wagyu *Bos indicus* cross cattle (WYXX) and purebred Wagyu (WYWY) cattle (F3 or higher). In total 18 heads of purebred Wagyu and 18 heads of F1 Wagyu were selected to be part of the cohort out of a much larger kill group. Animals were selected as case and control pairs with purebred and F1 Wagyu carcasses matched as close on marbling, ossification and hump height as possible. The purebreds were identified by genotyping all cattle in the pen prior to slaughter and were classified as anything that was F3 or higher ($\geq 87.5\%$ Wagyu). From these 36 heads, the striploin, bolar blade, outside flat, D-rump and chuck roll were collected and cut into grill samples.

2.1.3 Cohort 3

The 36 animals for this kill were sourced from a single feedlot and all cattle had been fed in three different pens for 300 days prior to processing a commercial abattoir on the same day. The cattle consisted of F1 Wagyu Angus cattle (WYAA) and purebred Wagyu cattle (WYWY). In total 18 heads of purebred and 18 heads of F1 Wagyu were selected to be part of the cohort. Six F1s and 6 purebreds were selected from each of the 3 pens. Animals were selected as case and control pairs with purebred and F1 Wagyu carcasses matched as close on marbling and ossification as possible. From these 36 heads, the striploin was collected and cut into grill samples.

2.2 Slaughter Procedure, Carcase Grading and Primal Collection

Each cohort group was slaughtered separately at commercial abattoirs in Queensland, Australia. A temperature and pH rate of decline was recorded for each carcass at hourly intervals, from the time of entry into the chiller until the muscle pH fell below 6. This was done to identify the potential risk of heat toughening (i.e. carcass reached pH 6 while the temperature was above 35 °C) or cold shortening. This ensured only carcasses that conformed to MSA pH and temperature decline requirements were selected for further use in the study. Only carcasses that dropped below pH 6 while the temperature ranged between 15°C and 35 °C were selected for these cohorts.

The carcasses were graded against the Aus-Meat Chiller Assessment Standards and the Meat Standards Australia Grading Standards (AUS-MEAT Limited, 2005) by a single grader at 20 hours post-slaughter. The measurements taken included hot standard carcass weight (HSCW), ossification, hump height (mm), eye muscle area (EMA; cm²), subcutaneous rib fat depth (mm), AUS-MEAT marble score, MSA marble score, meat colour and fat colour, and ultimate pH (pH_u).

The Bolar Blade (HAM #2302), Chuck Roll (HAM #2275), Striploin (HAM #2140), D-Rump (HAM #2100) and Outside Flat (HAM #2050) were collected for cohorts 1 and 2, whilst just the Striploin was collected from cohort 3 at boning. These primals were then packed into vacuum-sealed bags and chilled for 24hrs prior to collection from the abattoir. For further processing, these primals were transported at 1 °C to the processing site at the University of New England.

2.3 Sample preparation

On the 6th day from primal collection, the primals were trimmed of external fat and epimysium. Primals were broken down into individual muscles; Bolar Blade into BLD096 (*M. triceps brachii*

caput longum) and BLD097 (*M. triceps brachii caput mediale*); Chuck Roll into CHK081 (*M. spinalis dorsi*); Striploin into STR045 (*M. longissimus lumborum*); D-Rump into RMP005 (*M. biceps femoris*), RMP131 (eye rump centre; *M. gluteus medius*), and RMP231 (eye rump side; *M. gluteus medius*); OUT005 (*M. biceps femoris*). Table 2 outlines the cuts utilised for grill cook method.

Sensory samples were prepared according to MSA protocols, as reported by (Watson, Gee, et al., 2008). The grill (GRL) samples were prepared from a 75 x 25 x 150 mm block. Each sample was individually wrapped in freezer film and then vacuum packed. All samples were aged 7 d, frozen and stored at ~-20 °C until sensory testing. If more than 1 sample was coming from a muscle, the samples were balanced for muscle position across the treatment groups.

Table 2. Muscles tested by cook (grill) method

Muscle
BLD096 (<i>M. triceps brachii caput longum</i>)
CHK078 (<i>M. serratus ventralis</i>)
CHK081 (<i>M. spinalis dorsi</i>)
OUT005 (<i>M. biceps femoris</i>)
RMP005 (<i>M. biceps femoris</i>)
RMP131 (<i>M. gluteus medius</i>)
RMP231 (<i>M. gluteus medius</i>)
STR045 (<i>M. longissimus lumborum</i>)

2.4 Fatty acid samples

Three different types of fatty acids were measured: saturated (myristic (C14:0), palmitic (C16:0), and stearic (C18:0)), monosaturated (oleic (C18:1) palmitoleic (C16:1)) and polyunsaturated (linoleic acid (C18:2) and linolenic acid (C18:3)).

2.4.1 Fatty acids extraction

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on extracts of fatty acids.

2.4.1.1 Reagents required

- **10N KOH in water (Caution! Extremely Caustic)** - Add 561.1 g of KOH slowly into 700 mL water in a glass beaker while mixing on a magnetic stirrer in a fume hood. When the KOH has dissolved and the solution has cooled, diluted to 1.0 L in a volumetric flask. Store at room temperature for 1 year. 14*4
- **24 N sulphuric acid in water (Caution! Extremely Corrosive)**-Dilute 66.66 mL of sulphuric acid to 100 mL with water. Add the acid slowly to the water while mixing on a magnetic stirrer in a fume hood. Store at room temperature for 1 year.
- **Internal Standard Solution (ISS)** - NONADECANOIC ACID METHYL ESTER 200mg/ 100ml of HPLC grade Hexane. Use a Volumetric flask and record the exact mass used. (2ml/sample). Store in Freezer when not in use.
- **Anhydrous or Dried Sodium Sulphate** (powder) (<0.2g/sample)

- HPLC Grade Hexane (800ul/Sample)
- HPLC Grade Methanol (1.1mls/sample)

2.4.1.2 Methods

1. Weigh approximately 100mg of meat sample into Pyrex culture tubes (Reaction Tubes). Record exact mass. These can be re-capped and stored in a freezer until needed.
2. Add **140uL of 10 N KOH** in water and **1100ul methanol** to each tube. Cap tightly and incubate at 55-60°C for 1.5 hours. Mix the tubes vigorously for 5 seconds every 20 minutes.
3. Cool the tubes to below room temperature in a cold tap water bath.
4. Add **120uL of 24 N sulphuric acid** in water. (K₂SO₄ will precipitate) Cap tightly and heat at 55-60°C for 1.5 hours. Mix the tubes vigorously for 5 seconds every 20 minutes.
5. Cool the tubes to below room temperature in a cold tap water bath.
6. **In the fume hood.** Add **exactly 2000ul of Internal Standard Solution.**
7. **In the fume hood:** Recap and mix thoroughly to extract FAME into the upper hexane layer.
8. Transfer mixture to 10-14ml centrifuge tubes with caps and centrifuge until 2 clear layers appear. (*This step may not be needed if layers separate spontaneously*)
9. **In the fume hood:** Transfer about 1ml of top layers to individual microfuge tubes.
10. Add a small amount of anhydrous sodium sulphate and mix thoroughly. If the white solid turns to liquid add more sodium sulphate.
11. Centrifuge to remove any suspended solid.
12. **In the fume hood** -Transfer exactly **200ul** of upper liquid to GC Vial and add **800ul** of Hexane (1 in 5 Dilution)

2.5 Consumer sensory testing

The consumer sensory testing procedures were conducted in line with the MSA protocols, as reported by (Watson, Gee, et al., 2008).

Briefly, groups (picks) of 60 untrained consumers were recruited. Each consumer tasted 7 samples with the first sample being a standard sample among the consumers. The following 6 samples were controlled in a 6 x 6 Latin Square design which ensured each piece was eaten equally before and after each other piece.

Twenty (20) GRL picks were collected for sensory testing, totalling 1,200 consumers.

The consumers scored each sample on a 0 to 100 scale line for tenderness, juiciness, flavour, and overall liking. From this, the meat quality score (MQ4) was calculated using a weighted average, where tenderness was rated 0.3, juiciness 0.1, flavour 0.3 and overall liking 0.3.

Equation 1. Calculation of Meat Quality Score (MQ4)

$$\text{Meat Quality (MQ4)} = 0.3 \times \text{tenderness} + 0.1 \times \text{juiciness} + 0.3 \times \text{flavour} + 0.3 \times \text{overall liking}$$

The consumers also marked each sample whether it was unsatisfactory, good every day, better than every day or premium.

2.5.1 Grill (GRL)

Samples were grilled on a Silex Clamshell Grill (Silex, Hamburg, Germany) set at 195°C for the top cast iron plate and 210°C for the bottom cast iron plate to produce a medium steak (Watson, Polkinghorne, et al., 2008). The grill was turned on and left to reach the desired temperature 45 minutes prior to cooking. A set of 10 “starter” steaks were placed on the grill to create a stable temperature before the cooking cycle commenced for the samples used in the study. Steaks were placed on the grill in accordance with their order on the sheets; link steaks were cooked in the first round, followed by the 6 sample steaks specific to the study.

The grill cooking procedure followed a strict time schedule to ensure that the time spent cooking was uniform to achieve medium doneness and in the correct sequence. In each round of cooking, 10 steak samples were loaded onto the bottom plate within 45 seconds before closing the lid of the Silex grill. Once the cooking time interval of 5 minutes and 15 seconds was completed samples were placed on a cutting board and left to rest for 3 minutes. At this time the next round of samples was placed on the bottom grill to start the cooking process. Once the rest period was completed the samples were cut through the middle to yield two equal-sized portions that were placed onto paper plates. These plates contained a corresponding sample reference code and consumer number and were used to serve the samples to consumers. The sample reference code and consumer number were also on the top of the consumer surveys so the plate could be cross-checked by staff when samples were placed before the consumer.

