Final report

AMPC
AUSTRALIAN MEAT PROCESSOR CORPORATION

Packaged and aged lamb

The data-based confirmation of chilled lamb (held for up to 20 weeks) quality and safety, using novel smart packaging and spectroscopic technologies

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Contents

1.0 Executive summary

The project investigated if sustainable packaging could preserve chilled lamb meat quality, safety, and oxidative status across a 20 week storage period. The study also investigated the potential for Raman spectroscopy to predict in-pack microbial counts of aged lamb meat.

384 lamb *longissimus lumborum* muscles (LL) were selected at random from the boning room of a commercial Australian abattoir. These were used to complete 2 independent repeats of an 8 × 4 factorial designed experiment that captured the effects of 8 storage periods (Week 0, 1, 2, 6, 10, 14, 18, and 20) and 4 packaging types (PACK 1, Eco-tite® Recycle Ready Shrink Bag; PACK 2, Cryovac® Barrier Shrink Bag; PACK 3, a foil pouch; and PACK 4, Biovac™ Recyclable Vacuum Pouch.

PACK 1 was selected due to its material properties respond to new standards for recyclable plastic film and it was untested for lamb meat preservation; PACK 2 was selected due to its representation of conventional and current industry practice; PACK 3 was selected due to its oxygen transmission rate, with < 1 CC/m^2/24 h offering a proof of concept packaging to determine whether 20 weeks of chilled storage was a valid hypothesis; and PACK 4 was selected due to its 'biodegradability' and being marketed for red meat preservation.

It was apparent that TVC increased with storage period, but not to levels that exceed the upper limits defined for microbial spoilage in domestic and export markets (log 7 CFU/g) and regardless of the storage period by packaging type effects on lamb meat.

The diversity of in-pack microorganism populations tended to decline as storage period increased, particularly after 6 weeks of chilled storage. Packaging type was found to affect the pH and TVC of lamb meat, although the differences were of little practical importance. Packaging type did not substantively affect microorganism populations. TVB-N concentrations increased between Week 1 and Week 14, but again remained within defined limits for fresh lamb meat .

There were few storage period by packaging type interactions found to impact on lamb meat and the effect of those that did were nugatory on practical terms, i.e., unperceivable to consumers or inferring minimal 'real' change to the lamb meat. A possible exception was PACK 4, as the lamb meat held under this packaging type discoloured rapidly after long-term chilled storage periods and to a greater extent that was observed in PACK 1-3.

The lamb meat stored for > 6 weeks became unacceptably discoloured increasingly sooner into the retail display period, with few practical differences apparent from the storage period by packaging type interactions on colour parameters.

The concentration of some fatty acids changed with long-term chilled storage, mostly between Weeks 6-18. The fatty acid indices and EPA+DHA confirmed that lamb meat supported health outcomes, associated with fatty acid consumption, irrespective to storage period or packaging type. Lamb held under PACK 3 generally had higher concentrations of omega-3 and omega-6 fatty acids. MUFA concentrations were affected, but to a lesser extent, by packaging type. In addition, it was found that biomarkers for oxidative stability were inconsistent or unaffected by storage period.

Raman spectra provided modest predictions (R^2 = 0.29; RMSE = 1.34) of in-pack total viable count (TVC) of microorganisms. It could, however, differentiate between lamb meat with high and low TVC (based on log 5 CFU/g) with relatively high accuracy (92.5%) and sensitivity (88.0%).

This project has demonstrated that new and sustainable plastic packaging can deliver the shelf-life of conventional practice for long-term chilled lamb meat products. It demonstrated that vacuum packaged lamb meat can be held chilled for up to 20 weeks and therefore 10-15% longer than has been previously recommended in the literature. In addition, it demonstrated that Raman spectroscopy can provide non-destructive information of the in-pack microbial status of lamb meat.

Future investigation is necessary to quantify the microbial load of microorganisms particularly associated with foodborne illnesses. Objective measures for aroma and taste (flavour) were not directly investigated and future study should quantify these to ensure consumer satisfaction. The transference of these findings to alternative cuts of lamb, including bone-in product, as well as to alternative storage temperatures merits further investigation. In addition, consideration should be given to the end-user of the chilled lamb meat *viz*. confinement odour is characteristic of vacuum packaged meats, being malodours that develop and disappear within a short period of time after the pack is opened. Future studies should investigate the changes in smell and aroma of lamb meat held under different packaging types for long-term chilled storage periods.

2.0 Introduction

Plastic packaging is an important issue for lamb processors and industry stakeholders. Its disposal represents a cost, in economic and environmental terms, and community expectations have shifted so that 'sustainable practice is also considered to be best practice' (King et al., 2021, Kan and Miller, 2022). Key Australian export markets (e.g., South Korea, European Union) are moving to adopt more stringent requirements for meat packaging, namely that it adheres to a high standard of recyclability (i.e., LDPE/RIC2 recycling streams) and can otherwise participate in a circular economy (Matthews et al., 2021, Song and Park, 2024). This has prompted the development of innovative packaging types for meat (Holman et al., 2018b, Holman et al., 2018a, Kolluru et al., 2024, Nobile et al., 2024). If adopted, some of these may reduce some negative environmental effects associated with the conventional packaging of lamb meat (Kan and Miller, 2022). However, meat packaging and its disposal are not the only factors underpinning 'sustainable practice'. Extended periods of preservation, wherein lamb meat retains its safety and quality, are required to reduce food waste and to improve supply chain efficiencies (Mills et al., 2014, Bogataj et al., 2020, Toomik et al., 2023). Within this context, new and sustainable plastic packaging must also deliver a shelf-life that meets or exceeds conventional practice for long-term chilled lamb meat products.

The duration of chilled storage will affect microorganism populations and thereby the safety and spoilage of vacuum packaged lamb meat. Kaur et al. (2017), for example, reported that aerobic plate (TVC) and lactic acid bacterium (LAB) counts for vacuum packaged lamb were log 7.5 CFU/cm^2 and log 7.0 CFU/cm^2, respectively, after 42 days of chilled storage at -1.2 °C. These same authors also reported consistency in TVC and LAB between Days 42 and 124 of chilled storage, as well as an initially diverse microbiome that became distinctive and stable as the chilled storage period increased (Kaur et al., 2017). Using sterile samples of lamb meat, inoculated with combinations of obligate and facultative anaerobes as well as *Pseudomonas* sp., it was found that *Clostridium* spp. played a major role in quality deterioration in vacuum packaged lamb meat that was held for chilled for 10 weeks at 2 °C (Rood et al., 2022). For vacuum packaged lamb meat held chilled for up to 8 weeks, Coombs et al. (2017a) reported an increase to LAB count between Weeks 2-4; *Brochothrix thermosphacta* counts between Weeks 0-2, 2-4, and 4-6; and *Enterobacteriaceae* sp. between Weeks 0-4 – although all final counts (Week 8) were below recommendations from the literature (Gill, 2014, Bell, 2001). The apparent chilled storage effects on microorganism populations are a function of initial populations, physiochemical meat properties, and storage environment (Mills et al., 2014, Pennacchia et al., 2011). Specifically, the ability for

microorganisms to proliferate under anaerobic conditions and low storage temperatures, as well as their capacity to compete with other microorganisms and react to pH, water activity, and substrate biochemistry (e.g., glycogen) will contribute to their dominance and the spoilage potential for lamb meat (Rood et al., 2022, Toomik et al., 2023). That said, the visual appearance and odour scores for vacuum packaged (boneless) lamb shoulder cuts were reported as acceptable throughout a chilled storage period of 85 days, even though TVC and LAB counts were often high (up to log 8 CFU/g) (Kiermeier et al., 2013). Shaw et al. (1980) instead recommended a maximum of 4 weeks chilled storage for bone-in lamb cuts, a recommendation based the population of microorganisms upon retail display. Noting that not all microorganisms are detrimental to lamb meat safety and shelf-life (Bekhit et al., 2021b), it was important that the effects of storage period on microorganism populations and community diversity are understood.

Research Question 1: Can 'sustainable' packaging preserve microorganism populations to acceptable levels in lamb meat held chilled for up to 20 weeks?

The duration of chilled storage will affect the water-holding capacity, texture, and colour of vacuum packaged lamb meat. These changes will affect the quality and retail potential of lamb meat and have been widely reported in the literature. For example, Ponnampalam et al. (2017) reported the retail colour stability of vacuum packaged lamb meat chilled for 40 days as being substantively less acceptable than was observed after 5 days, a finding based on consumer thresholds for *a** values and brownness (reflectance ratio of 630 to 580 nm). Ponnampalam et al. (2021a) reported the rate of *a** decline, across a 72 hour retail display period, was greater for vacuum packaged lamb meat stored for 90, 45, and 2 days, respectively. Rant et al. (2019) reported that the shear force and expressed juice percentage values were lower and the cooking loss value was higher for 14 days wet aged lamb, compared with unaged samples. Coombs et al. (2017a) reported that initial shear force values declined and then stabilised when the chilled storage period exceeded 32 days. These same authors reported lamb purge and cooking loss values increased across the 56 day period of chilled storage; ultimate pH was reportedly unchanged by storage period; and the rate of colour change across 3 days of retail display accelerated when lamb was held chilled for > 14 days (Coombs et al., 2017a). Calnan et al. (2019) reported the lamb meat colour stability during retail display decreased as chilled storage period increased from 5 days to 35-70 days, observations proposed to be the result of vitamin E and oxidative status of the meat. Understanding the variability in oxygen permeability between vacuum packaging films (Gill and Penney, 1985)

and the effects of oxygen on the quality and colour of lamb meat is important to understanding the effects of storage period on quality and colour parameters investigated.

Research Question 2: Can 'sustainable' packaging preserve the quality and consumer appeal of lamb meat held chilled for up to 20 weeks?

Consumers prefer lamb meat that is 'healthy' and includes many of the macro- and micronutrients required for a balanced diet. Eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are important to human health, being associated with positive cardiovascular, cognitive, and optical health outcomes, but they are often limited within a typical Western diet (Swanson et al., 2012). Lamb meat is a source of these 'health-claimable' fatty acids, meaning that its nutritional value can be characterised by its fatty acid profile and the concentration of these long-chain omega-3 polyunsaturated fatty acids (Ponnampalam et al., 2021b). Due to their biochemistry, these fatty acids have a higher susceptible to oxidation compared to other fatty acids (Cosgrove et al., 1987, Porter et al., 1995) and therefore, their availability at the point of consumption is relative to the oxidative status/stability of the meat (Ponnampalam et al., 2022). Oxidative stability can change with chilled storage, with biomarkers for lipid oxidation shown to increase when vacuum packaged beef was held for 20 weeks under chilled storage (Hughes et al., 2015). Shorter chilled storage durations may also increase the amount of lipid oxidation, with Dou et al. (2022) reporting reduced oxidative stability in lamb *biceps femoris* and *longissimus* muscles held chilled for 4 days at 4 °C; and Adeyemi et al. (2016) reported reduced concentrations of health claimable fatty acids, total omega-3 and omega-6 fatty acids, and PUFA were found to reduce when chevon was held chilled for 12 days at 4 °C. Other studies have found little variation to peroxidation in chilled lamb meat, with Fernandes et al. (2014) observed that lipid oxidation was restricted when lamb meat was vacuum packaged, being < 1 mg malonaldehyde per kg serve (as-is) after 4 weeks of chilled storage. Likewise, (Coombs et al., 2018a) and Coombs et al. (2018b) reported changes to the oxidative status of lamb meat that was vacuum packaged and chilled for 8 weeks, although no corresponding change to the fatty acid profile was found. The differences between these studies could be the oxidative potential of the lamb meat investigated. Vitamin E has been identified as key to the preservation of fatty acids and lipids in lamb meat across a chilled storage period (Ponnampalam et al., 2014). Alternatively, variation to the oxygen permeability of vacuum packaging material may have impacted on presence of oxygen, its free-radicals, and the antioxidants available to preserve the nutritional value of chilled lamb.

Research Question 3: Can 'sustainable' packaging preserve the fatty acid and oxidative status of lamb meat held chilled for up to 20 weeks?

From these aforementioned examples, the literature does not seem to support a shelf-life of > 90 days for chilled lamb meat (Coombs et al., 2017b, Mills et al., 2014). This should be explored, especially given the shelf-life of 14-20 weeks reported for chilled and vacuum packaged beef (Holman et al., 2022a, Frank et al., 2019, Small et al., 2012, Youssef et al., 2014, Brightwell et al., 2009, Hughes et al., 2015). Identifying the point of spoilage for chilled lamb meat could support decisions that reduce food wastage, assure consumer safety, and promote sustainable industry practices. Likewise, there is little evidence in the literature as to the effects of different vacuum packaging materials on chilled lamb meat properties after extended storage periods. Kowalczyk et al. (2024), for example, reported that beef steaks packaged in biodegradable polylactic acid (NAT/PLA) film had lower purge loss and expressible water values after 14 and 21 days of chilled storage, compared to control samples held under conventional packaging for these same periods. This same study also reported that beef colour was enhanced when stored in Mater-Bi (NAT/MBI) biodegradable film, although all the 'sustainable packaging materials' preserved lipid oxidation and tenderness measurements across the 28 day chilled storage period (Kowalczyk et al., 2024). Few other studies have compared packaging films that can participate in a circular economy, have application to meat preservation, and that could be used for long-term chilled storage (Cutter, 2006, Motelica et al., 2020, Holman et al., 2018a). This is limiting factor to industry adoption. It is necessary, therefore, to provide a direct comparison of recyclable packaging films and by doing so confirm their functionality and practical value.

Research Question 4: Is there a storage period by packaging type interaction that could impact on the lamb meat?

Australian lamb is an important food for many, because of sociocultural and culinary preferences and the hygienic processing methods adhered to by the industry. The interval between processing and consumption is, however, not standard – as vacuum packaged lamb meat is often held under chilled storage for extended periods. The proliferation of microorganisms is restricted, but not halted, by applying anaerobic and cold storage conditions (Mills et al., 2014). This can have a detrimental effect on the rate of spoilage and safety of vacuum packaged lamb meat (Coombs et al., 2017b). Consequently, there is an imperative for lamb meat processors and retailers to assure microbial loads are within acceptable limits and food standards (CSIRO, 1995, Kim et al., 2018).

Tradition methods for quantifying the microorganisms present on a meat product are destructive and relatively expensive (Capita et al., 2004). Raman spectroscopy is a non-invasive technique that can detailed information of chemical structure, crystallinity, and molecular interactions associated with microorganisms (Li et al., 2024) as well as physiochemical properties of lamb meat (Fowler et al., 2015, Fowler et al., 2017, Fowler et al., 2021). This technology has been used to detect changes in the microbial count for pork stored chilled for 20-30 days, with measurements made through the packaging material (in-pack) (Jordan et al., 2009). The development of a portable Raman device resulted in its application to class poultry fillets held chilled for 9 days as fresh, semi-fresh, and spoiled, based on spectra recorded on the fillet surface (Jaafreh et al., 2018). From these preliminary studies and the literature, the application of Raman spectroscopy to quantify the in-pack microbial status of packaged lamb meat is untested and, if viable, of significant value as a tool for quality assurance.

Research Question 5: Can Raman spectroscopy provide real-time information of the microbial load present on vacuum packaged lamb meat?

Collectively, this project aimed to answer five research questions. By doing so, in collaboration with industry, this project will provide evidence as to the practical advantages to using 'sustainable' packaging in place of conventional packaging systems. This is expected to provide industry stakeholders and policy makers the knowledge to protect Australia's clean and green brand, leverage a more competitive market position, deliver upon market expectations, and affirm sustainable practices that reduce meat wastage and spoilage over long-term storage periods. The concurrent investigation of spectroscopy is expected to provide evidence for its application as a non-destructive means to quantify the in-pack population of microorganisms, thereby inform supply chain efficiencies and ensure consumer wellbeing.

3.0 Project objectives

The objectives of the project are:

- To confirm that smart packaging can extend chilled lamb preservation across a 20 week period and provide value to industry, comparative to conventional packaging.
- To determine whether spectroscopic technology can provide real-time insight into chilled lamb microbial and shelf-life (spoilage) status.
- To develop a data-based reference against which industry stakeholders can benchmark their production systems, in terms of modelling quality and safety trait variation over a long-term chilled storage period.

4.0 Methodology

4.1 Study design and samples

This study used a total of 384 lamb *longissimus lumborum* muscles (LL) to complete 2 independent repeats of an 8 × 4 factorial designed experiment, used to capture the effects of 8 storage periods (Week 0, 1, 2, 6, 10, 14, 18, and 20) and 4 packaging types (PACK 1-4). Within each repeat, 192 LL were collected at random and on a single day from the boning room of a commercial Australian abattoir (Dubbo, AUS). From these, 6 were randomly allocated to each combination of storage period by packaging type. The packaging types were PACK 1, Eco-tite® Recycle Ready Shrink Bag (AMCOR, AUS); PACK 2, Cryovac® Barrier Shrink Bag (SealedAir, AUS); PACK 3, a foil pouch (unbranded); and PACK 4, Biovac™ Recyclable Vacuum Pouch (Grounded Packaging Co., AUS) (Table 1). Consultation with industry stakeholders resulted in the selection of these packaging types, with the following rationale: PACK 1 was selected due to its material properties respond to EU standards for recyclable plastic film and it was untested for lamb meat preservation; PACK 2 was selected due to its representation of conventional and current industry practice; PACK 3 was selected due to its 'biodegradability' and being marketed for red meat preservation; and PACK 4 was selected due to its oxygen transmission rate, with < 1 CC/m^2/24 h offering a proof of concept packaging to determine whether 20 weeks of chilled storage was valid.