2.6 Statistical analysis

All statistical analyses including Pearson correlations were conducted in R (R Core Team, 20121). Data cleaning, visualization and summary were found using the “tidyverse” (Wickham et al., 2019), and “emmeans” (Length, 2021) respectively. Australian consumer sensory scores were analysed using a linear model, with MQ4 score as the dependant variables, and muscle, IMF% rib fat, hot standard carcass weight (covariate), as well as higher order polynomials plus relevant interactions (muscle*IMF%) as response variables. Kill groups are included as the random term. Non-significant terms were removed in a stepwise manner for each trait.

3 Results

3.1 Carcass traits

Table 3 shows the mean (\pm SEM) along with the minimum and maximum values for the hot standard carcass weight (HSCW, kg), Rib fat depth (rib_fat, mm) and IMF (%).

Table 3. Mean (\pm SEM), minimum and maximum for the carcass characteristics of hot standard carcass weight (HSCW), rib fat depth, IMF (%).

Carcass Trait	Mean (\pm SEM)	Min	Max
HSCW (kg)*	393.1 \pm 5.2	334	472
rib_fat (mm)	13.2 \pm 0.7	8	28
IMF(%)	12.0 \pm 0.4	2.9	46.2

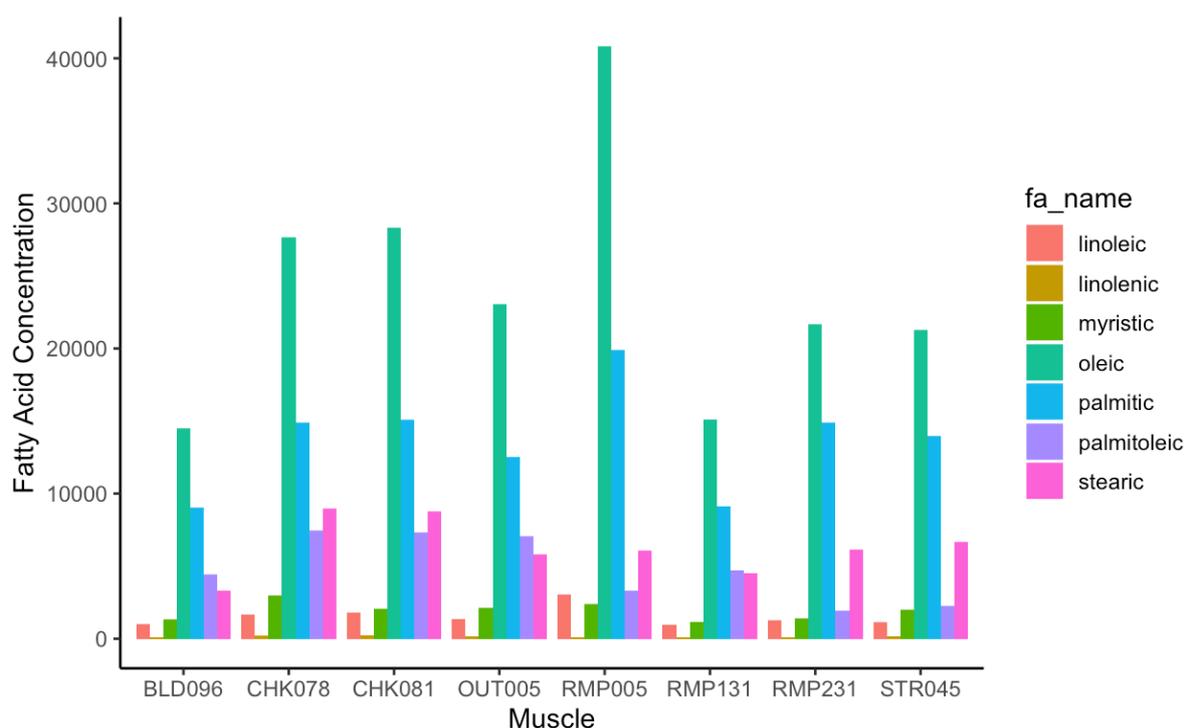
3.2 Fatty acids (FA)

Total FA content in the beef samples was highly variable and ranged from 65.4 (Linolenic) to 9856 (Oleic) mg/100g beef (Table 4). Oleic acid is typically found in greater proportions followed by Palmitic acid. Figure 1 shows the fatty acids concentration in different muscles.

Table 4. Mean (\pm SEM), minimum and maximum for the fatty Acids mg/100g)

Fatty acid	Carbon	Mean (\pm SEM)	Min	Max
Myristic	14:0	761.9 \pm 35.2	292.6	1229.5
Palmitic	16:0	6836 \pm 264.2	3287	10786
Steric	18:0	3327 \pm 149.3	1883	5470
Palmitoleic	16:1	840.9 \pm 40.7	306.1	1454.7
Oleic	18:1	9856 \pm 455.6	4910	18264
Linoleic	18:2	429.9 \pm 21.0	173.9	642.0
Linolenic	18:3	65.4 \pm 4.4	34.2	166.4

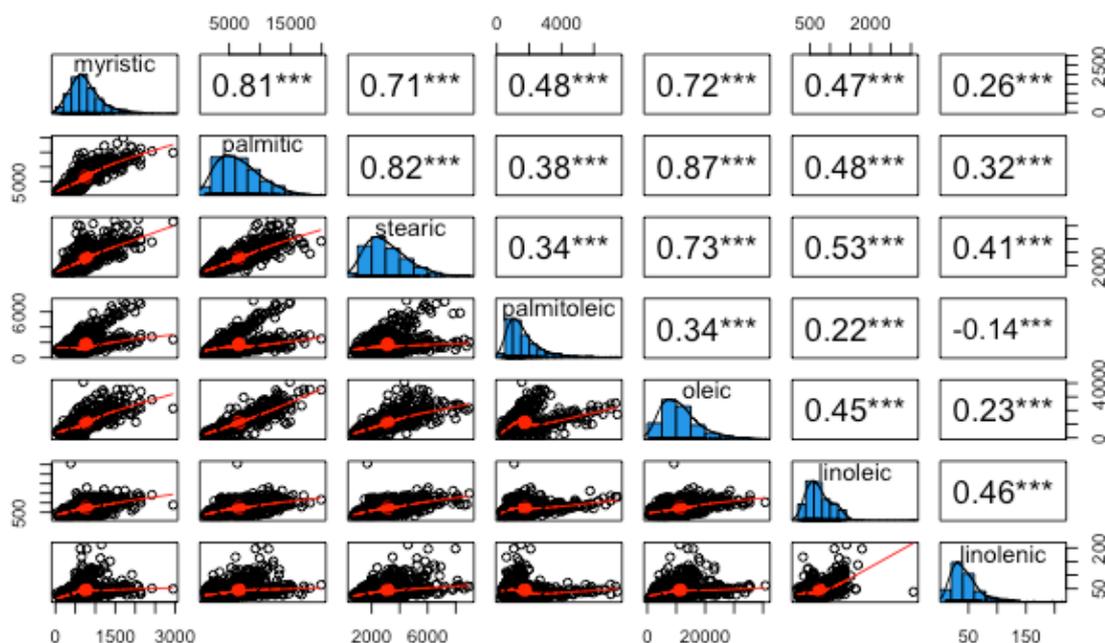
Figure 1 Fatty acids (FA) concentration in different muscles



3.2.1 Pearson correlation (r) across fatty acids

The correlation among fatty acids ranged from 0.45 to 0.87 (Figure 2). Saturated fatty acids (myristic, palmitic and stearic) were highly correlated (0.71 to 0.82) compared to monosaturated (0.34) and polyunsaturated (0.46) fatty acids. The correlation between palmitoleic with other fatty acids ranged from -0.14 to 0.48, whereas the correlation between linolenic with other fatty acids ranged from -0.14 to 0.46.