Table 1. The packaging type specifications.[1]

Packaging	Brand	Manufacturer	Material structure	Thickness, µm	Oxygen transfer rate, CC/m^2/24 h
PACK 1	Eco-tite® Recycle Ready Shrink Bag	AMCOR Pty Ltd., AUS	PE/EVOH/PE	50	< 12
PACK 2	Cryovac® Barrier Shrink Bag	SealedAir Pty Ltd., AUS	PE/PVdC/PE	50	< 20
PACK 3	Foil Pouch	Unbranded	PA/Foil/PE	155	< 1
PACK 4	Biovac™ Recyclable Vacuum Pouch	Grounded Packaging Co., AUS	BioPE/BioPE EVOH	100	2

[1]Other abbreviations include polyethylene (PE), polyvinylidene chloride (PVDC), polyamide (PA), biopolyethylene (BioPE), and ethylene-vinyl alcohol (EVOH).

All the LL were packaged at 24 hours *post-mortem* and visually inspected to assure packaging integrity. As per industry practice, these LL were placed into cardboard boxes and held on-site under chilled storage temperatures of -1.2 ± 1.1 °C (mean ± standard deviation). Storage temperatures were recorded at 30 minute intervals using temperature loggers (iButton DS19221G, Thermochron Temperature Loggers, AUS) that included within each cardboard box. At the end of each storage period, the corresponding LL were transported to the DPI Meat Laboratory (Wagga Wagga, AUS) for dissection under aseptic conditions (Figure 1). Samples not tested immediately (as-is) for colour, drip loss, and purge loss, were frozen at -25 °C until analysis. These analyses are described in the below sections.

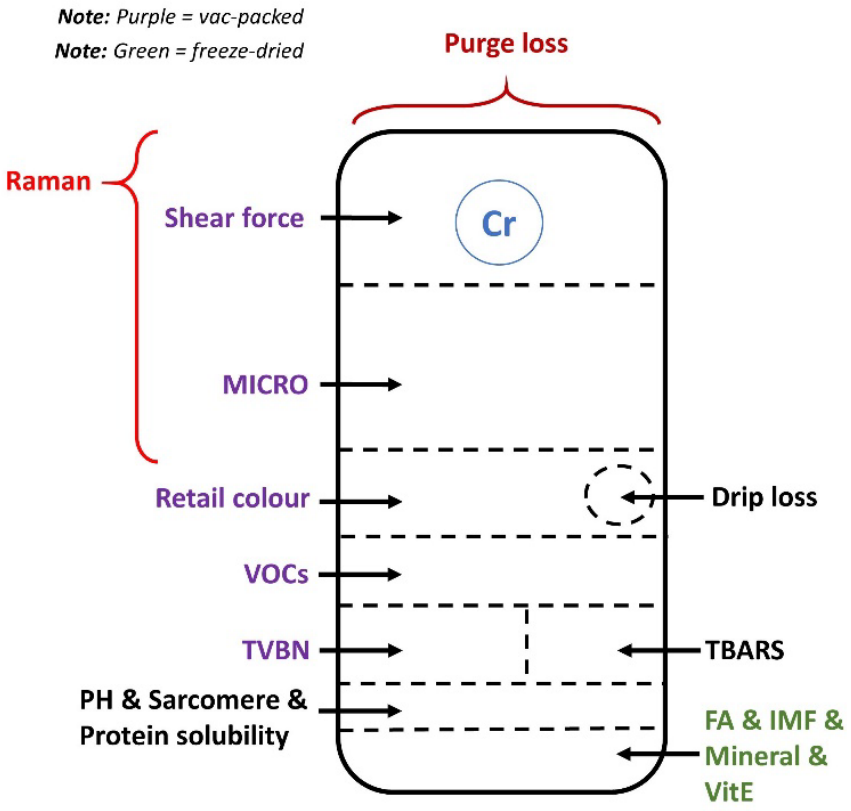

Figure 1. Dissection schematic for the lamb *longissimus lumborum* muscles. Abbreviations included microorganism analysis (MICRO), volatile compounds (VOC), total volatile basic nitrogen (TVBN), thiobarbituric reactive substances (TBARS), ultimate pH (PH), fatty acids (FA), intramuscular fat (IMF), vitamin E (VitE), and Cranial end (Cr). The cranial end of the muscle was scanned using Raman spectroscopy.

4.2 Microorganisms and freshness biomarkers

4.2.1 Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) was determined as per Holman et al. (2021) using a steam distillation method. Samples of 10 g were first homogenised in 100 mL of distilled water, and then filtered using Whatman No. 1 Filter Paper. A 10 mL aliquot of the filtrate was analysed using a Kjeldahl automated distillation unit (Kjeltec 8400, FOSS, DEN). TVB-N as mg per 100 g sample (as-is) was calculated according to the consumption of HCl (0.1 mol per L).

4.2.2 Ultimate pH

Samples of 1 g were homogenised with 6.0 mL of buffer (150 mL KCl, 5 mM iodoacetate) and then incubated in a 20 °C circulating water bath (TWBC-24-TU3, Thermoline Scientific, AUS) until temperatures had equilibrated. A pH meter fitted with a polypropylene spear-type gel electrode (WP-80 and IJ-44 Iodone™, respectively, TPS, AUS) was used to record the ultimate pH (PHU). The pH meter was first calibrated at 20 °C and using pH 4.01 and 7.00 standards. Two measurements were recorded for each sample as per Hopkins et al. (2018).

4.2.3 Aerobic plate count

Aerobic plate count (TVC) was determined at a commercial laboratory (Symbio Laboratories, AUS) and in accordance the protocol of Standards Australia (2016). Samples of 10 g were removed from the surface of each sample using a scalpel and aseptic conditions (Yang et al., 2023). This was combined with 90 mL of 0.1% peptone salt solution and homogenised within individual stomacher bags (BagFilter 400P, Interscience Co., FRA). Serial dilutions were prepared and used to inoculate petri dishes of Plate Count Agar. These were incubated at 30 ± 1 °C for 72 hours and the bacterial colonies were then counted. TVC were reported as colony forming units (CFU) per of sample (as-is).

4.2.4 Metagenomic analysis of microorganism populations

Metagenomic data was determined at a commercial laboratory (Symbio Laboratories, AUS) and using the protocol of Yang et al. (2023). Samples of 50 g were removed from the surface of each sample using a scalpel and aseptic conditions. This was combined with 100 mL BPW and homogenised within individual stomacher bags (BagFilter 400P, Interscience Co., FRA). These were incubated at 37 ± 1 °C for 24 hours before a 1.5 mL aliquot was removed and centrifuged at

1,500 × *g* for 2 mins, to remove any sample debris. The supernatant was transferred to a new microtube and centrifuged at 6,000 × *g* for 5 minutes. The supernatant was then discarded. The pellets were resuspended in 200 µL of TE buffer (10 mM Tris, 1 mM EDA, pH 8.0). The total genome DNA was extracted as per the protocol of All Prep Bacterial DNA Kit (No. 47054, AllPrep®, Qiagen, GER). Subsequently, the V3 - V4 region of the bacterial 16S rRNA gene was PCR-amplified with Illumina adaptors:

Forward primer:

5′ - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG - 3′

Forward overhang:

5′ - CGTCGGCAGCGTCAGATGTGTATAAGAGACAG - 3′

Reverse primer:

5′ - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC - 3′

Reverse overhang:

5′ - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG - 3′

PCR amplification was performed with the following conditions: 1 cycle of 95 °C for 3 minutes; 25 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds; and then 1 cycle of 72 °C for 5 minutes. The PCR products were detected with 2% agarose gel electrophoresis, purified using a Gel Extraction Kit (QIAquick, Qiagen, Germany), and sequenced using a MiSeq® Reagent Kit V3 (Illumina, USA). At last, 300 bp paired-end reads were generated on the Illumina NovaSeq platform. Data were analyzed using Basespace sequencing hub app software (16S Metagenomics, Version 1.1.1).

4.3 Meat quality and colour stability

4.3.1 Purge loss

The percentage change in sample weight after chilled storage was calculated as purge loss (%). The final sample weights were recorded once excess moisture had been blotted away using paper towel.

4.3.2 Drip loss

Sample cores of 2.5 cm (diameter) were prepared and placed into EZ-Driploss tubes (Danish Meat Research Institute, DEN). The percentage change in sample core weight after 72 hours of refrigeration at 3-4 °C was calculated as drip loss (%) (Holman et al., 2020).

4.3.3 Cooking loss and shear force

Vacuum packaged and frozen sample blocks of 62.9 ± 7.1 g (mean ± standard deviation) were cooked in batches of 16 (within repeat and balanced by storage period and packaging type) to an internal temperature of 71 °C (35 minutes) using a water bath (TWBC-24-TU3, Thermoline Scientific, AUS) (Hopkins and Thompson, 2001). Temperatures were verified using an infrared HACCP thermometer with probe attachment (model 8838, AZ Instruments Corp., TAI). The cooking process was halted as samples were submerged and held in an ice slurry for 30 minutes. The percentage change in sample block weight after cooking was calculated as cooking loss (%). The final cooked sample weights were recorded once excess moisture blotted away using paper towel. Cooked samples were placed into resealable plastic bags and refrigerated at 3-4 °C until their temperatures had stabilised.

Cuboidal strips of 1 cm^2 (cross-sectional area) were removed from each sample and analysed using a texture analyser (XT-Plus 100C, Stable Micro Systems, UK) fitted with a 50 kg load cell and a Warner-Bratzler Vee-shaped blade set to a 20 cm/minute crosshead speed. The cutting line was perpendicular to the muscle fibre orientation and positioned so as to avoid fatty deposits and connective tissue. The average peak shear force (Newtons) of 6 technical replicates was recorded (Holman et al., 2015a).

4.3.4 Chemical proximate and intramuscular fat content

Samples of ~ 25 g were lyophilised at -50 °C using a freeze-drying unit (FD-10N-60A, Faithful Instruments, PRC). The percentage change in sample weight after lyophilisation was calculated as the dry matter (%) and total moisture (%). The final weights were recorded once sample weight had stabilised (± 0.02 mg when measured after a 24 hour interval). The intramuscular fat (IMF) and crude protein concentration of ground lyophilised samples were determined using the near infrared (NIR) method of Bailes et al. (2022) and a multipurpose NIR analyser (MPA II, Bruker, USA). IMF data were calculated to % sample (as-is).

4.3.5 Protein solubility

Using a method modified from Farouk and Swan (1998), duplicate minced samples of ~ 100 mg were combined with 1 mL of either total (25 mM KH_2PO_4, pH 7.2) or sarcoplasmic (1.1 M KI, 0.1 M KH_2PO_4, pH 7.2) protein buffer. These were homogenised using microtube pestles and refrigerated overnight at 3-4 °C. Samples were centrifuged for 15 minutes at 2,400 × *g* and the supernatant analysed using the colorimetric protocol of the Bicinchoninic Acid Kit for Protein Determination (BCA1, Sigma-Aldrich, AUS). Absorbance was measured at 560 nm using a benchtop spectrophotometer (Multiskan Skyhigh, ThermoFisher Scientific, AUS). The concentration of total and sarcoplasmic protein solubility were calculated as mg per g of sample (as-is). Myofibril protein solubility was calculated by subtracting sarcoplasmic protein from corresponding total protein solubility value.

4.3.6 Total myoglobin

Total myoglobin content was determined using an isobestic point assay (AMSA, 2012). Samples of ~ 2 g were homogenised with 10 mL of buffer (40 mM potassium phosphate, pH 6.8) and centrifuged for 30 minutes. The supernatant was removed and analysed a benchtop spectrophotometer (Multiskan Skyhigh, ThermoFisher Scientific, AUS) set to record absorbance at 525 nm. Total myoglobin concentrations were then calculated as mg per g of sample (as-is) (Faustman and Phillips, 2001).

4.3.7 Colour stability

Sample slices of 3-4 cm (depth) were placed onto individual black Styrofoam trays, arranged so that the muscle fibres had a perpendicular orientation to the exposed surface. These were overwrapped with clear PVC meat wrap film (thickness: 12 µm), allowed to bloom for ~ 45 minutes at room temperature, and measured in duplicate using a calibrated spectrophotometer (model 45/0, HunterLab Associates Laboratory Inc., PRC). Illuminant and standard observer settings of D65 and 10°, respectively, were used as well as a 25 mm aperture size (Holman et al., 2015b). Following this first measurement (Day 0), a further 3 measurements were made at daily intervals (Day 1-3), between which the samples were held refrigerated at 2.2 ± 2.6 °C (mean ± standard deviation) and under continuous lighting. The colour coordinates (*L**, *a** and *b**) were recorded (CIE, 1977) and used to calculate hue and chroma values (AMSA, 2012). The ratio of 630 to 580 nm reflectance values were calculated *post hoc* using the spectral data. Surface proportions for

the myoglobin redox forms of metmyoglobin (MMB), deoxymyoglobin (DMB), and oxymyoglobin (OMB) were calculated post hoc using the spectral data (Krzywicki, 1979).

4.4 Fatty acids and oxidation biomarkers

4.4.1 Fatty acids

Fatty acid concentrations were determined at a commercial laboratory (Feed Quality Service Laboratory, AUS) as per Clayton et al. (2024) and using a method modified from Clayton et al. (2012). First, lyophilised samples of 10 mg were combined with 2 mL of methanol and toluene (4:1 v/v) that contained 10 µg of C13:0 internal standard. Fatty acids were methylated with the addition of acetyl chloride and 60 minutes of incubation at 100 °C. The separation of layers and partitioning of fatty acid methyl esters (FAME) into the toluene solvent was achieved when 6 mL of a 6% potassium carbonate solution was added. Samples were centrifuged at 1,500 × *g* for 10 minutes before the upper toluene supernatant was transferred into a 2 mL glass vial with a crew-cap lid.

The FAME were quantified using an Agilent 6890A gas chromatograph (GC) fitted with a BPX70 capillary column (120 m column length × 0.25 mm internal diameter × 0.25 µm film thickness, Trajan Scientific, AUS) and a flame detector (FID). Helium was used as the carrier gas with a split ratio of 10:1, total flow rate of 23.3 mL per minute, and a constant column pressure of 420 kPa (estimated flow rate = 1.9 per minute). The inlet temperature was 250 °C with an injection volume of 2.0 µL into a focus inlet liner (4 mm internal diameter, no. 092,002, Trajan Scientific, AUS). The following oven temperature program was employed; initial temperature = 130 °C for 1 minute, 5 °C per minute to 180 °C then held for 30 minutes; 5 °C per minute to 170 °C then held for 22 minutes; 1 °C per minute to 200 °C then held for 30 minutes; and 40 °C per minute to 260 °C then held for 10 minutes to give a total run time of 136.5 minutes. The FID temperature was 280 °C with the following gas flow rates: hydrogen = 35 mL per minute, instrument air = 350 mL per minute, nitrogen make-up gas = 25 mL per minute. FAME were identified in comparison with the retention times of commercial standards and published data (Clayton et al., 2012, Or-Rashid et al., 2010). Concentrations were calculated against a 3-point standard curve. Cis- and trans-double bond geometries and conjugated linoleic acids (CLA) are described. Fatty acid data were calculated to mg per 100 g of sample (as-is).

4.4.2 Alpha-tocopherol

Alpha-tocopherol concentrations were determined at a commercial laboratory (Feed Quality Services Laboratory, Wagga Wagga, AUS) using the method of McMurray et al. (2020). Lyophilised samples of ~ 1 g were combined with 1 mL of 60% KOH and 10 mL of 6% pyrogallol. These were incubated at 70 °C in a dry heating block for 30 mins. Once cooled, samples were combined (vortexed) with 5 mL of water and 20 mL of hexane – to facilitate extraction. A 5 mL aliquot of the hexane layer was taken from each sample. These was evaporated under nitrogen gas, reconstituted in 0.5 mL of methanol, and then analysed using an Agilent high performance liquid chromatography (1260) fitted with a Zorbax SB-C18 column (150 mm length × 3.0 mm internal diameter × 3.5 µm film thickness). Data were compared against a standard curve using fluorescence detection and a benchtop spectrophotometer set to measure emission at 330 nm and excitation at 296 nm. Data were transformed and reported as mg alpha-tocopherol per kg of sample (as-is).

4.4.3 Thiobarbituric reactive substances

Samples of ~ 100 mg were combined with Samples (~ 100 mg) were combined with 250 µL of RIPA buffer (no. 10010263, RIPA Buffer Concentrate, Cayman Chemicals, USA) and homogenised (Zhang et al., 2019). These were centrifuged at 2,100 × *g* and the supernatant analysed using the colorimetric protocol of the TBARS (TCA Method) Assay Kit (no. 700870, Cayman Chemicals, USA). Absorbance was measured at 530 nm using a benchtop spectrophotometer (Multiskan Skyhigh, ThermoFisher Scientific, AUS). The concentration of thiobarbituric reactive substances (TBARS) was calculated as mg malondialdehyde (MDA) per kg of sample (as-is).