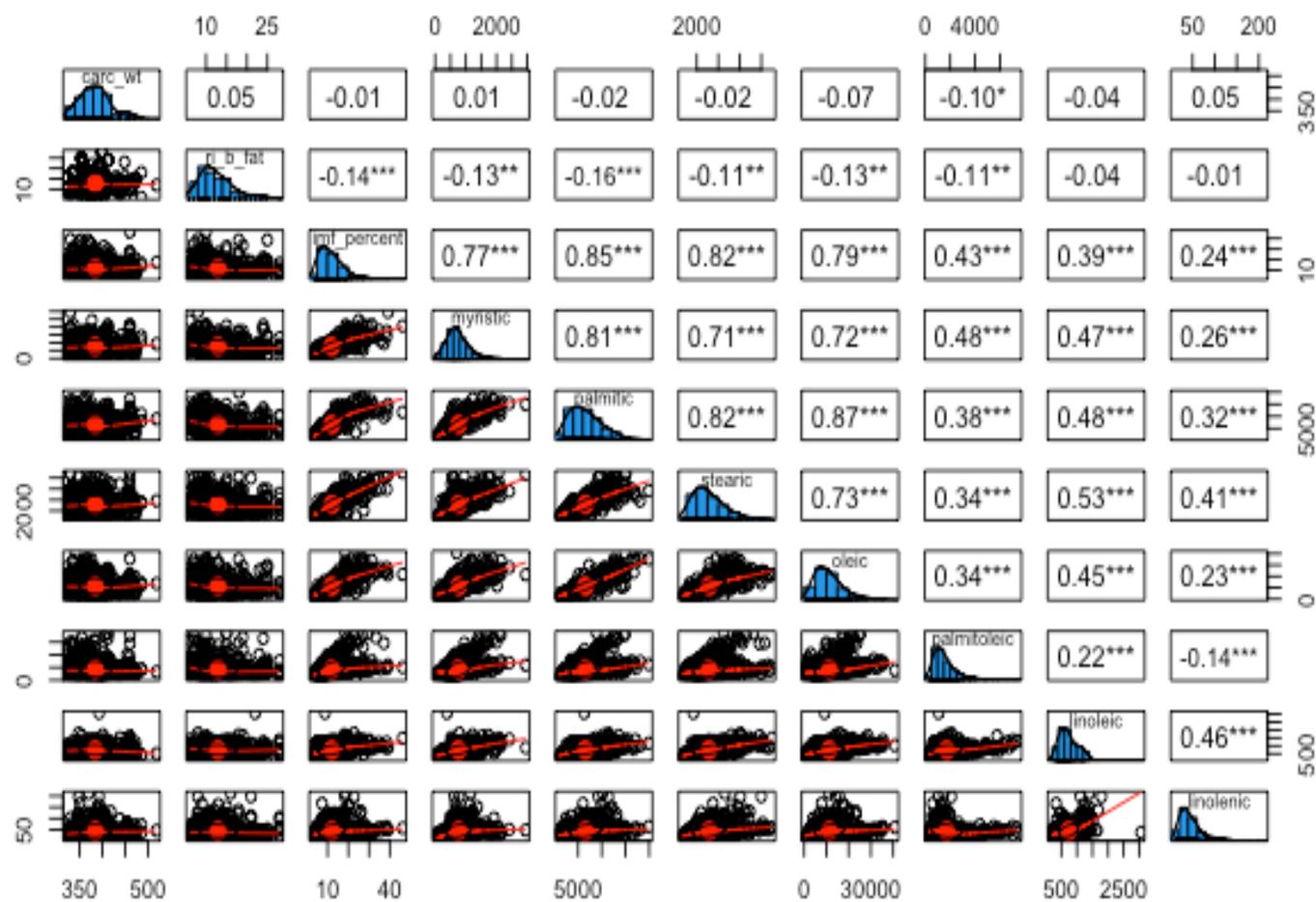
Figure 2: Pearson correlation (r) across fatty acids



3.2.2 Pearson correlation (r) between fatty acids and carcass traits

The IMF% was highly correlated with palmitic (0.85), followed by stearic (0.82), oleic (0.79) and myristic (0.77) and moderately correlated with palmitoleic, linoleic and linolenic (0.24 to 0.43 (Figure 3). Rib fat was negatively correlated with all fatty acids. Carcass weight was negatively correlated with fatty acids, although not significant.

Figure 3: Pearson correlation (r) between fatty acids and carcass traits



3.3 Sensory

The following section outlines all fixed effects used to model the CMQ4 score and the F-value for each factor that composes the models to predict CMQ4 for the grill samples. The main drivers of CMQ4 for grill samples were good predictors of CMQ4 ($R^2 = 0.54$). The main predictor of CMQ4 was muscle ($P < 0.001$), followed by IMF% ($R^2 = 0.12$, Figure 4). Muscle by IMF% had a positive and Rib fat had a negative impact on CMQ4 (Figure 5). The inclusion of total saturated fatty acids (SFA) was not significant; however, the inclusion of Mono-unsaturated Fatty acids (MUFA) and Poly-unsaturated Fatty acids (PUFA) had significant effects on CMQ4.

Figure 7 to Figure 13 show the scores of CMQ4 explained by myristic ($P < 0.001$), palmitic ($P < 0.001$), stearic, ($P < 0.001$), oleic ($P < 0.001$), and linolenic ($P < 0.05$), whereas palmitoleic ($P < 0.01$) had a negative impact and linoleic had no significant effect on CMQ4.

Table 5: The F-value for each factor that composes the models to predict CMQ4 for the grill samples

Model terms	R2	F- Value										
			Myristic	Palmitic	Stearic	Oleic	Palmitoleic	Linoleic	Linolenic	SFA	MUFA	PUFA
Muscle	0.47	71.76	80.99	80.69	81.28	81.02	81.12	81.96	84.85	80.9	81.1	81.8
Carcass weight (kg)	- 0.00	0.23	0.51	0.50	0.50	0.50	0.50	0.51	0.53	0.50	0.50	0.51
IMF (%)	0.12	75.24	53.27	53.07	53.46	53.29	53.36	53.91	55.81	53.2	53.4	53.8
Rib fat (mm)	0.01	9.20	10.51	10.47	10.54	10.51	10.52	10.63	11.01	10.5	10.5	10.6
Muscle*IMF%	0.53		3.45	3.52	3.47	3.26	3.22	3.89	3.87	3.55	3.14	3.67
Myristic ² (mg/100g)	0.54		2.81									
Palmitic ² (mg/100g)	0.53			0.18								
Stearic ² ((mg/100g)	0.54				4.61							
Oleic ²	0.54					4.36						
Palmitoleic ² (mg/100g)	0.54						5.26					
Linoleic ² (mg/100g)	0.54							6.45				
Linolenic (mg/100g)	0.56								2.29			
Linolenic *muscle	0.56								4.46			
*SFA	0.54									1.54		
*MUFA ²											6.05	
*PUFA												6.74

*SFA: Total saturated fatty acids; MUFA: Mono-unsaturated Fatty acids; PUFA: Poly-unsaturated Fatty acid