4.4.4 Protein carbonyls

Samples of ~ 100 mg were homogenised with 250 µL RIPA buffer (no. 10010263, RIPA Buffer Concentrate, Cayman Chemicals, USA), centrifuged at 6,708 × *g* for 10 min, and their supernatant analysed using the colorimetric protocol of the Protein Carbonyl Assay Kit (MAK094, Merck, USA). For this analysis, absorbance was measured at 375 nm using a benchtop spectrophotometer (Multiskan Skyhigh, ThermoFisher Scientific, AUS). The supernatant was also analysed using the colorimetric protocol of the Bicinchoninic Acid Kit for Protein Determination (BCA1, Sigma-Aldrich,

AUS) as well as the sample benchtop spectrometer, set to measure absorbance at 540 nm. These data allowed carbonyl content to be calculated as nmol per mg of protein.

4.4.5 Ferric reducing antioxidant potential

Samples of ~ 100 mg were combined with 200 µL of RIPA buffer (no. 10010263, RIPA Buffer Concentrate, Cayman Chemicals, USA) and homogenised using microtube pestles Samples were then centrifuged at 6,708 × *g* for 10 min and their supernatant analysed using the colorimetric protocol of the Ferric Reducing Antioxidant Power (FRAP) Assay Kit (MAK509, Sigma-Aldrich, AUS). A benchtop spectrophotometer (Multiskan Skyhigh, ThermoFisher Scientific, AUS) was used to measure absorbance at 590 nm. FRAP was calculated as µM Fe^{2+} reduction potential per mg of sample (Fe^{2+} eq., µM/mg).

4.5 Raman spectroscopy

Only the samples held under PACK 1, PACK 2, and PACK 4 were measured using a hand-held Raman spectroscopy device (Mira, Metrohm, AUS) using a 785.0 ± 0.5 nm laser, 8-10 cm^{-1} spectral resolution, and fitted with a universal attachment. Measurements were made through the packaging material of each unopened sample (in-pack) at the end of the storage period. Triplicate measures were made for each sample, and on different sites, with an integration time of 3 seconds and 5 accumulations (Logan et al., 2022).

4.6 Statistical analysis

Data were analysed in GenStat (23rd Edition, VSN International, www.vsni.co.uk) after having first visualised data for errors and outliers and tested data for skewness and normality. The exception being spectra data which were analysed in *R* (R Core Team, 2023).

4.6.1 Microorganisms and freshness biomarkers

TVC data were first log-transformed for normality. Metagenomic data were used to calculate the diversity indices, including CHAOS1, Goods coverage, Log series alpha, Shannon, Simpson (1-D), and operational taxonomic units (OTU). Data were analysed using multiple linear mixed models fitted with the fixed effects of storage period, packaging type, and their interaction, and the random effect of repeat. Means were compared using Bonferroni's multiple comparison test and a level of significance level set at $P < 0.05$.

To minimise the false discovery rate metagenomic data were filtered to only include microorganisms with > 100 detections. Frequency analysis was then used to rank and summarise the relative abundance of microorganisms by storage period and packaging type.

4.6.2 Meat quality and colour stability

Unless stated, meat quality data were analysed using multiple linear mixed models fitted with the fixed effects of storage period, packaging type, and their interaction, and the random effect of repeat. Cooking batch was included as an additional random term for the analysis of cooking loss and shear force data. Retail display was included as an additional fixed term for the analysis of colour stability and myoglobin redox form data, as well as the effects of its first and second order interactions with storage period and packaging type. Means were compared using Bonferroni's multiple comparison test and a level of significance level set at $P < 0.05$.

4.6.3 Fatty acids and oxidation biomarkers

Atherogenic and thrombogenic indices were calculated as per Ulbricht and Southgate (1991). The peroxidability index values were calculated as per Arakawa and Sagai (1986). The ratios of omega-6 to omega-3 fatty acids (n-6:n-3), linoleic acid to alpha-linolenic acid (LA:ALA), arachidonic acid to eicosapentaenoic acid (ARA:EPA), polyunsaturated to saturated fatty acids (PUFA:SFA), and the sum of eicosapentaenoic and docosahexaenoic acids (EPA+DHA) were calculated. Fatty acids, fatty acid indices, and oxidation biomarker data were analysed using multiple linear mixed models fitted with the fixed effects of storage period, packaging type, and their interaction, and the random effect of repeat. Means were compared using Bonferroni's multiple comparison test and a level of significance set at $P < 0.05$.

4.6.4 Spectroscopic prediction of in-pack microorganism populations

Spectra were averaged prior to background correction using baseline with an iterative restricted least square method. Partial least square regression models were then used to predict the absolute TVC value. The optimal number of latent variables selected for the model was based on the minimal root mean squared error of prediction (RMSEP). The capacity for spectra to classify samples based on TVC being high (TVC > log 5 CFU/g) or low (TVC < log 5 CFU/g) was also investigated using a one class partial least square discriminate analysis (PLS-DA) model. Again, for this model the optimal number of latent variables was based on the minimal RMSEP. No samples had TVC > 7 CFU/g, therefore log 5 CFU/g was applied as the threshold value for samples

at high risk of spoilage. All models were cross validated using leave on out cross validation methods.

5.0 Project outcomes

5.1 Microbiology and freshness biomarkers

There were no significant packaging type by storage period effects on spoilage and microbial parameters ($P > 0.05$).

TVC levels were lower for PACK 1 than was found for PACK 2 and PACK 4; and lower for PACK 3 than was found for PACK 2 ($P < 0.001$; Table 2). The ultimate pH level was higher for PACK 1 and PACK 4 than was found for PACK 3 ($P = 0.010$; Table 2).

Table 2. Predicted means and standard error (SEM) for the packaging type effects on lamb *longissimus lumborum* muscle spoilage and microbial parameters.[1]

Spoilage parameter	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
Microbial diversity indices						
CHAOS1	715.0	711.6	745.5	726.6	30.3	0.691
Goods coverage, %	99.89	99.97	99.96	99.98	0.05	0.248
Log Series alpha	89.2	90.1	95.0	94.1	3.8	0.321
OTU	415.0	419.0	445.6	427.8	18.5	0.372
Shannon	2.38	2.45	2.46	2.47	0.06	0.378
Simpson (1-D)	0.82	0.84	0.83	0.84	0.01	0.179
Total readings, ×1,000,000	319.2	322.8	355.4	317.0	21.5	0.255
Total viable count, log CFU/g	4.39^{a}	4.85^{c}	4.41^{ab}	4.72^{bc}	0.12	< 0.001
Total volatile basic nitrogen, mg/100g	6.07	6.05	5.92	5.95	0.20	0.835
Ultimate pH	5.83^{b}	5.81^{ab}	5.71^{a}	5.82^{b}	0.04	0.010

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include operational taxonomic units (OTU) and colony forming units (CFU).

The CHAOS1 and OTU values were higher for Weeks 0-2 than was found for Weeks 18-20 ($P < 0.001$; Table 3). Log series alpha values were higher for Weeks 0-2 than was found for Week 18; and higher for Weeks 0-1 than was found for Week 20 ($P < 0.001$; Table 3). Shannon values were higher for Weeks 0-6 than were found for Week 18; and higher for Weeks 0-2 than were found for Weeks 10-20 ($P < 0.001$; Table 3). Simpson (1-D) values were higher for Week 1 than was found for Weeks 10-20; higher for Week 0 than was found for Week 10 and Weeks 18-20; and higher for Week 2 than was found for Week 10 ($P < 0.001$; Table 3). The total number of reading value was higher for Weeks 10-14 than was found for Week 6 ($P = 0.002$; Table 3). TVC levels were lower for Weeks 0-2 than was found for all other storage periods; lower for Week 6 than was found for Weeks 10-20; and lower for Week 10 than was found for Week 20 ($P < 0.001$; Table 3). TVB-N levels were higher for Week 14 and Week 20 than was found for Weeks 1-2; and higher Week 0 than was found for Week 1 ($P < 0.001$; Table 3).

Table 3. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle spoilage and microbial parameters.[1]

Spoilage parameter	Storage period, weeks								SEM	*P*-value
	0	1	2	6	10	14	18	20		
Microbial diversity indices										
CHAOS1	791.4^{b}	797.1^{b}	789.1^{b}	960.9^{ab}	725.7^{ab}	736.8^{ab}	625.1^{a}	641.2^{a}	42.9	< 0.001
Goods coverage, %	99.92	99.95	99.95	99.95	99.95	99.99	99.99	99.86	0.08	0.618
Log Series alpha	100.8^{c}	102.3^{c}	100.4^{bc}	87.8^{abc}	91.3^{abc}	87.1^{abc}	83.6^{a}	83.6^{ab}	5.3	< 0.001
OTU	472.1^{b}	471.8^{b}	467.4^{b}	408.7^{ab}	431.5^{ab}	426.6^{ab}	360.1^{a}	376.4^{a}	26.1	< 0.001
Shannon	2.62^{c}	2.71^{c}	2.61^{c}	2.48^{bc}	2.29^{ab}	2.33^{ab}	2.20^{a}	2.30^{ab}	0.09	< 0.001
Simpson (1-D)	0.86^{cd}	0.89^{d}	0.86^{bcd}	0.83^{abcd}	0.79^{a}	0.81^{abc}	0.80^{ab}	0.80^{ab}	0.02	< 0.001
Total readings, ×1,000,000	331.3^{ab}	306.9^{ab}	318.8^{ab}	278.9^{a}	392.8^{b}	382.4^{b}	298.8^{ab}	319.0^{ab}	30.4	0.002
Total viable count, log CFU/g	3.06^{a}	3.13^{a}	3.15^{a}	4.39^{b}	5.40^{c}	5.74^{cd}	5.72^{cd}	6.14^{d}	0.17	< 0.001
Total volatile basic nitrogen, mg/100g	6.24^{bc}	5.26^{a}	5.52^{ab}	6.01^{abc}	6.02^{abc}	6.53^{c}	5.77^{abc}	6.63^{c}	0.29	< 0.001
Ultimate pH	5.70	5.80	5.73	5.73	5.70	5.81	5.74	5.83	0.05	0.126

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include operational taxonomic units (OTU) and colony forming units (CFU).

The 5 most prominent *phylum* were found to account for ~ 98% of the total relative abundance of microorganisms. These phyla were selected to evaluate storage period effects on microorganism community dynamics (Figure 2). The relative abundance of *Actinobacteria* was highest for Week 1 (2.0%). *Bacteroidetes* were found to be the most prominent phylum for Week 0 (37.6%), Week 1 (31.9%), and Week 2 (37.2%), with the relative abundance of this phylum observed to decrease when storage period was > 6 weeks. *Firmicutes* were the most prominent *phylum* for Week 6 (34.9%) and Week 20 (42.2%), with the relative abundance of this *phylum* generally observed to increase as storage period increased. Fusobacteria was found to have a relative abundance > 0.1% for all of the storage periods, except for Week 1 (6.6%) and Week 6 (5.9%). *Proteobacteria* were the most prolific *phylum* for Week 10 (36.2%), Week 14 (41.2%), and Week 18 (50.3%).

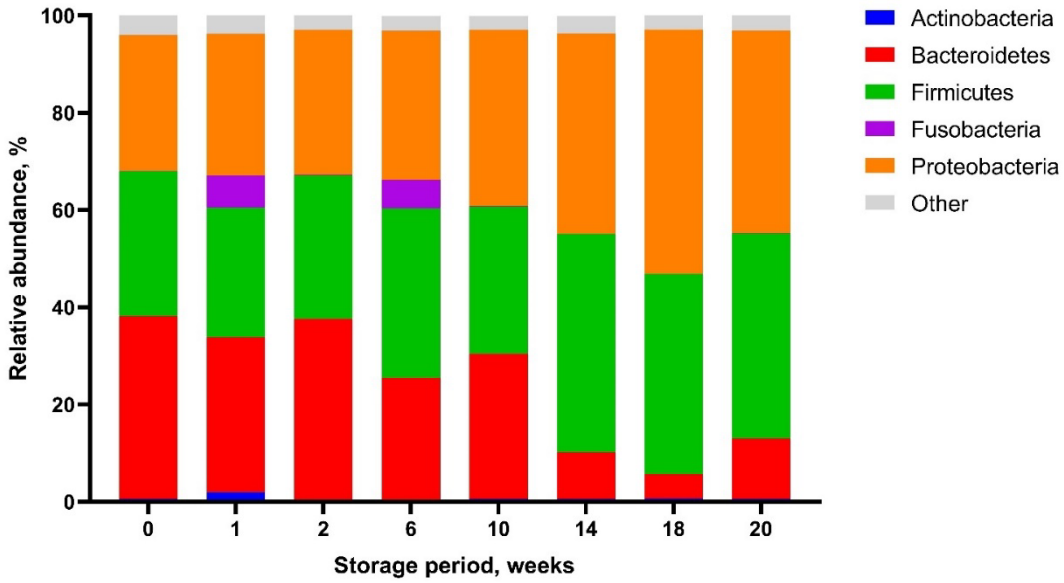

Figure 2. The relative abundance of the most prominent *phylum* detected for the lamb *longissimus lumborum* muscles, when categorised by storage period.

The 18 most prominent families were found to account for 90-98% of the total relative abundance of microorganisms. These families were selected to evaluate storage period effects on microorganism community dynamics (Figure 3). *Peptaoniphilaceae* (13.0-29.2%) was found to be the most prolific family of microorganisms for each of the storage periods, being observed to be lowest for Week 6 (13.0%). The relative abundance of *Aeromonadeceae* was < 1% for Weeks 0-2 and Week 18 and observed to be highest for Week 6 (4.0%) and Week 10 (3.7%). *Bacteriodaceae* was most prominent in Week 0 (9.9%), Week 1 (8.1%), and Week 2 (7.7%), with the relative abundance of this family < 1% for Weeks 6-20. *Campylobacteraceae* was most prominent for Week 0 (3.0%), with the relative abundance of this family observed to be < 1% for Weeks 6-20. *Carobacteriaceae* was most prominent for Weeks 6-20 (10.9-16.1%), with the relative abundance of this family observed to be < 1% for Weeks 0-2. *Clostridiaceae* was most prominent for Weeks 10-18 (5.2-6.9%). The relative abundance of Enterobacteriaceae (5.2-8.8%), *Enterococcaceae* (1.9-5.1%), *Moraxellaceae* (1.1-2.7%), *Peptostreptococcaceae* (3.5-8.6%), and *Planococcaceae* (2.5-9.7%) were observed to be somewhat consistent for all of the storage periods. *Lactobacillaceae* was most prominent for Week 20 (2.4%), followed sequentially by Week 18 (1.2%), and all the other storage periods (< 1%). The relative abundance of *Porphyromonadaceae* was found to be most prominent for Week 10 (11.6%) and least prominent for Weeks 14-20 (1.4-4.3%). Pseudomonadaceae was observed to increase with storage period, being least prominent for Weeks 0-2 (2.3-6.2%) and most prominent for Weeks 18-20 (10.3-10.9%). *Streptococcaceae* was most prominent for Week 0 (10.8%), Week 1 (7.8%), and Week 2 (9.0%), with the relative abundance of this family observed to be < 1% for Weeks 6-20. The relative abundance of *Xanthomonadaceae* was least for Weeks 18-20 (0.8-1.5%). The relative abundance of other families of microorganisms, not included in the top 18, was highest for Week 18 (15.2%), followed sequentially by Week 1 (12.6%), Week 6 (10.1%), Weeks 10-14 (7.5-8.1%), Week 0 and Week 2 (6.0-6.6%), and Week 20 (1.2%).

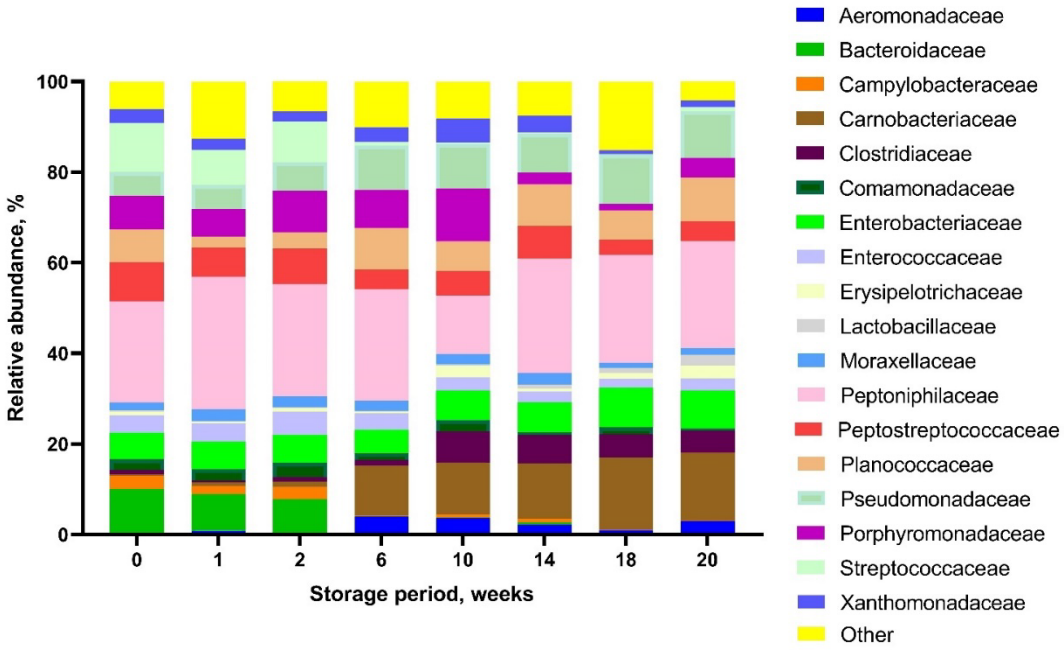

Figure 3. The relative abundance of the most prominent *family* detected for the lamb *longissimus lumborum* muscles, when categorised by storage period.