Figure 4 CMQ4 vs IMF% correlation

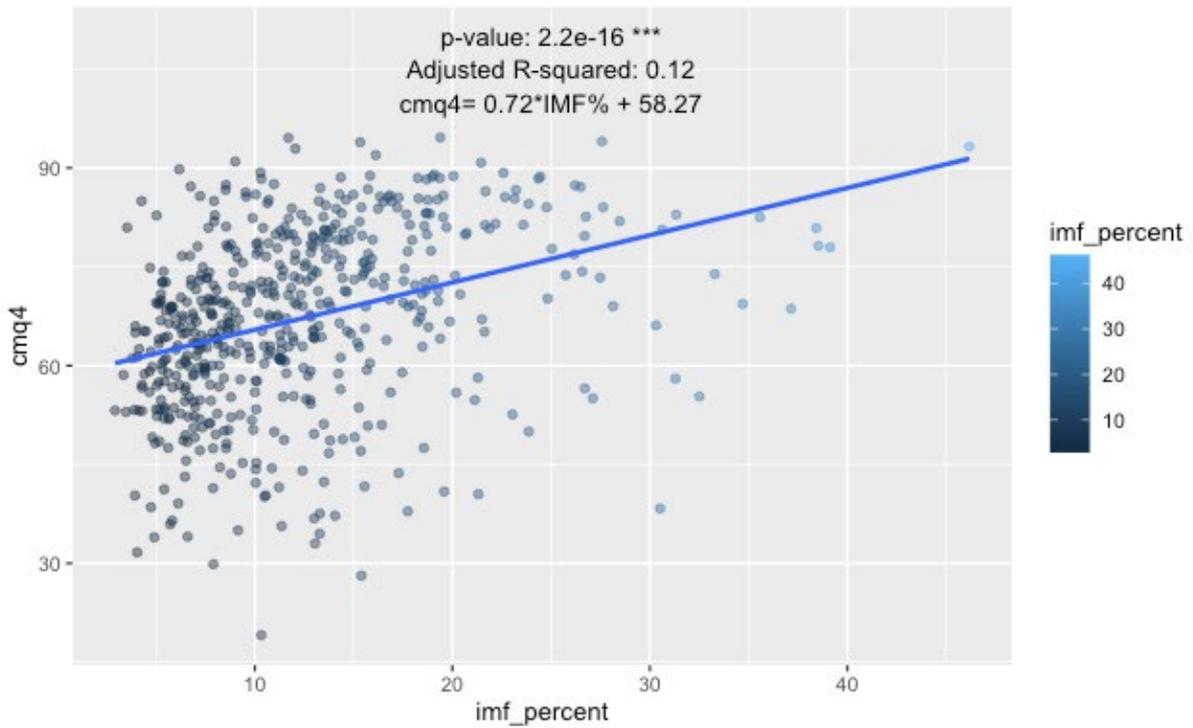


Figure 5: CMQ4 vs Rib fat correlation

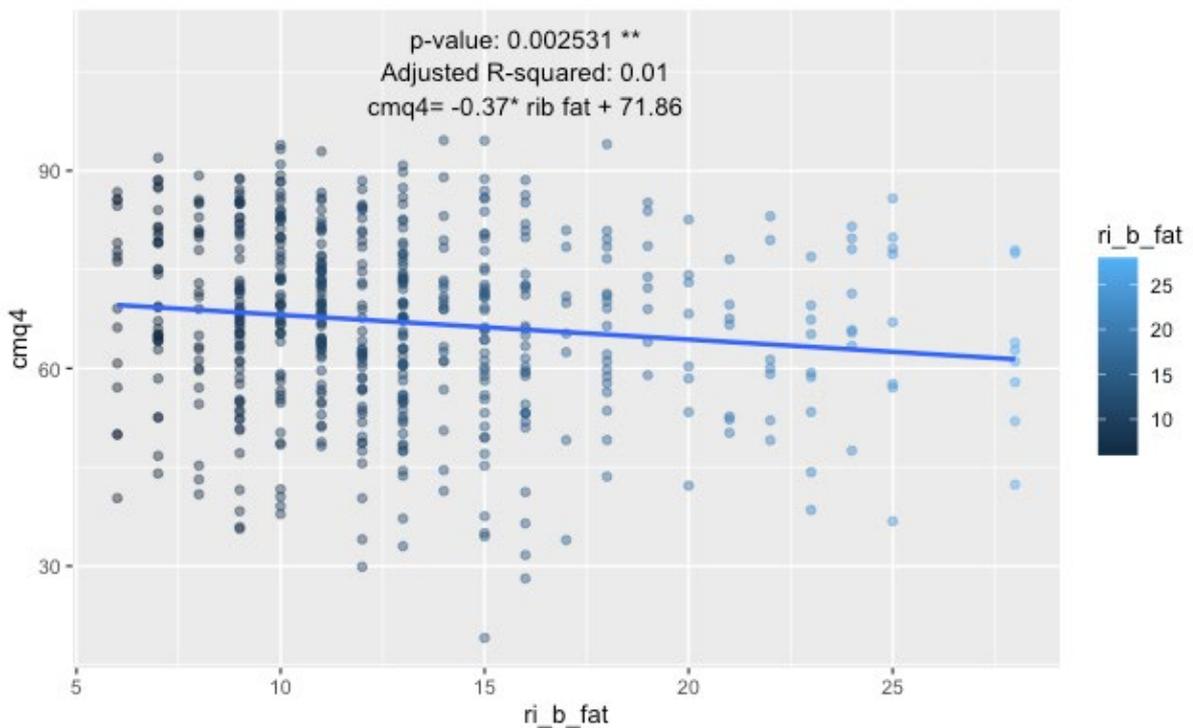


Figure 6: CMQ4 vs hot standard carcass weight (HSCW) correlation

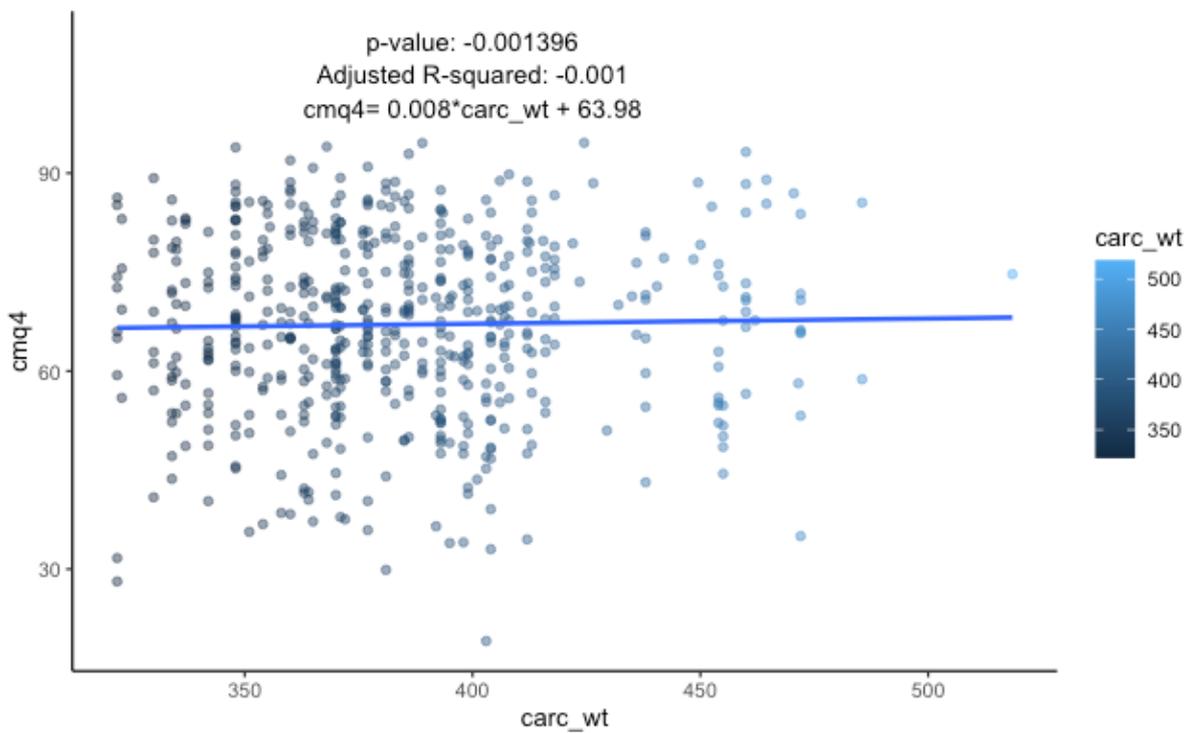


Figure 7: CMQ4 vs myristic acid correlation

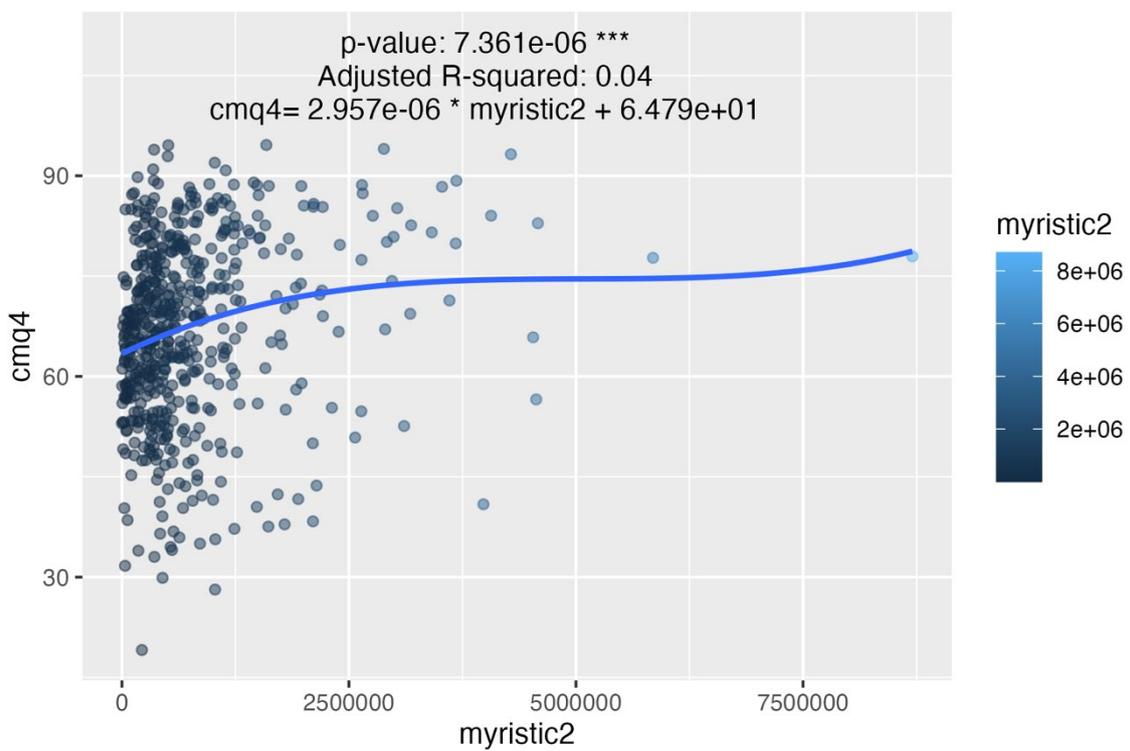


Figure 8: CMQ4 vs palmitic acid correlation

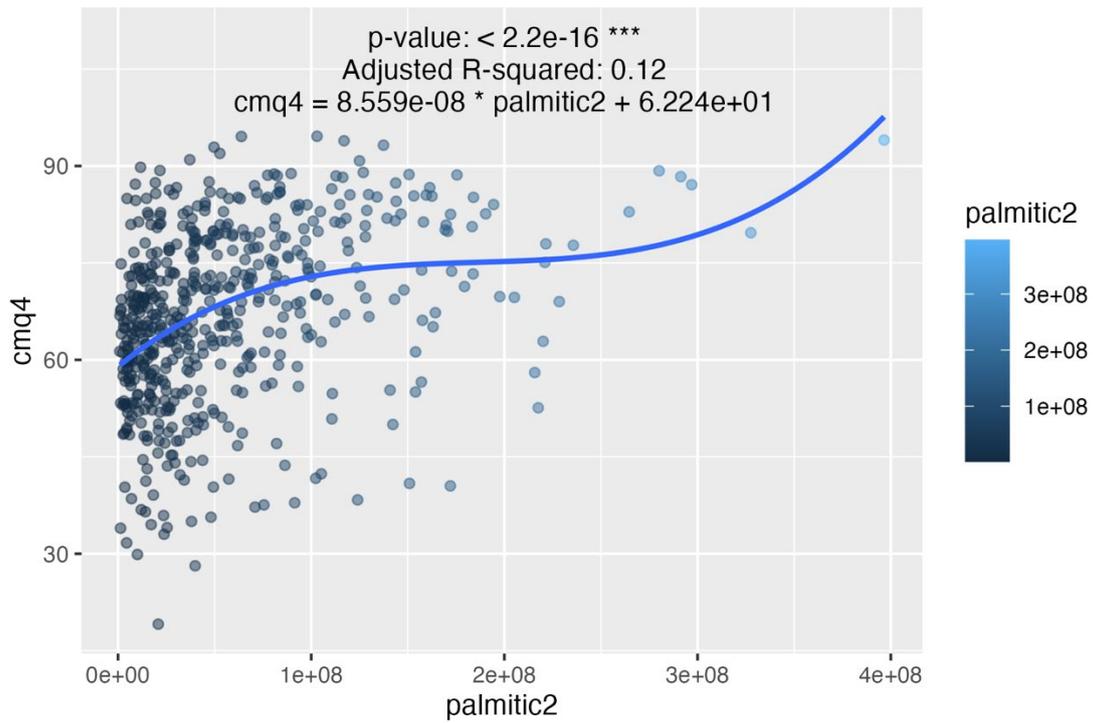


Figure 9: CMQ4 vs stearic acid correlation

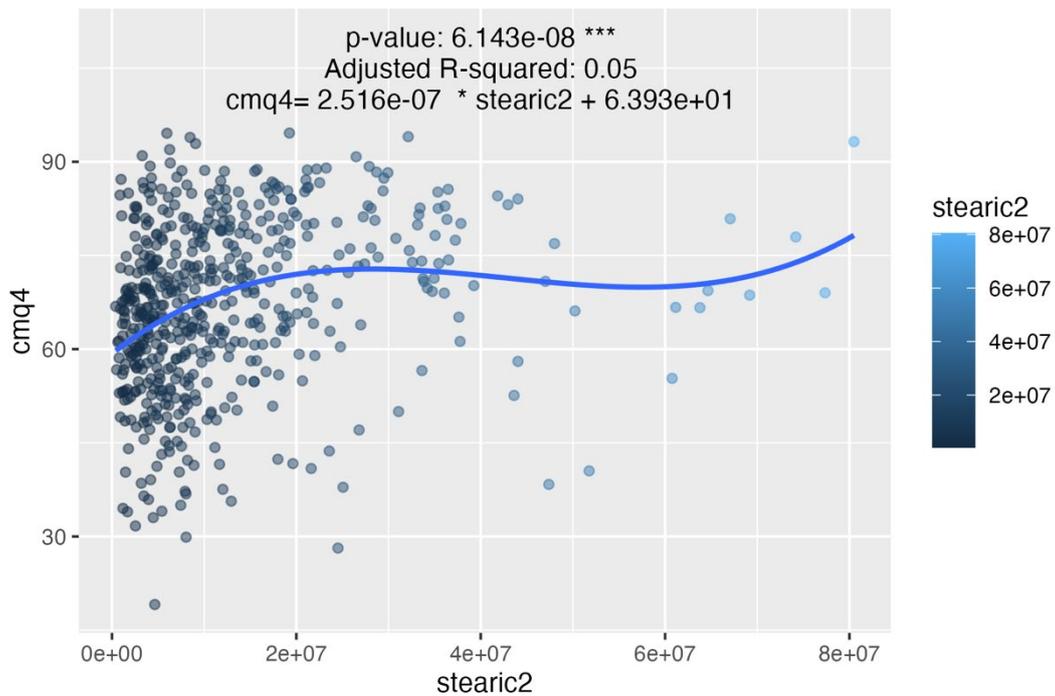


Figure 10: CMQ4 vs oleic acid correlation

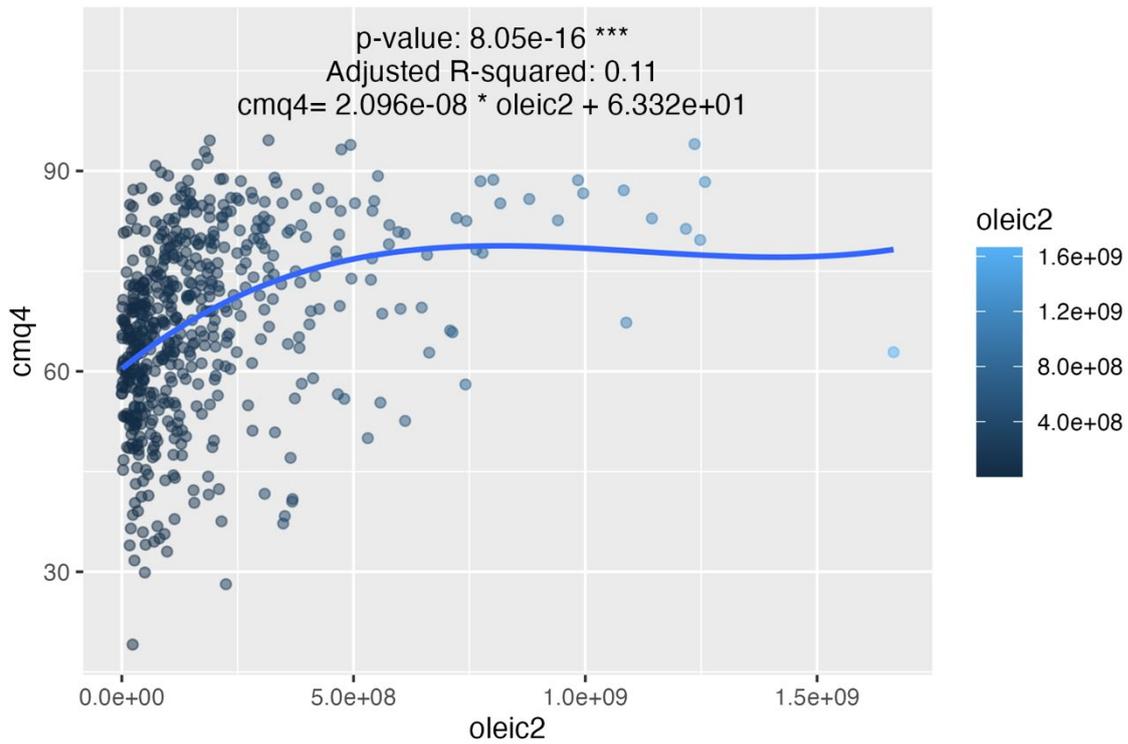


Figure 11: CMQ4 vs palmitoleic acid correlation

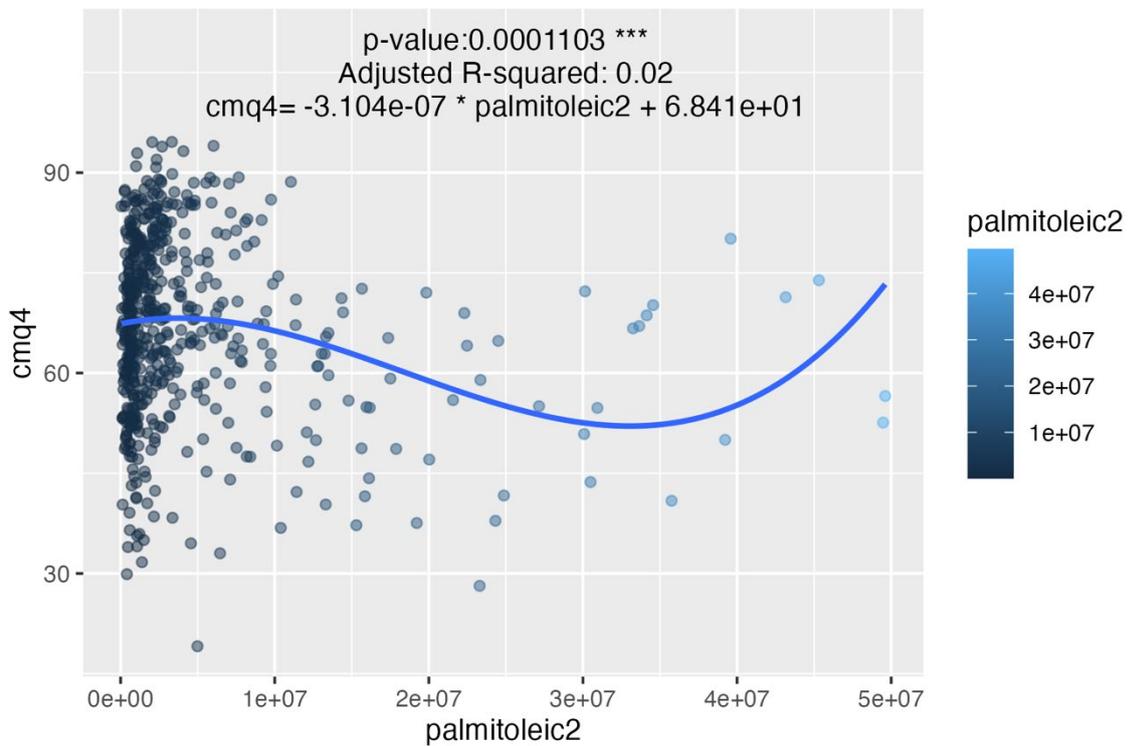


Figure 12: CMQ4 vs linoleic acid correlation

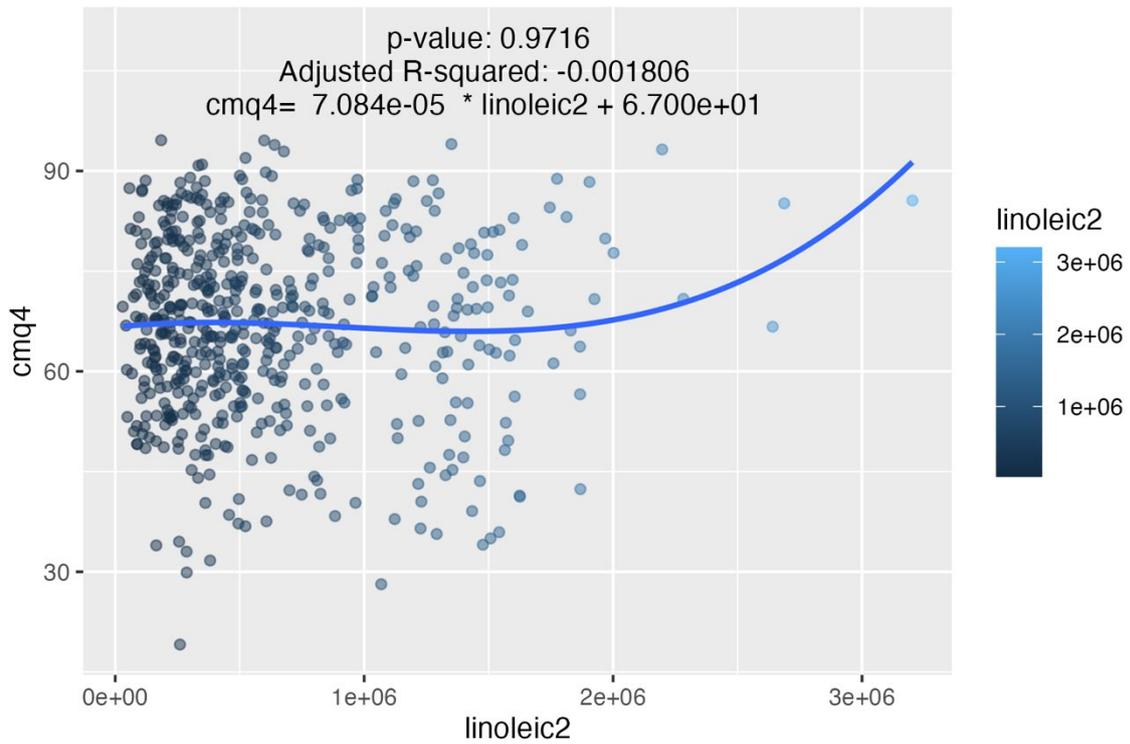


Figure 13: CMQ4 vs linolenic acid correlation

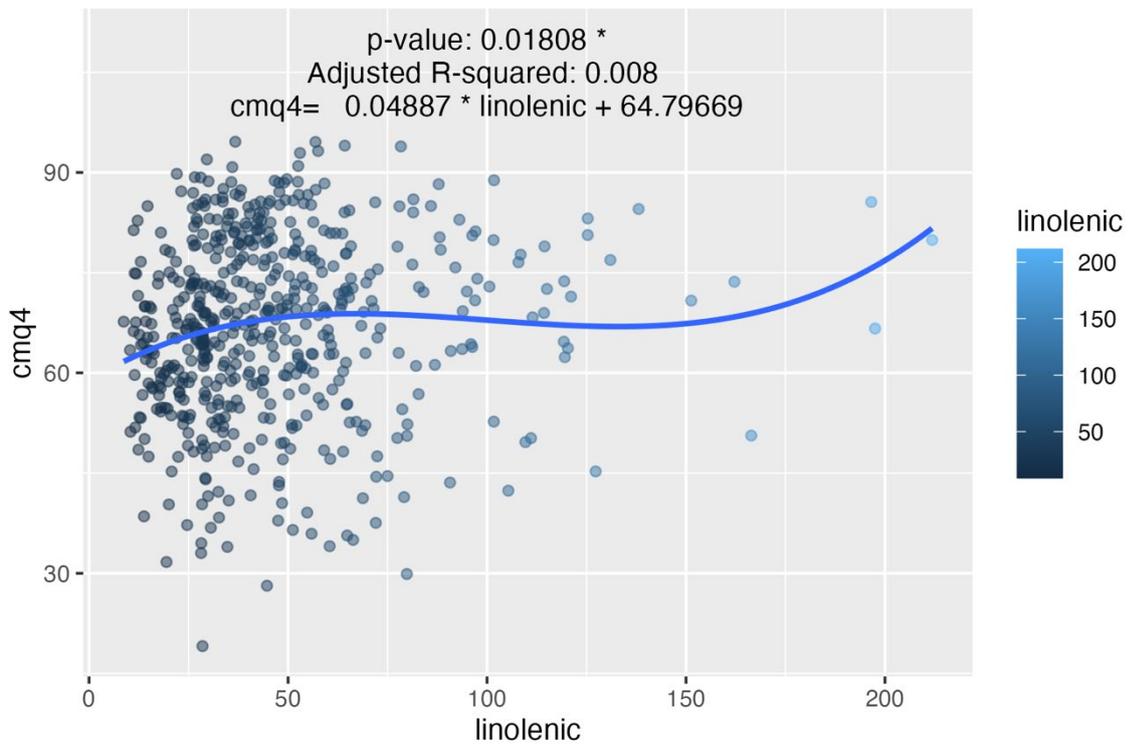


Figure 14: CMQ4 vs saturated fatty acid (SFA) correlation

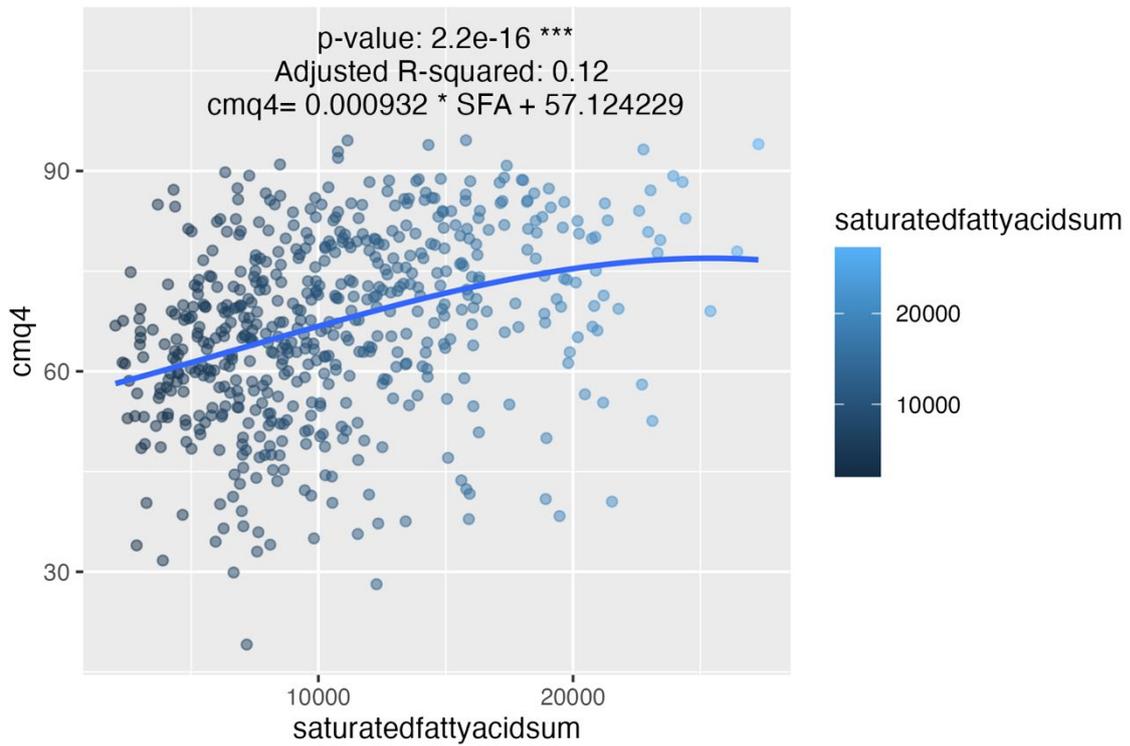


Figure 15: CMQ4 vs monounsaturated fatty acid (MUFA) correlation