The 5 most prominent phylum were found to account for ~ 98% of the total relative abundance of microorganisms. These phyla were selected to evaluate packaging type effects on microorganism community dynamics (Figure 4). The relative abundance of Proteobacteria (30.6-36.1%), Firmicutes (31.3-39.1%), and Bacteroidetes 20.9-30.7%) were observed to be somewhat consistent for all the packaging types. Actinobacteria was most prominent for PACK 2 (1.4%) as other packaging types had a relative abundance of < 1%. Fusobacteria was most prominent for PACK 2 (3.0%) and PACK 4 (3.5%), with PACK1 and PACK 3 having a relative abundance of < 1%.

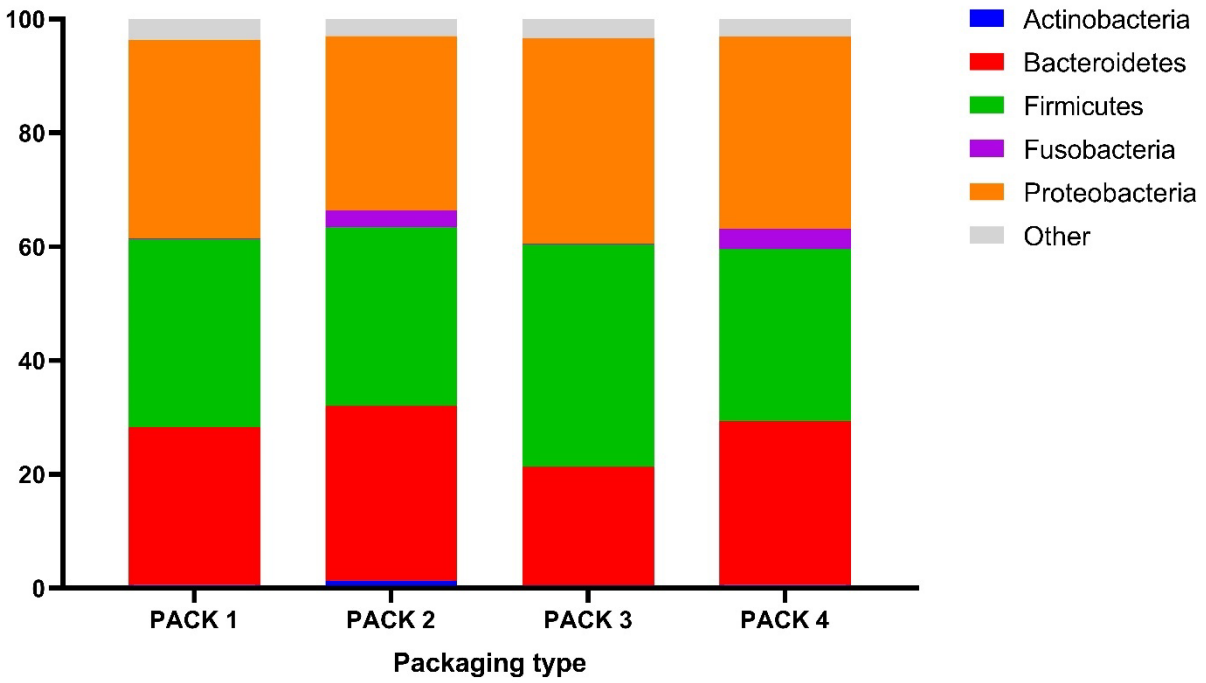

Figure 4. The relative abundance of the most prominent *phylum* detected for the lamb *longissimus lumborum* muscles, when categorised by packaging type.

The 18 most prominent families were found to account for ~ 90% of the total relative abundance of microorganisms. These families were selected to evaluate packaging type effects on microorganism community dynamics (Figure 5). *Carnobacteriaceae* was most prominent for PACK 2 (9.5%) compared to the other packaging types (7.5-7.7%). *Peptoniphilaceae* (25.1%) and *Bacteriodaceae* (8.3%) were most abundant for PACK 4 compared to the other packaging types (20.5-23.0% and 3.5-6.7%, respectively). The relative abundance of Enterobacteriaceae (5.8-6.6%), *Lactobacillaceae* (0.7-0.9%), and *Clostridiaceae* (1.1-3.2%) were observed to be somewhat consistent for all the packaging types. Aeromonadaceae had a relative abundance of < 1% for PACK 3, whereas the other packaging types had a relative abundance of 1.8-2.5%.

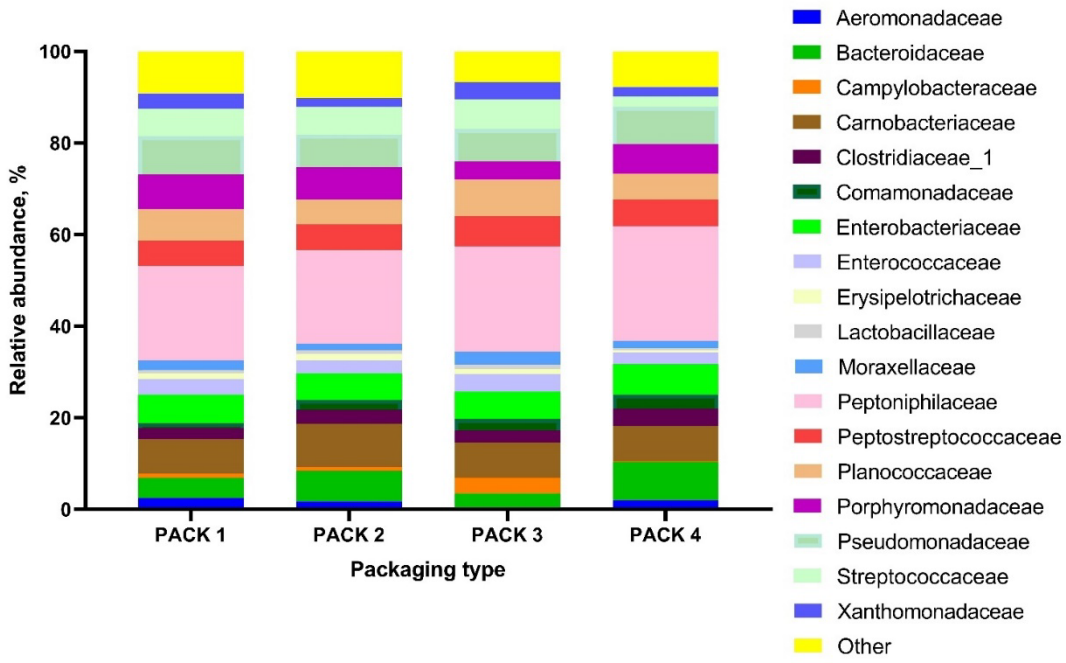

Figure 5. The relative abundance of the most prominent *family* detected for the lamb *longissimus lumborum* muscles, when categorised by packaging type.

5.2 Meat quality and colour stability

There was a significant packaging type by storage period effect on cooking loss ($P = 0.020$; Figure 6). Cooking loss levels were higher for PACK 2 at Weeks 0-1 and PACK 4 at Week 1 than was found for PACK 1 at Week 10 and Weeks 18-20; PACK 2 and PACK 4 at Week 20; and PACK 3 at Week 10 and Weeks 18-20 ($P < 0.05$). The cooking loss level was higher for PACK 4 at Week 6 than was found for PACK 1 at Week 18 and PACK 2 and PACK 4 at Week 20 ($P < 0.05$). The cooking loss level was lower for PACK 2 at Week 20 than was found for PACK 1 at Weeks 0-1; PACK 2 at Weeks 0-6; PACK 3 at Weeks 0-2; and PACK 4 at Weeks 0-10 ($P < 0.05$). The cooking loss level was lower for PACK 4 at Week 20 than was found for all other packaging type by storage period combinations, except for PACK 1 and PACK 3 at Week 10 and Weeks 18-20; and PACK 2 at Weeks 18-20 ($P < 0.05$).

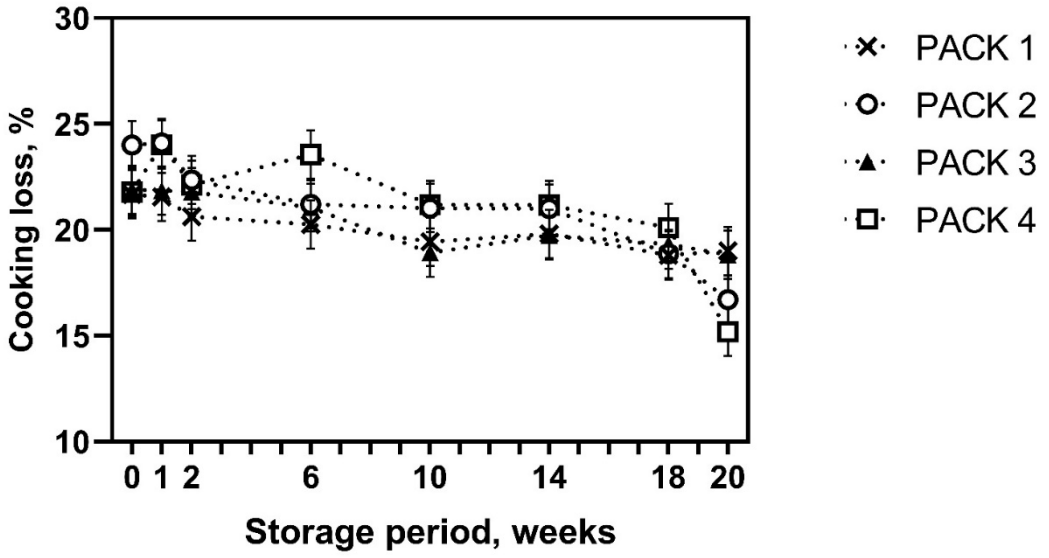

Figure 6. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* cooking loss (%). Means and standard error bars are plotted.

There was a significant packaging type by storage period effect on purge loss ($P < 0.001$; Figure 7). The purge loss level was higher for PACK 3 at Week 20 and PACK 4 at Week 18 than was found for PACK 1 at Weeks 0-18; PACK 2 and PACK 3 at Weeks 0-14; and PACK 4 at Weeks 0-2 ($P < 0.05$). The purge loss level was higher for PACK 4 at Week 10 than was found for PACK 1 at Weeks 0-14; PACK 2 at Weeks 0-2 and Week 14; PACK 3 at Weeks 1-2; and PACK 4 at Weeks 0-2 (P < 0.05). The purge loss level was higher for PACK 4 at Week 10 and Week 14 than was found for PACK 1 at Weeks 0-14; PACK 2 at Weeks 0-2; PACK 3 at Weeks 1-2; and PACK 4 at Weeks 0-1 (P < 0.05). The purge loss level was lower for PACK 1 at Weeks 0-1 than was found for PACK 1 at Week 20; PACK 2 at Weeks 18-20; PACK 3 at Weeks 18-20; and PACK 4 at Weeks 6-20 (P < 0.05).

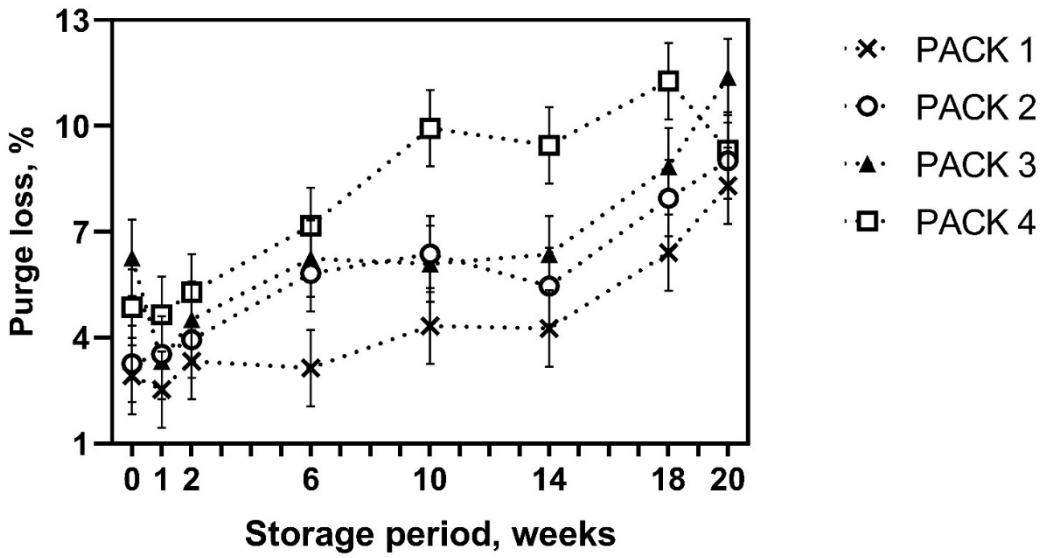

Figure 7. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* purge loss (%). Means and standard error bars are plotted.

The crude protein values was higher for PACK 3 than was found for PACK 2 (P = 0.008; Table 4). The dry matter values was higher for PACK 3 than was found for PACK 2 and PACK 4 (P = 0.002; Table 4). The total moisture values was higher for PACK 2 and PACK 4 than was found for PACK 3 (P = 0.002; Table 4). The drip loss values was higher for PACK 2 than was found for PACK 3 (P = 0.005; Table 4). The shear force values was higher for PACK 3 and PACK 4 than was found for PACK 1 (P = 0.001; Table 4). The total myoglobin values was higher for PACK 1 than was found for PACK 3 (P = 0.005; Table 4).

Table 4. Predicted means and standard error (SEM) for the packaging type effects on lamb *longissimus lumborum* muscle quality parameters.[1]

Quality parameter	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
Cooking loss, %	20.1^{a}	21.2^{b}	20.4^{ab}	21.2^{b}	0.4	< 0.001
Chemical proximate						
Crude protein, %	21.8^{ab}	21.7^{a}	22.1^{b}	21.7^{ab}	0.1	0.008
Dry matter, %	27.7^{ab}	27.2^{a}	28.1^{b}	27.4^{a}	0.3	0.002
Intramuscular fat, %	4.84	4.57	4.79	4.72	0.22	0.625
Total moisture, %	72.3^{ab}	72.8^{b}	71.9^{a}	72.6^{b}	0.3	0.002
Drip loss, %	0.93^{ab}	0.98^{b}	0.71^{a}	0.78^{ab}	0.09	0.005
Protein solubility						
Myofibrillar, mg/g	128.2	130.1	132.4	127.0	4.2	0.604
Sarcoplasmic, mg/g	73.9	71.4	72.1	70.8	1.4	0.120
Total, mg/g	202.1	201.5	204.5	197.8	4.3	0.468
Purge loss, %	4.41^{a}	5.67^{b}	6.63^{b}	7.75^{c}	0.38	< 0.001
Shear force, N	32.6^{a}	34.3^{ab}	38.1^{b}	38.9^{b}	1.9	0.001
Total myoglobin, mg/g	5.36^{b}	5.13^{ab}	4.96^{a}	5.02^{a}	0.12	0.005

[1]Means within rows with different superscripts were significantly different (P < 0.05).

Drip loss values were higher for Weeks 0-2 and Week 20 than was found for Weeks 14-18; and higher for Weeks 6-10 than was found for Week 14 ($P < 0.001$; Table 5). The myofibrillar protein solubility values was higher for Weeks 6-10 than was found for Week 0 ($P = 0.004$; Table 5). The total protein solubility values was higher for Week 10 than was found for Week 0 ($P < 0.05$; Table 5). Shear force values were higher for Week 0 than was found for all other storage periods; and higher for Week 6 than was found for Week 14 ($P < 0.001$; Table 5). The total myoglobin values was lower for Week 14 than was found for Weeks 0-2 and Week 10 ($P = 0.046$; Table 5).

Table 5. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle quality parameters.[1]

Quality parameter	Storage period, weeks								SEM	*P*-value
	0	1	2	6	10	14	18	20		
Cooking loss, %	22.3^{d}	22.9^{d}	21.7^{cd}	21.5^{cd}	20.1^{bc}	20.4^{bc}	19.3^{ab}	17.4^{a}	0.6	< 0.001
Chemical proximate										
Crude protein, %	22.0	21.8	22.0	21.8	21.9	22.0	21.6	21.7	0.2	0.386
Dry matter, %	27.5	27.1	27.7	27.6	28.1	27.6	27.9	27.4	0.4	0.178
Intramuscular fat, %	4.77	4.63	4.87	4.91	4.61	4.72	4.87	4.35	0.31	0.633
Total moisture, %	72.5	72.9	72.3	72.4	72.0	72.4	72.1	72.7	0.4	0.178
Drip loss	1.05^{c}	1.00^{c}	1.09^{c}	0.83^{bc}	0.88^{bc}	0.42^{a}	0.50^{ab}	1.02^{c}	0.12	< 0.001
Protein solubility										
Myofibrillar, mg/g	118.4^{a}	120.1^{ab}	126.9^{ab}	138.3^{b}	138.3^{b}	128.7^{ab}	131.2^{ab}	133.3^{ab}	6.0	0.004
Sarcoplasmic, mg/g	73.4	72.9	72.1	72.1	72.8	72.9	70.0	70.3	1.9	0.549
Total, mg/g	191.8^{a}	193.0^{ab}	199.0^{ab}	210.4^{ab}	211.1^{b}	201.6^{ab}	201.3^{ab}	203.5^{ab}	6.0	0.008
Purge loss, %	4.33^{ab}	3.52^{a}	4.27^{ab}	5.6^{bc}	6.69^{c}	6.34^{c}	8.62^{d}	9.50^{d}	0.54	< 0.001
Shear force, N	49.6^{c}	37.3^{ab}	36.5^{ab}	38.1^{b}	32.2^{ab}	29.6^{a}	33.5^{ab}	31.2^{ab}	2.7	< 0.001
Total myoglobin, mg/g	5.02^{a}	4.92^{a}	4.98^{a}	5.25^{ab}	5.04^{a}	5.45^{b}	5.13^{ab}	5.15^{ab}	0.17	0.046

[1]Means within rows with different superscripts were significantly different ($P < 0.05$).