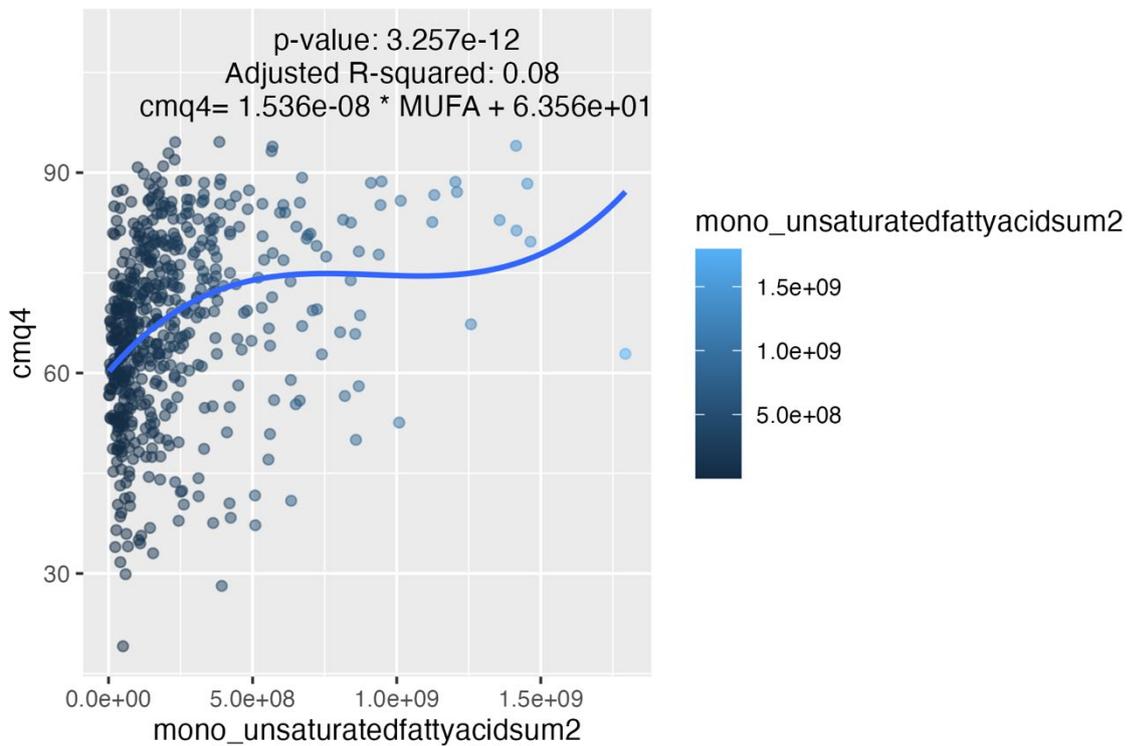


Figure 16: CMQ4 vs polyunsaturated fatty acid (PUFA) correlation

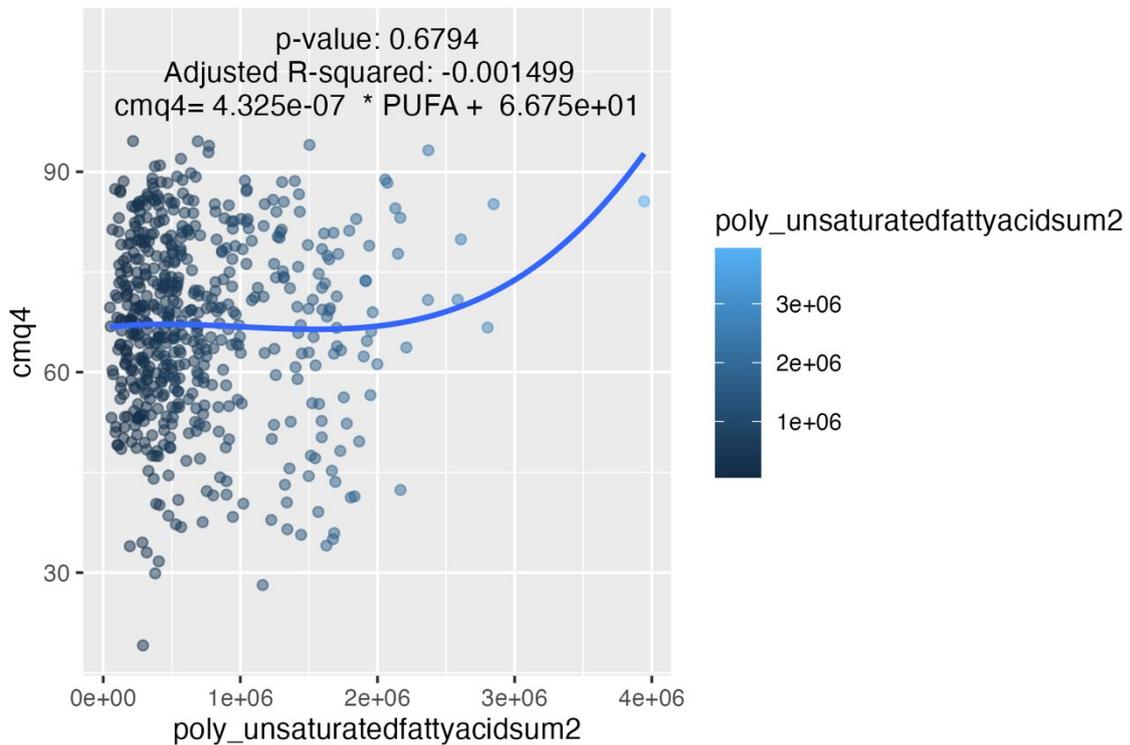


Figure 17 to Figure 23 represent estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 of fatty acids by muscle interaction. There were no significant differences in MCQ4 scores between the BLD096, CHK078, and CHK081; and between RMP131 and RMP231. The OUT005 had the lowest CMQ4 score and the RMP005 had the highest score, followed by STR045.