There was a significant storage period by packaging type effect on *L** ($P < 0.001$; Figure 8). The *L** was higher for PACK 3 at Week 20 and PACK 2 at Week 1 than was found for PACK 1 at Week 2; PACK 2 at Weeks 6-10; and for PACK 4 at Week 1 and Week 20 ($P < 0.05$). The *L** was higher for PACK 1 at Week 14 and Week 20 and PACK 4 at Week 18 than was found for PACK 1 at Week 2 and for PACK 4 at Week 20 ($P < 0.05$). The *L** was higher for PACK 1 at Week 18 than was found for PACK 4 at Week 20 ($P < 0.05$).

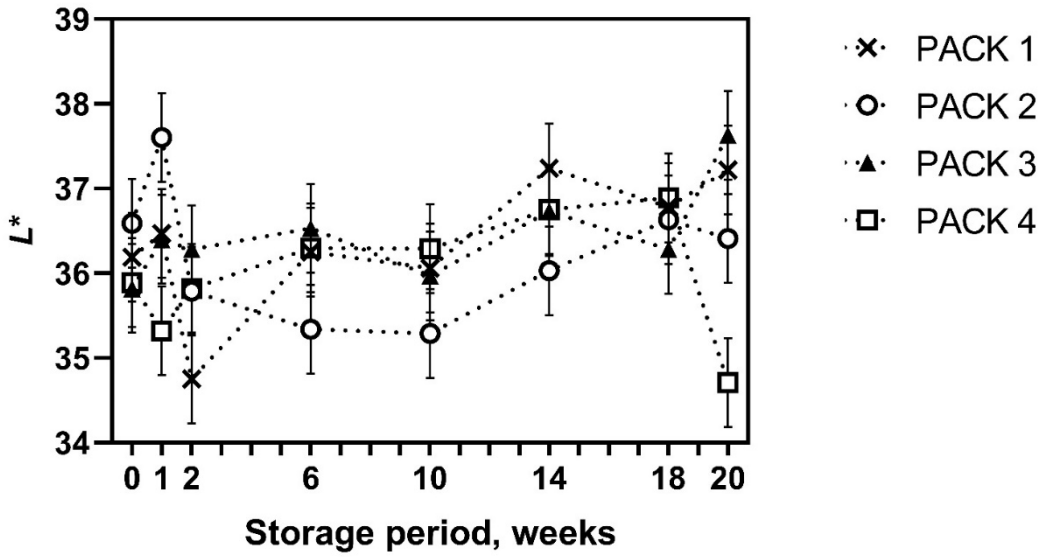

Figure 8. The effect of storage period by packaging type interactions on lamb *longissimus lumborum L** values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on *L** ($P < 0.001$; Figure 9). The *L** was higher for Week 0 at Day 1, Week 14 at Day 0, and Week 1 at Day 1 than was found for Week 2 at Day 3 and for Week 0 and Day 0 ($P < 0.05$). The *L** for Week 0 at Day 0 was lower than was found for all other storage period by display period combinations, except for Week 1 at Day 0 and Day 3; Week 1 at Day 0; Week 2 at Day 0 and Days 2-3; Week 6 at Days 2-3; and for Week 10 at Days 0-1 and Day 3 ($P < 0.05$).

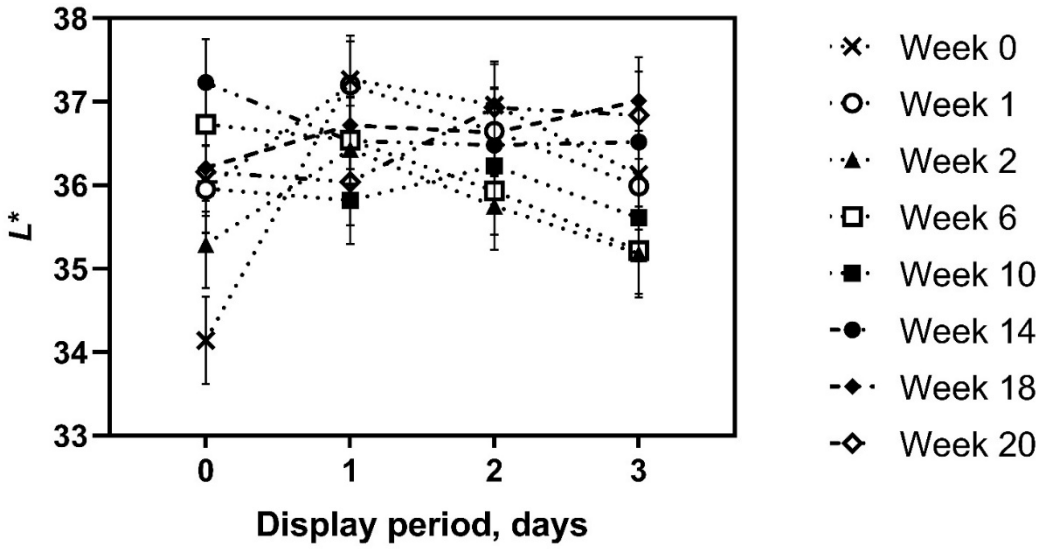

Figure 9. The effect of storage period by display period interactions on lamb *longissimus lumborum L** values. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on *a** ($P < 0.001$; Figure 10). The *a** was higher for PACK 3 at Week 2 than was found for all other packaging type by storage period combinations, except for PACK 1 at Week 2; PACK 2 at Week 2 and Week 6; PACK 3 at Week 1 and Week 6; and for PACK 4 at Weeks 1-6 ($P < 0.05$). The *a** was higher for PACK 3-4 at Week 1, PACK 1 at Week 6, and PACK 4 at Week 2 than was found for PACK 1-4 at Week 0 and Weeks 10-20 ($P < 0.05$). The *a** was higher for PACK 1-2 at Week 0 and for PACK 1 and PACK4 at Week 14 than was found for PACK 1-4 at Week 20 ($P < 0.05$). The *a** was higher for PACK 1 and for PACK 3 at Week 18 than was found for PACK 2-4 at Week 20 ($P < 0.05$). The *a** was higher for PACK 2 and PACK 4 at Week 18 and for PACK 4 at Week 0 than was found for PACK 2-4 at Week 20 ($P < 0.05$). The *a** was lower for PACK 4 at Week 20 for all other packaging type by storage combinations, except for PACK 1-3 at Week 20 ($P < 0.05$).

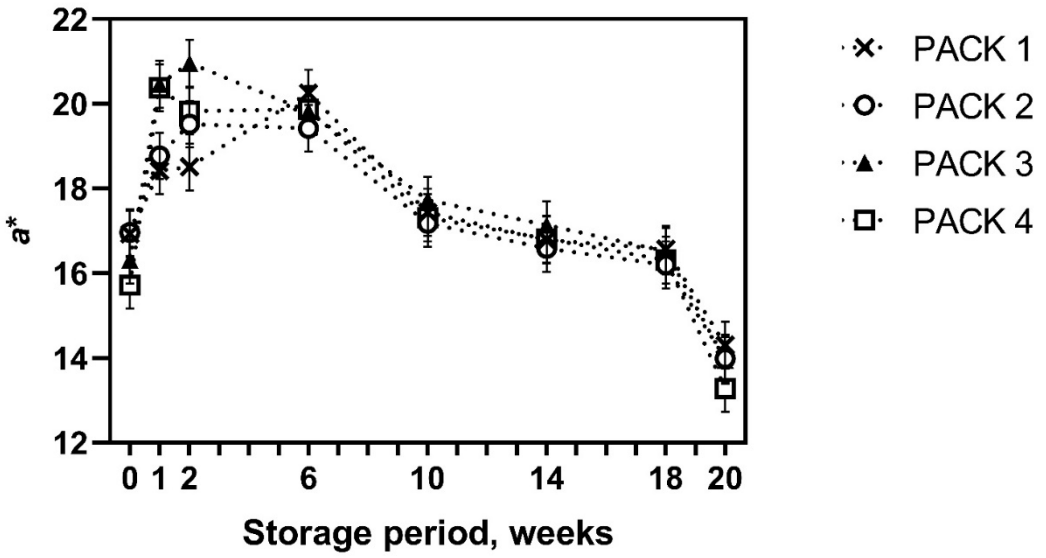

Figure 10. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* *a** values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on *a** ($P < 0.001$; Figure 11). The *a** was higher for Week 2 and Week 6 at Day 1 than was found for all other storage period by display period combinations, except for Week 6 and Weeks 10-18 at Day 0; Week 1 at Day 1; Week 2 and Day 2; and for Week 6 at Day 2 ($P < 0.05$). The *a** was higher for Week 1 at Day 1 than was found for Week 0 at Days 0-3; Week 1 at Day 3; Week 2 at Day 0; Week 10 at Days 2-3; and for Weeks 14-20 at Days 1-3 ($P < 0.05$). The *a** was higher for Week 2 at Day 2 and Week 14 at Day 0 than was found for Week 0 at Days 0-3; Weeks 10-14 at Days 2-3; and for Weeks 18-20 at Days 1-3 ($P < 0.05$). The *a** was higher for Week 2 at Day 2, Week 6 at Day 0 and Day 2, and Week 10 and Week 18 at Day 0 than was found for Week 0 at Day 0-3; Weeks 10-18 at Days 2-3; and for Week 20 at Days 1-3 ($P < 0.05$). The *a** was lower for Week 20 at Day 3 than was found for all other storage period by display period combinations ($P < 0.05$). The *a** was lower for Week 20 at Day 2 than was found for all other storage period by display period combinations, except for Weeks 10-18 at Day 3 ($P < 0.05$).

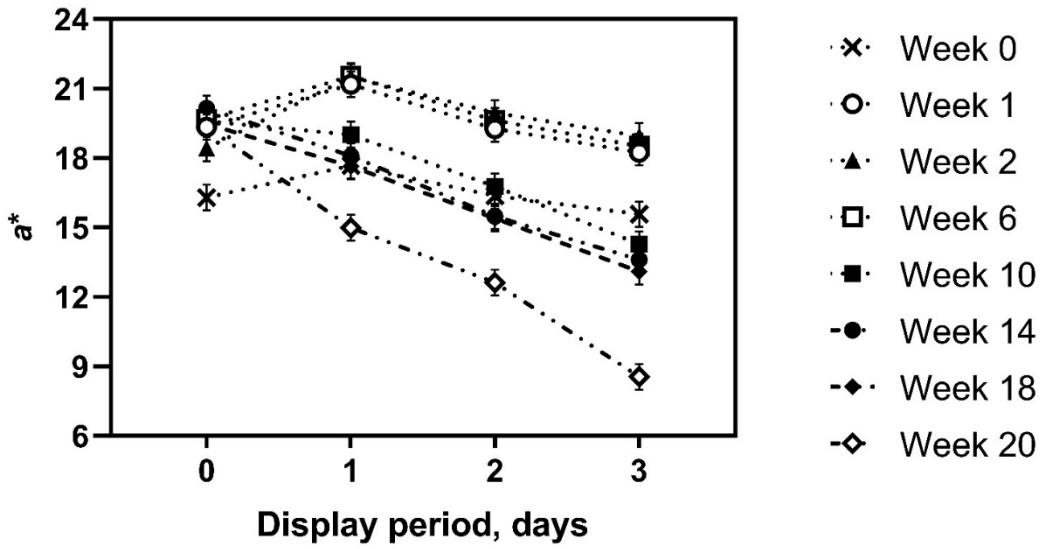

Figure 11. The effect of storage period by display period interactions on lamb *longissimus lumborum* *a** values. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on *b** ($P < 0.001$; Figure 12). The *b** was higher for PACK 4 at Week 1 than was found for PACK 1 at Weeks 0-10 and Week 20; PACK 2 at Week 0 and Weeks 6-20; PACK 3 at Weeks 0-1, Week 6, and Weeks 14-20; and for PACK 4 at Week 0 and Week 20 ($P < 0.05$). The *b** was higher for PACK 1 at Week 18, PACK 2 at Week 1, PACK 3 at Week 10, and PACK 4 at Week 2 than was found for PACK 1 at Weeks 0-2; PACK 2 at Week 0 and Week 10; PACK 3 at Week 0 and Week 20; and for PACK 4 at Week 0 and Week 20 ($P < 0.05$). The *b** was higher for PACK 1 at Weeks 10-14, PACK 2 at Week 2, and PACK 4 at Weeks 6-18 than was found at Week 0 for PACK 1-4; Week 2 for PACK 1; and Week 20 for PACK 2-4 ($P < 0.05$). The *b** was higher for PACK 1 at Week 6, PACK 2 at Week 18, and PACK 3 at Week 14 than was found at Week 0 for PACK 1 and PACK 3-4; Week 2 for PACK 1; and Week 0 and Week 20 for PACK 4 ($P < 0.05$). The *b** was lower for PACK 4 at Week 20 than was found for all storage period by packaging type combinations, except at Week 0 for PACK 1 and PACK 3-4 ($P < 0.05$).

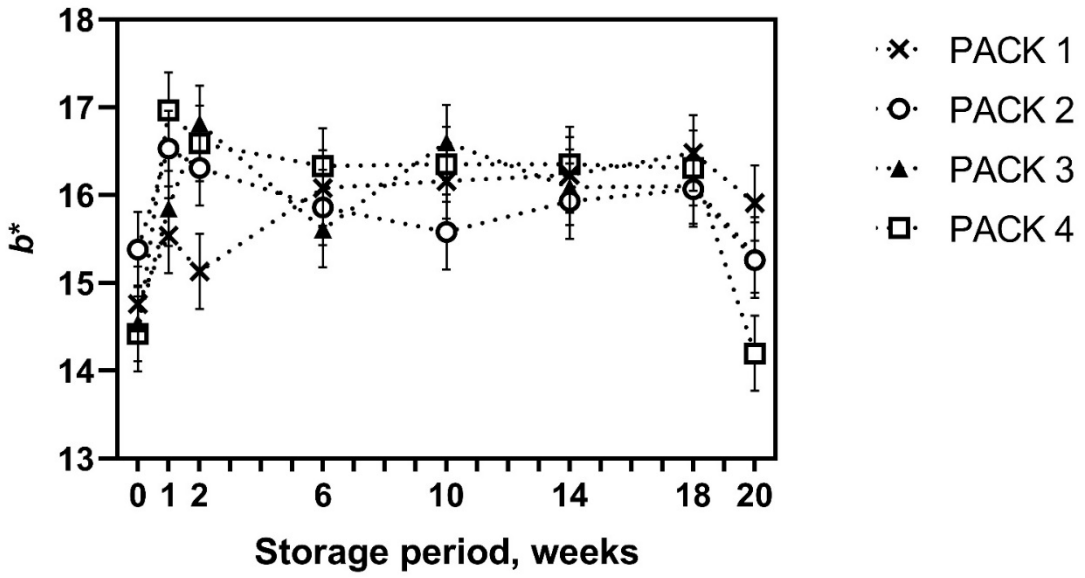

Figure 12. The effect of storage period by packaging type interactions on lamb *longissimus lumborum b** values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on *b** ($P < 0.001$; Figure 13). The *b** was higher for Weeks 1-2 and Week 10 at Day 1 and for Week 2 at Day 2 than was found for Weeks 0-2 and Week 6 at Day 0; Week 0 at Days 2-3; Weeks 10-14 at Day 3; and for Week 20 at Days 2-3 ($P < 0.05$). The *b** was higher for Week 14 at Day 1 than was found for Weeks 0-6 at Day 0; Week 14 at Day 3; and for Week 20 at Days 2-3 ($P < 0.05$). The *b** was higher for Week 14 at Day 0 and Week 18 at Day 1 than was found at Day 0 for Week 0, Week 2 and Week 6; and for Week 20 at Days 2-3 ($P < 0.05$). The *b** was lower than was found for all other storage period by display period combinations, except for Week 20 at Day 3 ($P < 0.05$). The *b** at Week 20 at Day 3 was lower than was found for all other storage period by display period combinations, except for Weeks 0-6 at Day 0; Weeks 10-14 at Day 3; and for Week 20 at Day 2 ($P < 0.05$).

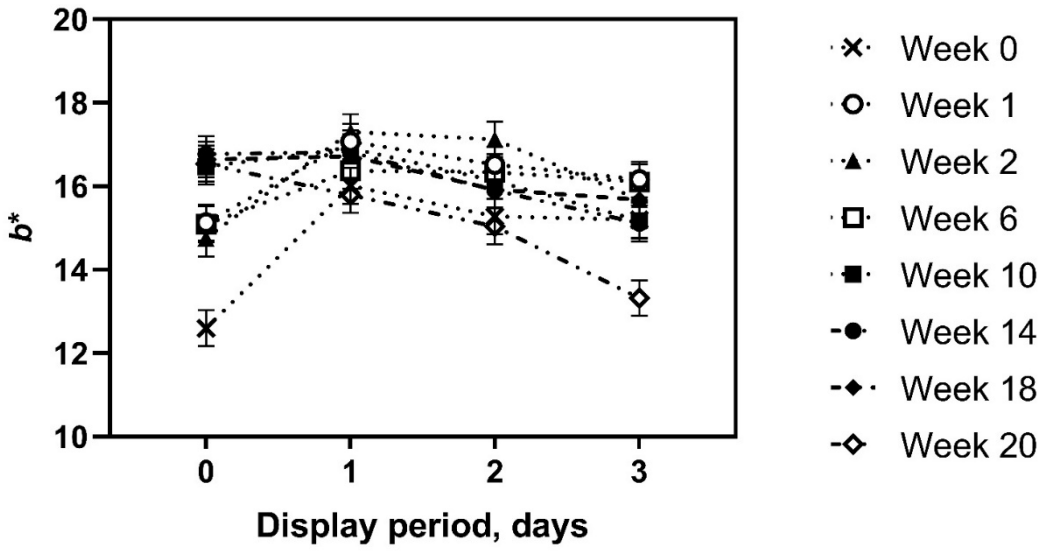

Figure 13. The effect of storage period by display period interactions on lamb *longissimus lumborum* *b** values. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on chroma ($P < 0.001$; Figure 14). The chroma was higher for PACK 3 at Week 2 than was found for PACK 1 at Weeks 0-2 and Weeks 10-20; PACK 2 at Week 0 and Weeks 10-20; PACK 3 at Week 0 and Weeks 14-20; and PACK 4 at Week 0 and Weeks 10-20 ($P < 0.05$). The chroma was higher for PACK 4 at Week 1 than was found for PACK 1 at Week 1, Week 2 and Weeks 10-20; PACK 2 at Week 0 and Weeks 10-20; PACK 3 at Week 0 and Weeks 14-20; and PACK 4 at Week 0 and Weeks 10-20 ($P < 0.05$). The chroma for PACK 3 at Week 1 was higher than was found for PACK 1 at Week 0, Week 14, and Week 20; PACK 2 at Week 0 and Weeks 10-20; PACK 3 at Week 0 and Weeks 18-20; and PACK 4 at Week 0 and Weeks 18-20 ($P < 0.05$). The chroma was higher for PACK 2 at Week 1 and Week 6 than was found at Week 0 for PACK 1 and PACK 3-4; and at Week 20 for PACK 1-2 and PACK 4 ($P < 0.05$). The chroma was lower for PACK 4 at Week 20 than was found for all other storage period by packaging type combinations, except at Week 0 for PACK 3-4 and Week 20 for PACK 1-4 ($P < 0.05$).

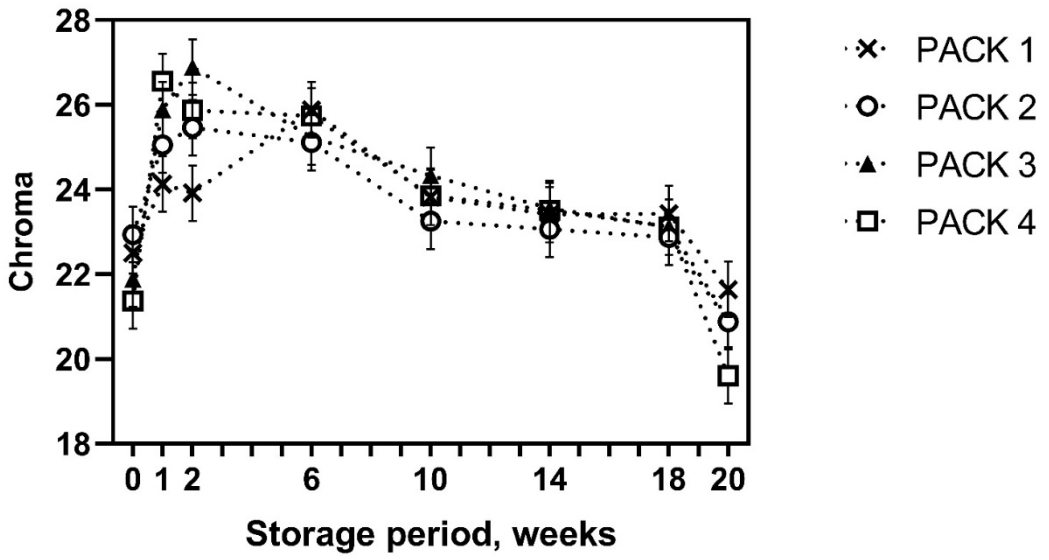

Figure 14. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* chroma values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on chroma ($P < 0.001$; Figure 15). The chroma was higher for Weeks 1-6 at Day 1 than was found for Week 0 at Day 0-3; Week 1 at Day 3; Week 2 at Day 0; Weeks 10-14 at Days 2-3; and for Weeks 18-20 at Days 1-3 ($P < 0.05$). The chroma was higher for Week 2 at Day 2 than was found for Week 0 at Days 0-3; Week 10-18 at Days 2-3; and for Week 20 at Days 1-3 ($P < 0.05$). The chroma was higher for Week 14 at Day 0 than was found for Week 0 at Days 1-3; Week 10-18 at Days 2-3; and for Week 20 at Days 1-3 ($P < 0.05$). The chroma was higher for Week 1 at Day 2, Week 6 at Day 2, and Week 10 and Weeks 18-20 at Day 0 than was found for Week 0 at Day 0 and Days 2-3; Week 10 at Day 3; Weeks 14-18 at Days 2-3; and for Week 20 at Days 1-3 ($P < 0.05$). The chroma was lower for Week 20 at Day 3 than was found for all other storage period by display period combinations ($P < 0.05$). The chroma for Week 20 at Day 2 was lower than was found for all other storage period by display period combinations, except for Week 0 at Day 0 and Days 2-3; Weeks 14-18 at Days 2-3; and Week 20 at Day 1 ($P < 0.05$).

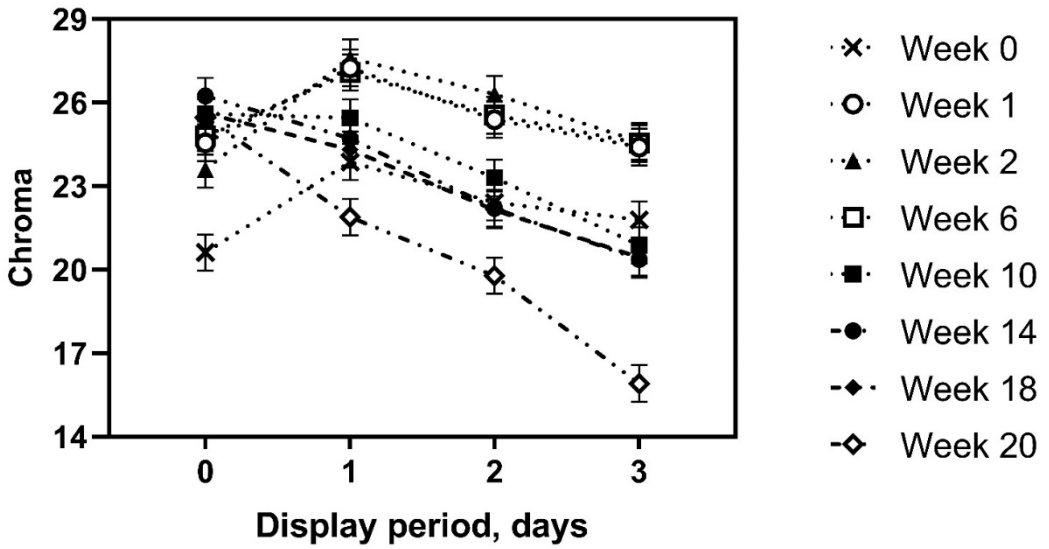

Figure 15. The effect of storage period by display period interactions on lamb *longissimus lumborum* chroma values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on hue ($P < 0.001$; Figure 16). The hue was higher for Week 20 at Day 3 than was found for all other storage period by display period combinations ($P < 0.05$). The hue was higher for Week 20 at Day 2 for all other storage period by display period combinations, except for Week 18 at Day 3 ($P < 0.05$). The hue was higher for Week 18 at Day 3 than was found for all other storage period by display period combinations, except for Week 20 at Day 1 and Week 14 at Day 3 ($P < 0.05$). The hue was higher for Weeks 14-18 at Day 2 than was found at Day 0 for Weeks 0-20; Day 1 for Week 0-14; Day 2 for Weeks 0-6; and at Day 3 for Week 1 and Week 6 ($P < 0.05$). The hue for Weeks 1-2 at Day 2 were higher than was found at Day 0 for Weeks 0-1 and Week 6; and for Week 6 at Day 1 ($P < 0.05$). The hue was lower for Week 6 at Day 1 than was found for all other storage period by display period combinations, except at Day 0 for Weeks 0-14; Day 1 for Weeks 1-6; Day 2 for Week 6; and for Day 3 for Week 2 ($P < 0.05$).

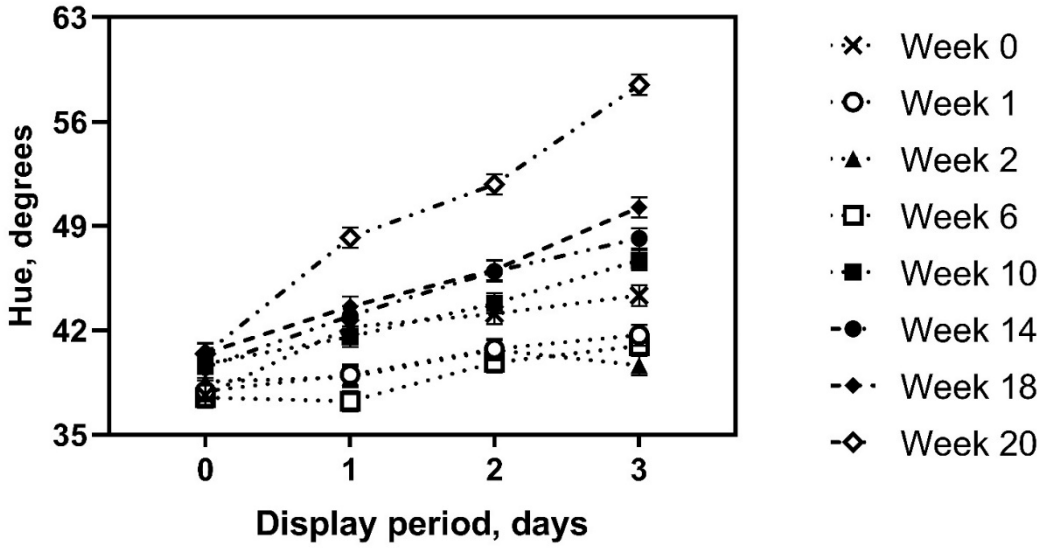

Figure 16. The effect of storage period by display period interactions on lamb *longissimus lumborum* hue values. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on hue ($P = 0.006$; Figure 17). The hue was higher for PACK 1-4 at Week 20 than was found for all other storage period by packaging type combinations ($P < 0.05$). The hue was higher for PACK 1-2 at Week 18 than was found for PACK 1 and PACK 3-4 at Weeks 0-6; and for PACK 2 at Weeks 0-10 ($P < 0.05$). The hue was higher for PACK 1-2 at Week 14 than was found for PACK 1 and PACK 3 at Weeks 0-6; and for PACK 2 and PACK 4 at Weeks 1-6 ($P < 0.05$). The hue was higher for PACK 1 and 3-4 at Week 10 and PACK 3 at Week 14 than was found for PACK 1 and PACK 3-4 at Weeks 1-6; and for PACK 2 at Week 2-6 ($P < 0.05$). The hue was lower for PACK 3 at Week 1 than was found for all other storage period by packaging type combinations, except for PACK 1 and PACK 3-4 at Weeks 1-6 and for PACK 2 at Week 2-6 ($P < 0.05$).

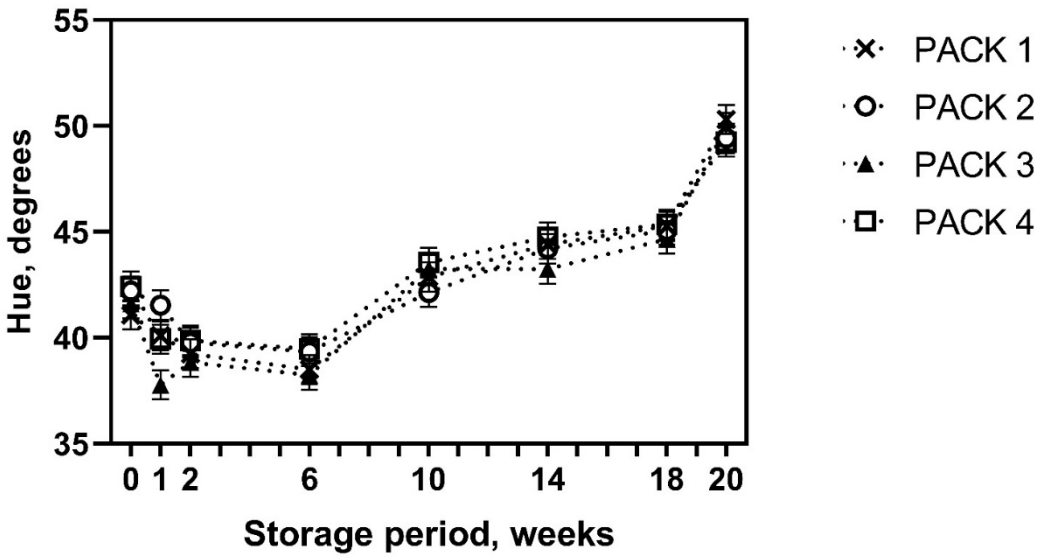

Figure 17. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* hue values. Means and standard error bars are plotted.

There was a significant storage period by display period effect on DMB ($P < 0.001$; Figure 18). The DMB was higher at Day 0 for Week 0 and Week 10 than was found for all other storage period by display period combinations, except at Day 0 for Weeks 14-20 ($P < 0.05$). The DMB was higher for Week 18 at Day 0 than was found for Weeks 0-6 at Days 1-3; Week 10 at Days 1-2; Week 14 at Day 1; Week 18 at Days 2-3; and Week 20 at Days 1-3 ($P < 0.05$). The DMB was higher for Week 2 at Day 0, Week 10 at Day 3, Week 14 at Days 2-3, and Week 18 at Day 1 than was found for Week 0 at Days 0-3; Weeks 1-6 at Days 1-3; Week 20 at Days 2-3 ($P < 0.05$). The DMB was higher for Week 18 at Day 2, Week 10 at Day 1, and Week 1 at Day 3 than was found for Week 0 at Day 1; Week 2 at Day 2; and Week 20 at Days 2-3 ($P < 0.05$). The DMB was higher for Week 14 at Day 1 than was found for Week 0 at Day 1 and Week 20 at Day 3 ($P < 0.05$). The DMB for Week 20 at Day 3 was lower than was found for all other storage period by display period combinations, except for Week 6 at Day 3 ($P < 0.05$).

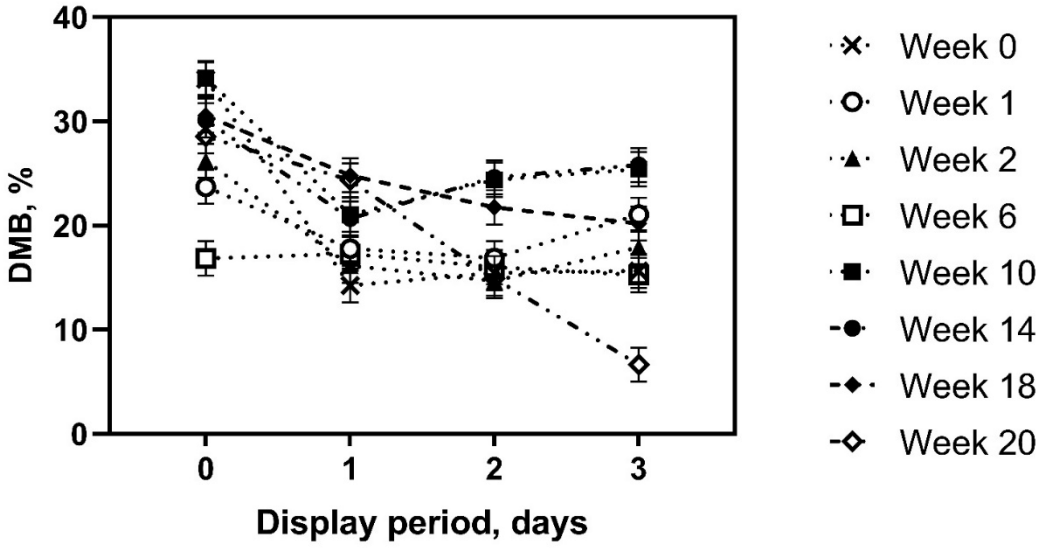

Figure 18. The effect of storage period by display period interactions on lamb *longissimus lumborum* deoxymyoglobin (DMB, %) proportion. Means and standard error bars are plotted.