Figure 17: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of Myristic by muscle interaction

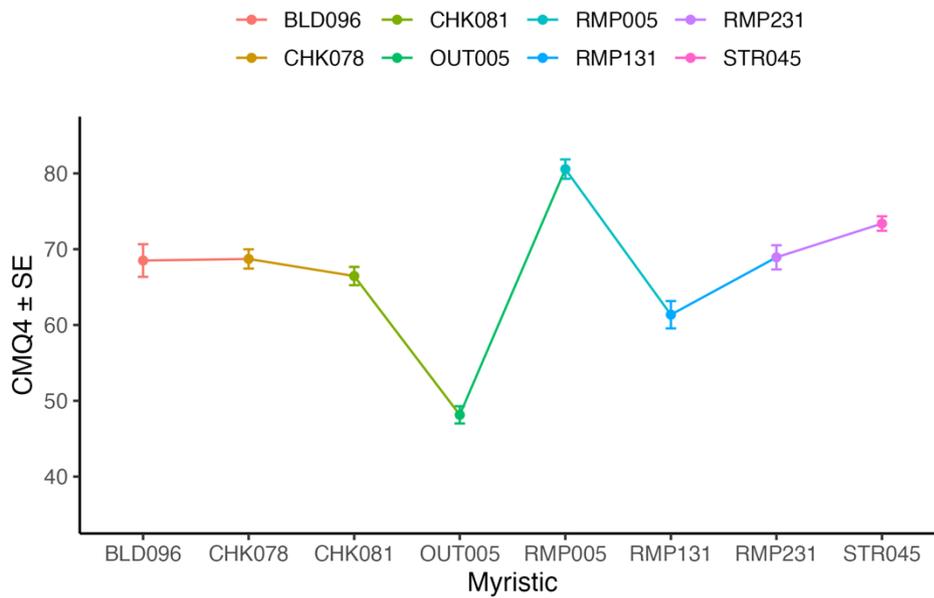


Figure 18: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of Palmitic by muscle interaction

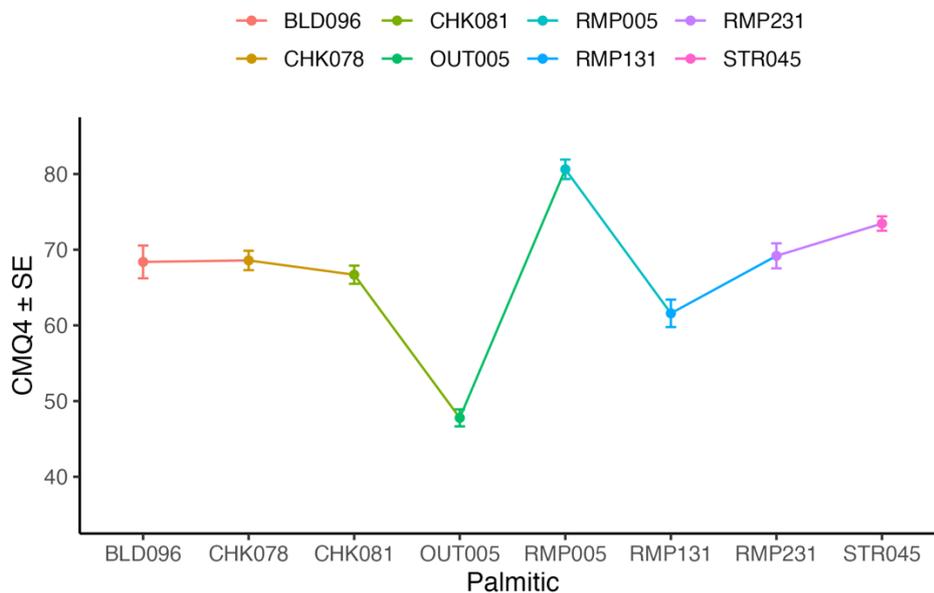


Figure 19: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of Stearic muscle interaction

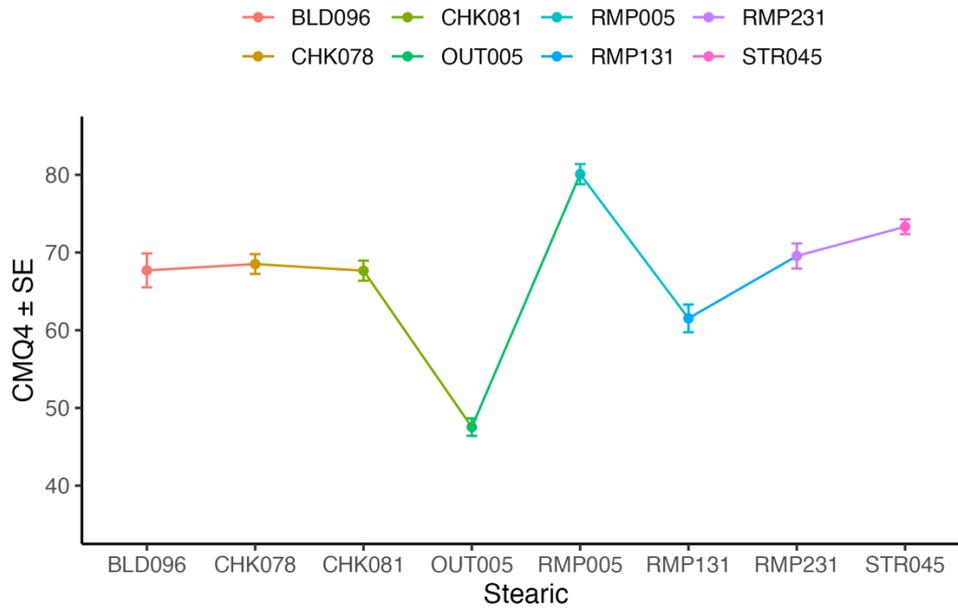


Figure 20: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of Oleic by muscle interaction