There was a significant storage period by display period effect on MMB ($P < 0.001$; Figure 19). The MMB was higher for Week 20 at Day 3 than was found for all other storage period by display period combinations ($P < 0.05$). The MMB for Week 20 at Day 2 was higher than was found for all other storage period by display period combinations, except for Week 18 at Day 3 ($P < 0.05$). The MMB was higher for Week 18 at Day 3 than was found for than was found for all other storage period by display period combinations, except for Week 0 at Days 2-3; Week 1 and Week 14 at Day 3; Week 20 at Day 1; and Week 6 at Day 2 ($P < 0.05$). The MMB was higher for Week 0 and Week 14 at Day 3, and Week 20 at Day 1 than was found at Day 0 for Weeks 0-20; Day 1 for Weeks 0-18; Day 2 for Weeks 20-14; and at Day 3 for Weeks 6-10 ($P < 0.05$). The MMB was higher for Week 0 at Day 2 than was found at Day 0 for Weeks 0-20; Day 1 for Weeks 0-18; Day 2 for Week 10; and Day 3 for Weeks 6-10 ($P < 0.05$). The MMB was lower for Week 10 at Day 0 than was found for all other storage period by display period combinations, except at Day 0 for Weeks 0-2 and Weeks 14-18; and for Week 10 at Day 1 ($P < 0.05$). The MMB was lower for Week 20 at Day 0 than was found for all other storage period by display period combinations, except for at Day 0 for Weeks 0-6 and Weeks 14-18; and at Day 1 for Week 10 ($P < 0.05$). The MMB was lower for Weeks 14-18 at Day 0 than was found at Day 1 for Weeks 0-6 and Week 20; Day 2 for Weeks 0-20; and Day 3 for Weeks 0-2 and Weeks 10-20 ($P < 0.05$).

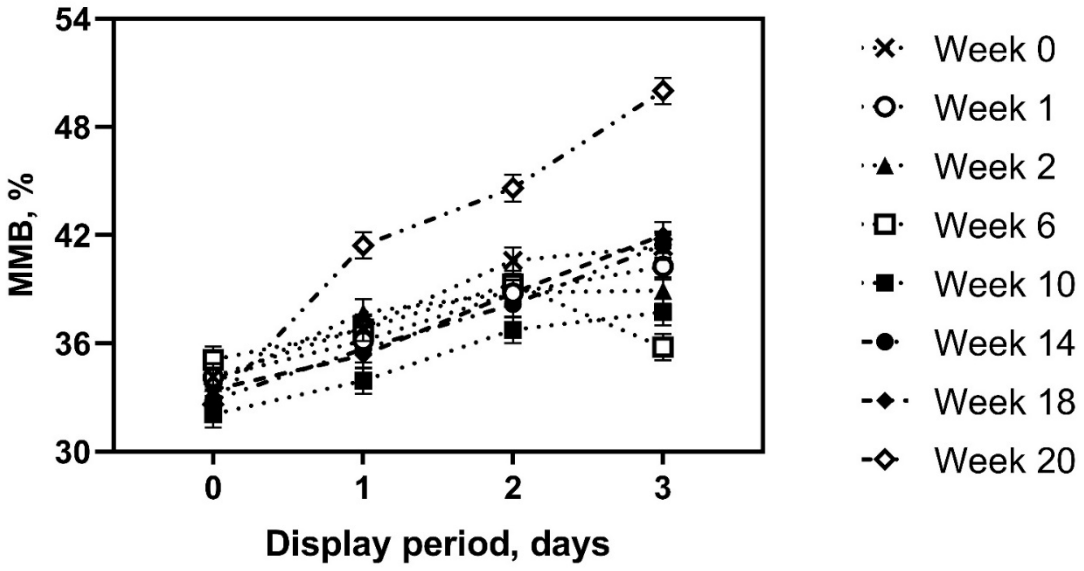

Figure 19. The effect of storage period by display period interactions on lamb *longissimus lumborum* metmyoglobin (MMB, %) proportion. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on MMB (P = 0.026; Figure 20). The MMB was higher for PACK 1-3 at Week 20 than was found for all other storage period by packaging type combinations, except for PACK 4 at Week 20 (P < 0.05). The MMB was higher for PACK 4 at Week 20 than was found for PACK 1 at Weeks 0-18; PACK 2 at Weeks 6-10; PACK 3 at Weeks 1-18; and for PACK 4 at Weeks 1-14 (P < 0.05). The MMB was higher for PACK 4 at Week 0 than was found for PACK 1 at Weeks 6-10; PACK 2 and PACK 4 at Week 10; and for PACK 3 at Week 1 and Weeks 10-14 (P < 0.05). The MMB was higher for PACK 2 at Week 1 than was found at Week 10 for PACK 1-4; and for PACK 3 at Week 10 (P < 0.05). The MMB was lower for PACK 4 at Week 10 than was found for PACK 1 at Week 0, Week 2, Week 14, and Week 20; PACK 2 at Weeks 0-6; PACK 3 at Week 0 and Week 20; and for PACK 4 at Week 0, Week 2, and Weeks 14-20 (P < 0.05). The MMB was lower for PACK 1 at Week 10 than was found for PACK 1 at Week 20; PACK 2 at Weeks 0-1 and Week 18; and for PACK 3-4 at Week 0 and Week 20 (P < 0.05).

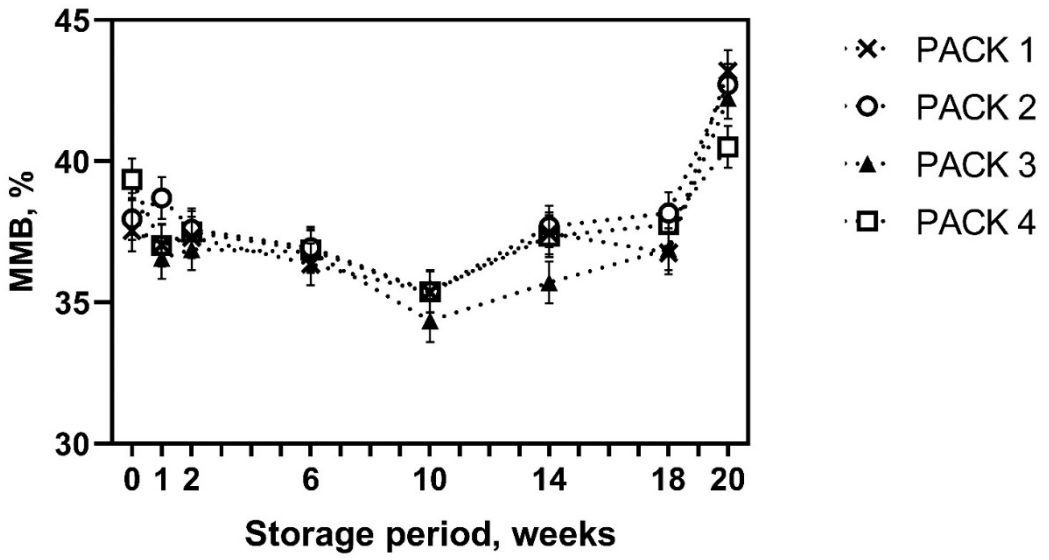

Figure 20. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* metmyoglobin (MMB, %) proportion. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on MMB (P = 0.026; Figure 20). The MMB was higher for PACK 1-3 at Week 20 than was found for all other storage period by packaging type combinations, except for PACK 4 at Week 20 (P < 0.05). The MMB was higher for PACK 4 at Week 20 than was found for PACK 1 at Weeks 0-18; PACK 2 at Weeks 6-10; PACK 3 at Weeks 1-18; and for PACK 4 at Weeks 1-14 (P < 0.05). The MMB was higher for PACK 4 at Week 0 than was found for PACK 1 at Weeks 6-10; PACK 2 and PACK 4 at Week 10; and for PACK 3 at Week 1 and Weeks 10-14 (P < 0.05). The MMB was higher for PACK 2 at Week 1 than was found at Week 10 for PACK 1-4; and for PACK 3 at Week 10 (P < 0.05). The MMB was lower for PACK 4 at Week 10 than was found for PACK 1 at Week 0, Week 2, Week 14, and Week 20; PACK 2 at Weeks 0-6; PACK 3 at Week 0 and Week 20; and for PACK 4 at Week 0, Week 2, and Weeks 14-20 (P < 0.05). The MMB was lower for PACK 1 at Week 10 than was found for PACK 1 at Week 20; PACK 2 at Weeks 0-1 and Week 18; and for PACK 3-4 at Week 0 and Week 20 (P < 0.05).

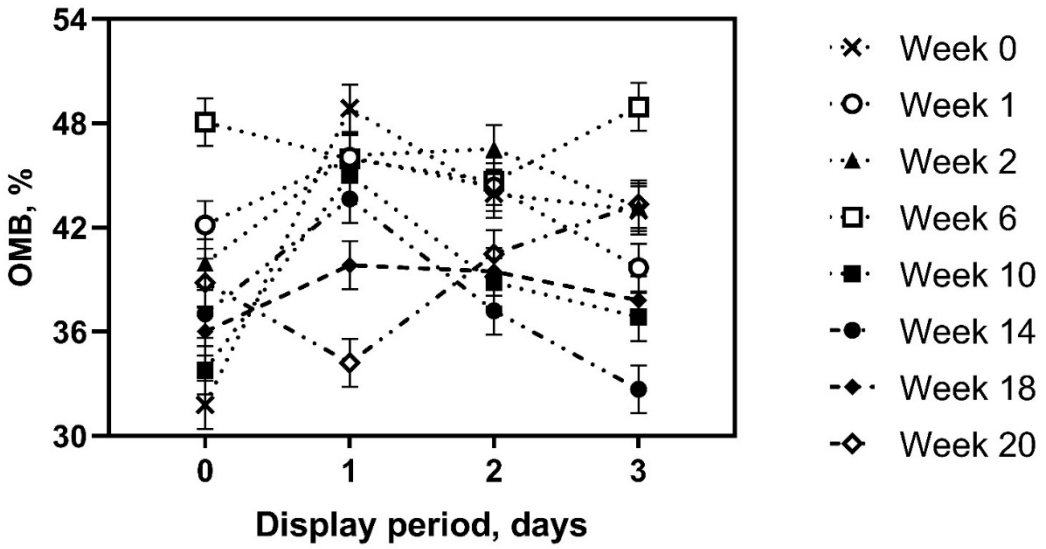

Figure 21. The effect of storage period by display period interactions on lamb *longissimus lumborum* oxymyoglobin (OMB, %) proportion. Means and standard error bars are plotted.

There was a significant storage period by packaging type effect on R630/580 ($P < 0.001$; Figure 22). The R630/580 was higher for PACK 3 at Week 2 and PACK 4 at Week 1 than was found for PACK 1-2 at Weeks 0-1 and Weeks 10-20; and PACK 3-4 at Week 0 and Weeks 10-20 ($P < 0.05$). The R630/580 was higher for PACK 1-2 at Week 6 than was found for PACK 1-4 at Week 0 ($P < 0.05$). The R630/580 was higher for PACK 1-2 and PACK 4 at Week 10 and PACK 3 at Week 0 and Week 14 than was found at Week 18 for PACK 2 and PACK 4; and at Week 20 for PACK 1-4 ($P < 0.05$). The R630/580 was higher for PACK 4 at Week 0 and Week 14 than was found for PACK 1-4 at Week 20 ($P < 0.05$). The R630/580 was lower for PACK 2 at Week 20 than was found for all other storage period by packaging type combinations, except at Week 20 for PACK 1 and PACK 3-4 ($P < 0.05$).

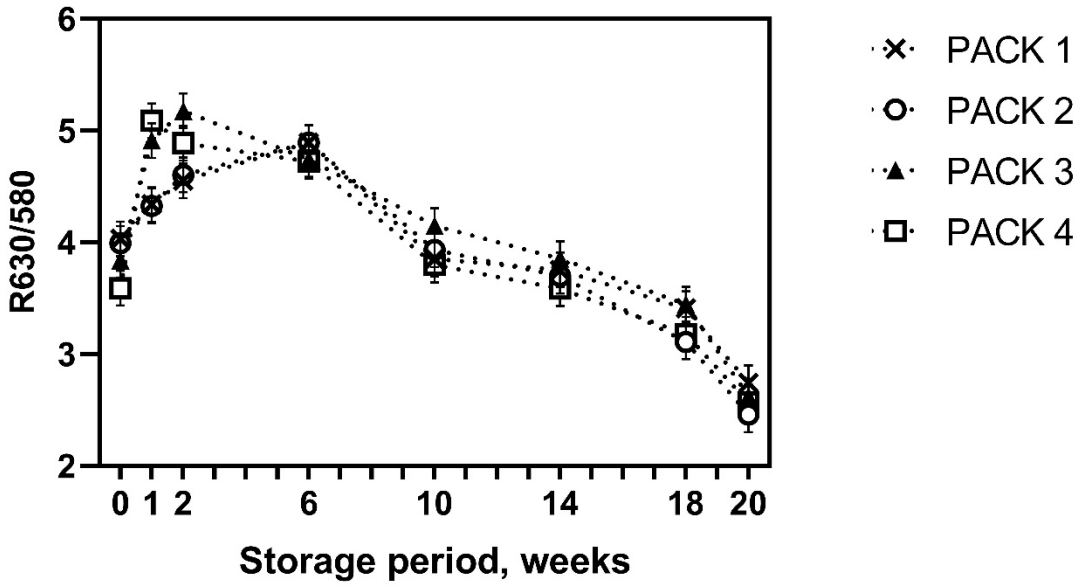

Figure 22. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* ratio of reflectance at 630 and 580 nm (R630/580). Means and standard error bars are plotted.

There was a significant storage period by display period effect on R630/580 ($P < 0.001$; Figure 23). The R630/580 was higher for Week 1 at Day 0 than was found at Day 0 for Week 0 and Weeks 10-20; Day 1 for Weeks 10-20; and Days 2-3 for Weeks 0-20 ($P < 0.05$). The R630/580 was higher for Week 2 and Week 6 at Days 0-1 than was found for all other storage period by display period combinations, except for Week 1 at Day 1 ($P < 0.05$). The R630/580 was higher for Weeks 10-14 at Day 0 than was found for Week 0 at Days 0-2; Week 10 at Day 3; Week 14 at Days 2-3; and Weeks 18-20 at Days 1-3 ($P < 0.05$). The R630/580 was higher for Week 0 and Week 10 at Day 3 than was found for Weeks 18-20 at Day 3 and Week 20 at Day 2 ($P < 0.05$). The R630/580 was higher for Week 18 at Day 2 and Week 20 at Day 1 than was found for Week 20 at Days 2-3 ($P < 0.05$). The R630/580 was lower for Week 20 at Day 3 than was found for all other storage period by display period combinations, except for Week 20 at Day 2 ($P < 0.05$).

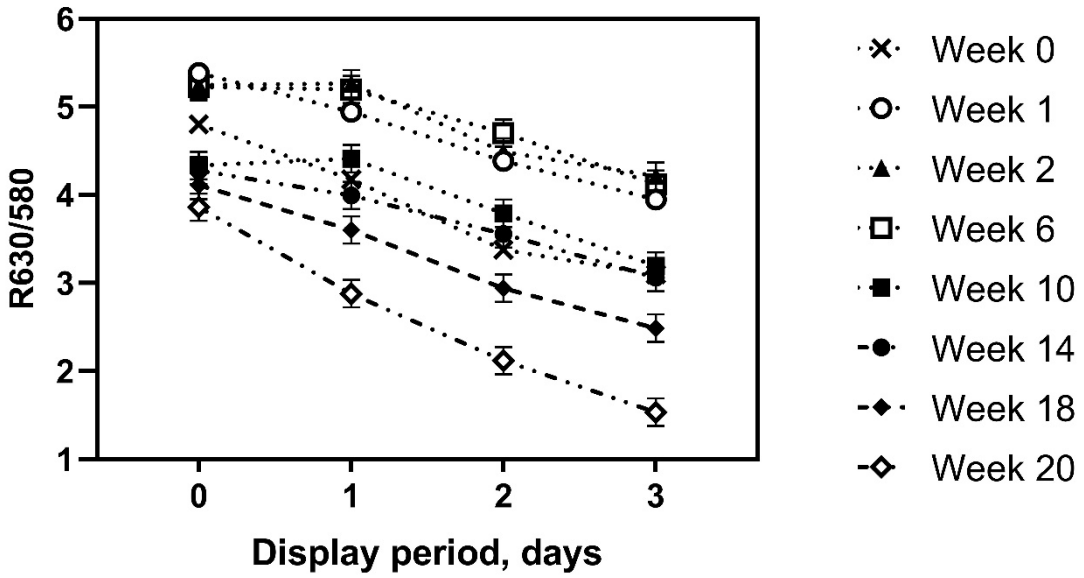

Figure 23. The effect of storage period by display period interactions on lamb *longissimus lumborum* ratio of reflectance at 630 and 580 nm (R630/580). Means and standard error bars are plotted.