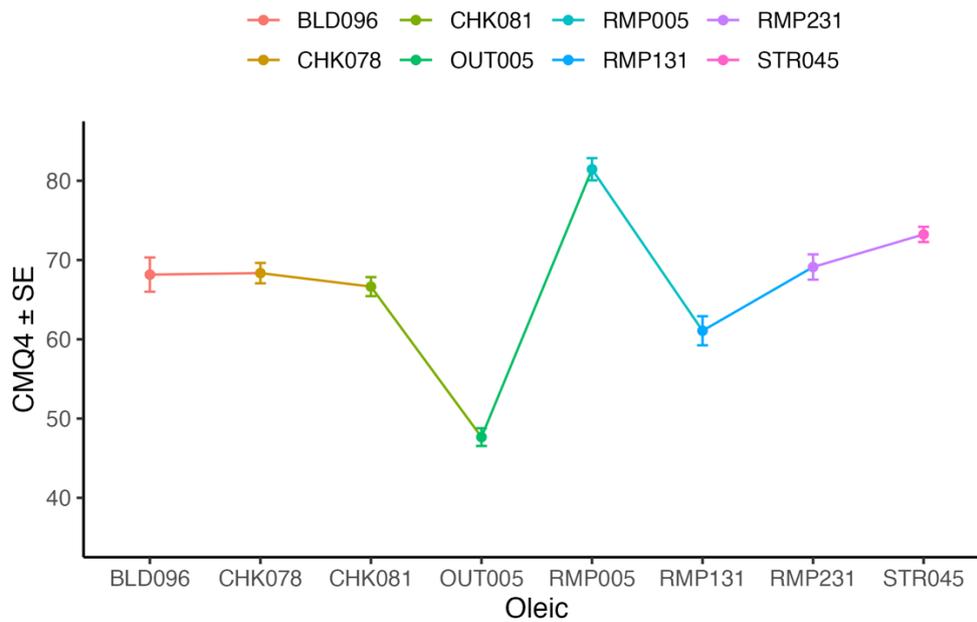


Figure 21: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of by Palmitoleic muscle interaction

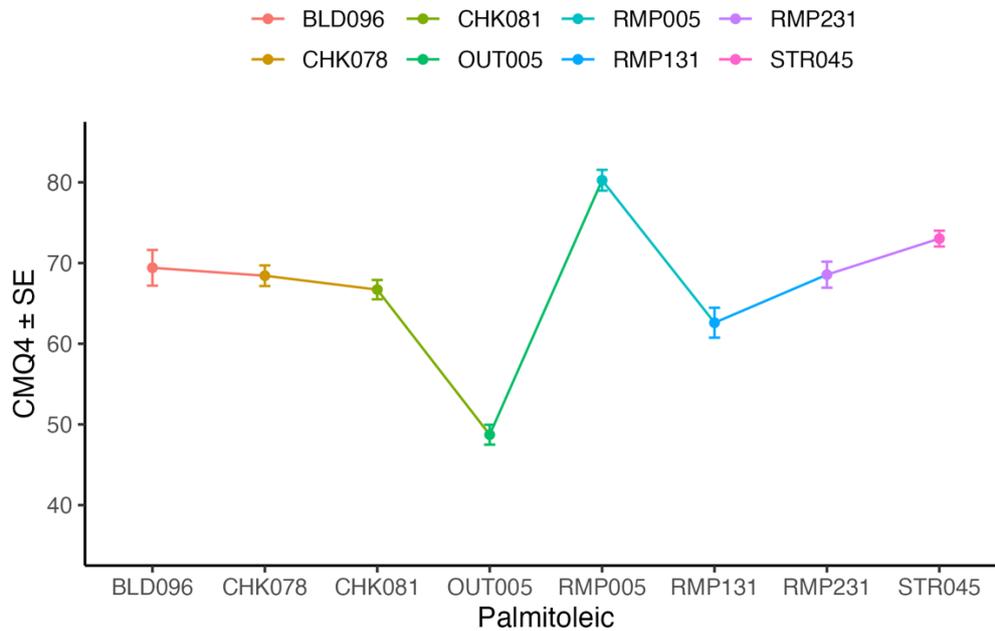


Figure 22: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of by Linoleic muscle interaction

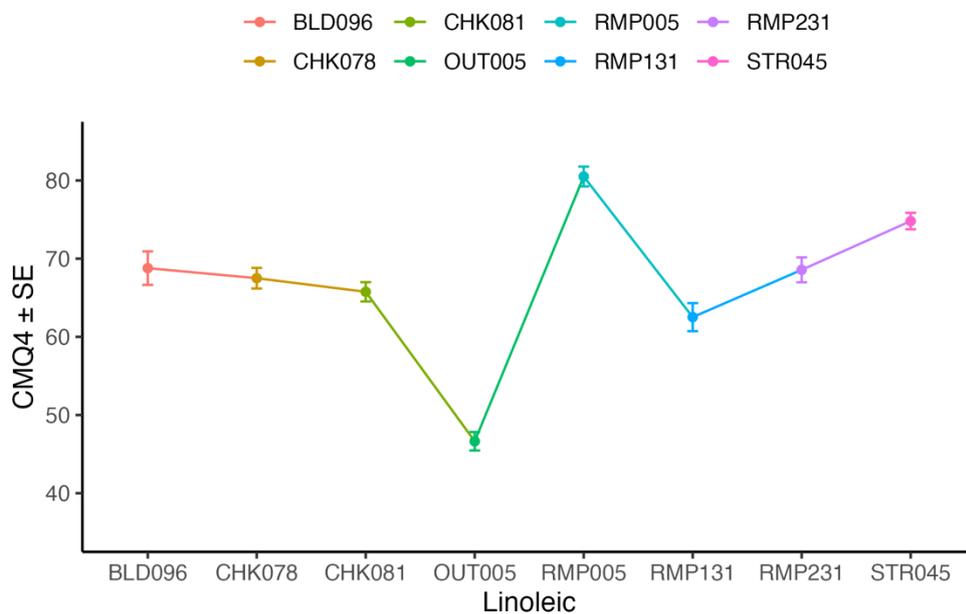
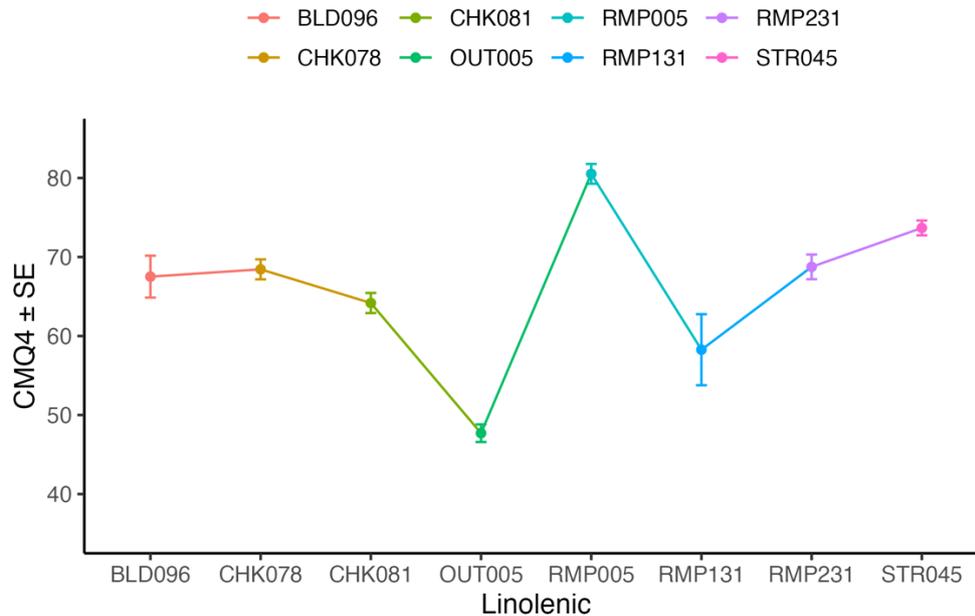


Figure 23: Estimated marginal means with 95% confidence intervals of consumer sensory for the CMQ4 ± SE of Linolenic by muscle interaction



4 Discussion

This study analysed the influence of fatty acid content on eating quality when balanced for carcass characteristics using the grill cooking method. The result showed that there is a positive significant effect of all fatty acids except a negative effect of palmitoleic and linoleic acid had no significant effect on CMQ4.

Wagyu-influenced cattle have been shown to produce higher monounsaturated fatty acids and less saturated fatty acids than other *Bos taurus* animals (May et al., 1993). This change in fatty acid profiles is hypothesised to affect eating quality aspects due to the lower melting points of unsaturated fatty acids.

4.1 Benefits to industry

- The data generated for consumer sensory scores on fatty acids will help to improve the accuracy of the MSA model for high-marbling carcasses across a broad range of cuts.
- The benefits to the wider industry are improved accuracy of eating quality predictions for premium long-fed carcasses aiding consistency within beef in premium brands and improving consumer satisfaction with Australian highly marbled beef.
- This project also clearly demonstrates that marble scores are not the only important determinants of eating quality but fatty acids can use as important terms used in marketing. Premium brands would benefit from MSA grading and segregation based on MSA eating quality predictions.

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