There was a significant packaging type by display period effect on R630/580 (P = 0.027; Figure 24). The R630/580 was higher for PACK 4 at Day 0 than was found for PACK 1-2 and PACK 3 at Day 1; and PACK 1-4 at Days 2-3 (P < 0.05). The R630/580 was higher for PACK 1-3 at Day 0 than was found for PACK 2 and PACK 4 at Day 1; and PACK 1-4 at Days 2-3 (P < 0.05). The R630/580 was higher for PACK 3 at Days 0-1 than was found for PACK 2 at Day 1; and PACK 1-4 at Days 2-3 (P < 0.05). The R630/580 was lower for PACK 1-2 and PACK 4 than was found for PACK 1-4 at Days 0-2 (P < 0.05). The R630/580 for PACK 3 at Day 3 was lower than all other packaging type by display period combinations, except for PACK 1-2 and PACK 4 at Day 2 (P < 0.05).

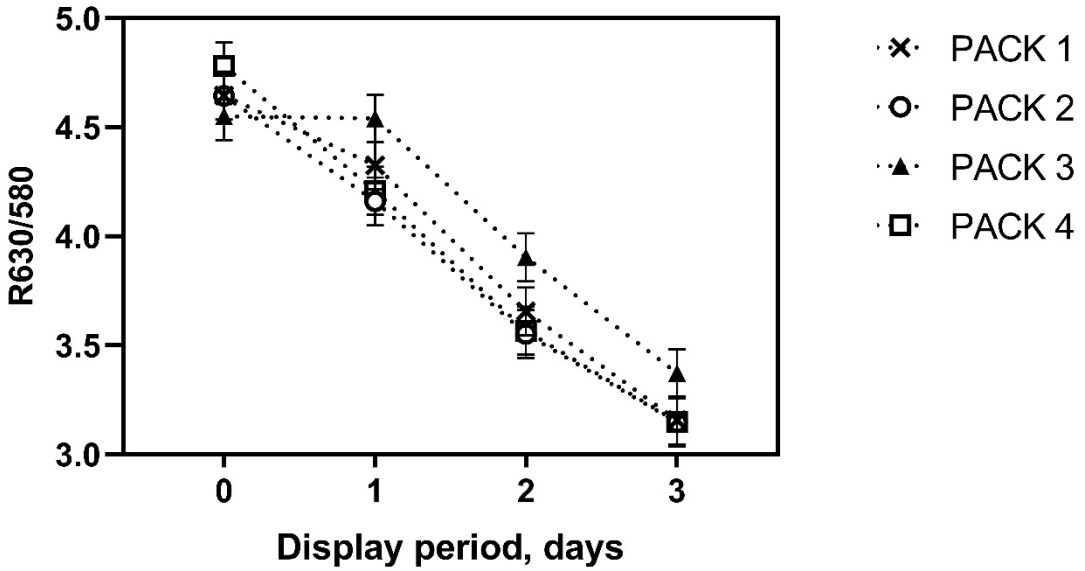

Figure 24. The effect of packaging type by display period interactions on lamb *longissimus lumborum* muscle ratio of reflectance at 630 and 580 nm (R630/580). Means and standard error bars are plotted.

5.3 Fatty acids and oxidation biomarkers

The C12:0 level was higher for PACK 3 than was found for PACK 2 (*P* = 0.032; Table 6). The anteiso-C19:0 level was higher for PACK 3 than was found for PACK 2 (*P* = 0.018; Table 6).

Table 6. Predicted means and standard error (SEM) for the packaging type effects on lamb longissimus lumborum muscle concentrations (mg/100 g serve as-is) of saturated (SFA) and branched chain (BCFA) fatty acids.[1]

Fatty acids	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
SFA	2047	1928	2095	2022	98.7	0.386
C10:0	6.98	6.40	6.91	6.27	0.39	0.168
C12:0	8.65^{ab}	7.82^{a}	9.26^{b}	8.06^{ab}	0.53	0.032
C14:0	120.9	111.5	120.2	114.8	7.1	0.482
C15:0	21.3	19.5	20.3	21.0	1.1	0.407
C16:0	1074	994	1040	1012	52.6	0.455
C17:0	62.9	58.8	58.6	63.4	3.4	0.319
C18:0	787.9	721.1	803.2	766.0	37.9	0.150
C20:0	4.00	3.73	4.03	4.01	0.21	0.424
C22:0	1.82	1.96	1.92	2.05	0.14	0.425
C24:0	2.00	2.28	2.32	2.27	0.21	0.205
BCFA	20.0	18.7	21.0	19.9	1.1	0.232
iso-C15:0	6.11	5.49	6.05	5.94	0.36	0.281
anteiso-C15:0	7.38	6.58	7.65	7.13	0.43	0.081
anteiso-C19:0	7.12^{ab}	6.09^{a}	7.41^{b}	6.56^{ab}	0.45	0.018

[1]Means within rows with different superscripts were significantly different (*P* < 0.05).

The BCFA level was higher for Week 18 than was found for Week 1, Weeks 10-14, and Week 20 (*P* = 0.035; Table 7). The anteiso-C15:0 level was higher for Week 18 than was found for Week 1, Week 10, and Week 20 (P = 0.004; Table 7).

Table 7. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle concentrations (mg/100 g serve as-is) of saturated (SFA) and branched chain (BCFA) fatty acids.[1]

Fatty acids	Storage period, weeks								SEM	*P*-value
	0	1	2	6	10	14	18	20		
SFA	2022	1978	2135	2097	1927	2074	2119	1832	139.4	0.338
C10:0	6.62	6.47	6.61	6.86	6.71	6.88	6.82	6.18	0.55	0.925
C12:0	8.85	8.00	8.82	8.69	8.29	8.05	9.46	7.42	0.75	0.195
C14:0	125.6	109.1	119.4	123.1	110.3	115.6	126.0	105.7	10.0	0.284
C15:0	20.5	19.0	21.3	21.4	19.4	20.8	22.8	18.8	1.6	0.176
C16:0	1056	1009	1065	1065	977	1062	1061	945	74.4	0.593
C17:0	59.9	59.7	65.5	61.2	59.9	63.6	63.1	54.7	4.8	0.487
C18:0	761.9	749.9	791.5	821.9	741.4	777.3	802.1	710.3	53.6	0.506
C20:0	3.84	3.52	3.93	4.15	4.00	4.11	4.26	3.72	0.29	0.214
C22:0	1.81	1.75	2.00	2.19	1.90	1.86	2.09	1.90	0.20	0.350
C24:0	2.04	1.96	2.34	2.55	2.12	2.10	2.47	2.18	0.29	0.377
BCFA	20.1^{ab}	18.2^{a}	20.5^{ab}	21.2^{ab}	18.4^{a}	19.7^{a}	22.9^{b}	18.1^{a}	1.6	0.035
iso-C15:0	6.15	5.34	5.69	6.39	5.39	6.05	6.66	5.51	0.50	0.073
anteiso-C15:0	7.10^{ab}	6.25^{a}	7.12^{ab}	7.87^{ab}	6.68^{a}	1.73^{ab}	8.63^{b}	6.70^{a}	0.61	0.004
anteiso-C19:0	7.17	6.52	7.13	7.34	5.97	6.81	7.34	6.10	0.64	0.190

[1]Means within rows with different superscripts were significantly different ($P < 0.05$).

The EPA+DHA level was higher for PACK 3 than was found for the other packaging types ($P < 0.001$; Table 8). The n-3 level was higher for PACK 3 than was found for PACK 2 and PACK 4 ($P = 0.005$; Table 8). C18:3n-3 levels were higher for PACK 3 than was found for PACK 2 and PACK 4; and higher for PACK 1 than was found for PACK 2 ($P < 0.001$; Table 8). C20:3n-3 levels were higher for PACK 1 and PACK 3 than was found for PACK 2 ($P = 0.005$; Table 8). The C20:5n-3 level was higher for PACK 3 than was found for the other packaging types ($P < 0.001$; Table 8). The C22:5n-3 level was higher for PACK 3 than was found for PACK 2 ($P = 0.046$; Table 8). The C20:3n-6 level was higher for PACK 4 than was found for PACK 3 ($P = 0.011$; Table 8). The C20:4n-6 level was higher for PACK 3 than was found for PACK 2 and PACK 4 ($P < 0.001$; Table 8).The C22:4n-6 level was higher for PACK 3 than was found for PACK 2 and PACK 4 (P = 0.003; Table 8). The C22:5n-6 level was higher for PACK 3 than was found for PACK 2 and PACK 4 ($P = 0.002$; Table 8). The CLA level was higher for PACK 3 than was found for PACK 2 ($P = 0.045$; Table 8). The c9,t11 CLA ($P = 0.005$), t10,c12 CLA ($P = 0.016$), and t9,t11 CLA ($P = 0.016$) levels were higher for PACK 3 than was found for PACK 2 (Table 8).

Table 8. Predicted means and standard error (SEM) for the packaging type effects on lamb longissimus lumborum muscle concentrations (mg/100 g serve as-is) of polyunsaturated (PUFA) fatty acids.[1]

Fatty acids	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
PUFA	412.6	403.7	410.0	410.3	13.5	0.601
C16:2n-4	0.20	0.22	0.20	0.20	0.02	0.810
C20:3n-9	7.47	8.05	7.81	7.99	0.33	0.304
EPA+DHA	41.23^{a}	41.92^{a}	50.49^{b}	43.14^{a}	2.02	< 0.001
n-3	130.5^{ab}	123.9^{b}	143.1^{b}	124.9^{b}	6.0	0.005
C18:3n-3	62.42^{bc}	52.55^{a}	68.57^{c}	56.58^{ab}	3.52	< 0.001
C18:4n-3	0.25	0.25	0.24	0.23	0.02	0.817
C20:3n-3	1.33^{b}	1.14^{a}	1.36^{b}	1.21^{ab}	0.07	0.005
C20:4n-3	0.40^{a}	0.42^{ab}	0.49^{b}	0.40^{a}	0.03	0.014
C20:5n-3	34.2^{a}	35.0^{a}	43.2^{b}	35.9^{a}	1.8	< 0.001
C22:5n-3	24.6^{ab}	23.2^{a}	25.4^{b}	24.6^{ab}	0.8	0.046
C22:6n-3	6.99	6.88	7.32	7.24	0.30	0.314

Table 8. Continued.

Fatty acids	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
n-6	232.3	234.0	224.1	235.1	8.9	0.585
C18:2n-6	177.9	176.7	166.1	183.1	7.4	0.127
C18:3n-6	1.89	1.88	1.82	2.04	0.09	0.057
C20:2n-6	1.45	1.43	1.33	1.49	0.07	0.121
C20:3n-6	5.01^{ab}	5.21^{ab}	4.88^{a}	5.37^{b}	0.16	0.011
C20:4n-6	43.0^{ab}	45.6^{b}	40.8^{a}	46.1^{b}	1.4	< 0.001
C22:4n-6	2.47^{ab}	2.63^{b}	2.22^{a}	2.59^{b}	0.12	0.003
C22:5n-6	0.60^{ab}	0.68^{b}	0.55^{a}	0.69^{b}	0.04	0.002
CLA	42.3^{ab}	37.7^{a}	44.7^{b}	41.8^{ab}	2.5	0.045
c9,t11 CLA	6.50^{ab}	5.48^{a}	6.92^{b}	6.02^{ab}	0.42	0.005
c9,t12 CLA	9.03	7.91	9.04	8.24	0.51	0.055
t10,c12 CLA	2.81^{ab}	2.46^{a}	3.03^{b}	2.65^{ab}	0.18	0.016
t9,t11 CLA	21.9^{ab}	18.7^{a}	23.3^{b}	21.2^{ab}	1.5	0.016
t9,c12 CLA	2.99	2.64	2.91	2.75	0.16	0.096

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), omega-3 (n-3), omega-6 (n-6), and conjugated linoleic acids (CLA).

The C16:2n-4 level was higher for Week 20 than was found for Week 0 (*P* = 0.026; Table 9). C22:5n-3 levels were higher for Week 6 than was found for Weeks 0-1 and Week 20; and higher for Weeks 14-18 than was found for Week 1 (*P* = 0.035; Table 9). The C22:4n-6 level was higher for Week 1 than was found for Week 14 (P = 0.022; Table 9). The CLA level was higher for Week 6 and Week 18 than was found for Week 20 (*P* = 0.002; Table 9). The c9,t11 CLA levels were higher for Week 18 than was found for Week 10 and Week 20; and higher for Week 1 than was found for Week 10 (*P* < 0.001; Table 9). The t10,c12 CLA levels were higher for Week 6 than was found for Week 10 and 20; and higher for Week 18 than was found for Week 20 (*P* < 0.001; Table 9). The t9,t11 CLA levels were higher for Week 6 than was found for Week 10 and Week 20; and higher for Week 18 than was found for Week 20 (P < 0.001; Table 9).

Table 9. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle concentrations (mg/100 g serve as-is) of polyunsaturated (PUFA) fatty acids.[1]

Fatty acids	Storage period, weeks								SEM	*P*-value
	0	1	2	6	10	14	18	20		
PUFA	413.5	420.1	414.3	418.2	390.9	421.9	419.1	395.0	19.1	0.601
C16:2n-4	0.14^{a}	0.18^{ab}	0.22^{ab}	0.20^{ab}	0.19^{ab}	0.21^{ab}	0.21^{ab}	0.27^{b}	0.03	0.026
C20:3n-9	7.39	7.20	7.54	8.33	7.97	8.05	8.21	7.95	0.47	0.172
EPA+DHA	43.59	42.78	42.64	47.26	42.08	45.36	43.25	43.59	2.85	0.530
n-3	125.0	126.4	130.1	139.7	121.1	134.3	141.5	126.7	8.5	0.181
C18:3n-3	57.91	58.52	58.73	66.15	52.88	61.60	67.56	56.86	4.98	0.065
C18:4n-3	0.28	0.22	0.25	0.26	0.25	0.22	0.26	0.22	0.03	0.184
C20:3n-3	1.19	1.22	1.28	1.31	1.18	1.35	1.39	1.18	0.10	0.249
C20:4n-3	0.22^{a}	0.38^{b}	0.48^{bc}	0.45^{bc}	0.44^{bc}	0.55^{c}	0.46^{bc}	0.44^{bc}	0.04	< 0.001
C20:5n-3	36.4	36.2	35.7	39.8	35.2	38.2	38.6	36.5	2.6	0.588
C22:5n-3	23.9^{ab}	23.2^{a}	24.2^{abc}	26.3^{c}	23.1^{a}	25.6^{bc}	25.4^{bc}	23.8^{ab}	1.1	0.035
C22:6n-3	7.15	6.54	6.92	7.44	6.93	7.18	7.61	7.10	0.43	0.314
n-6	239.8	245.6	231.4	222.4	224.8	239.7	222.4	225.0	12.5	0.379
C18:2n-6	182.5	189.2	178.7	166.5	170.7	178.5	171.3	170.1	10.4	0.381
C18:3n-6	1.92	1.92	1.96	1.95	1.95	1.88	1.83	1.83	0.12	0.923
C20:2n-6	1.60	1.49	1.43	1.34	1.37	1.42	1.41	1.36	0.10	0.164

Table 9. Continued.

Fatty acids	Storage period, weeks								SEM	*P*-value
	0	1	2	6	10	14	18	20		
C20:3n-6	5.15	5.14	5.07	5.01	5.25	5.27	4.98	5.08	0.22	0.866
C20:4n-6	43.4	44.9	42.9	42.2	45.5	45.5	42.6	44.2	2.0	0.565
C22:4n-6	2.47	2.67	2.44	2.34	2.64	2.59	2.36	2.34	0.17	0.259
C22:5n-6	0.70^{ab}	0.75^{b}	0.60^{ab}	0.60^{ab}	0.65^{ab}	0.55^{a}	0.59^{ab}	0.62^{ab}	0.06	0.022
CLA	41.3^{ab}	40.6^{ab}	45.0^{ab}	47.7^{b}	36.8^{ab}	39.8^{ab}	46.7^{b}	35.1^{a}	3.6	0.002
c9,t11 CLA	6.32^{abc}	6.88^{bc}	6.81^{abc}	6.77^{abc}	4.97^{a}	5.98^{abc}	7.04^{c}	5.07^{ab}	0.60	< 0.001
c9,t12 CLA	8.54	7.92	8.57	9.33	7.79	8.96	9.28	8.03	0.72	0.207
t10,c12 CLA	2.78^{abc}	2.91^{abc}	2.95^{abc}	3.24^{c}	2.30^{ab}	2.52^{abc}	3.02^{bc}	2.20^{a}	0.26	< 0.001
t9,t11 CLA	21.0^{abc}	21.3^{abc}	22.4^{abc}	26.3^{c}	18.6^{ab}	19.3^{ab}	24.0^{bc}	17.4^{a}	2.1	< 0.001
t9,c12 CLA	2.81	2.76	2.87	3.02	2.61	2.85	3.02	2.64	0.22	0.492

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), omega-3 (n-3), omega-6 (n-6), and conjugated linoleic acids (CLA).

There was a significant packaging type by storage period effect on C20:4n-3 (P = 0.027; Figure 25). The C20:4n-3 level was higher for PACK 3 at Week 2 than was found for PACK 1 at Week 0, Week 2, and Week 10; PACK 2 at Weeks 0-2; PACK 3 at Week 0; and PACK 4 at Week 0, Week 18, and Week 10 (P < 0.05). The C20:4n-3 level was higher for PACK 3 at Week 14 than was found for PACK 1, PACK 3 and PACK 4 at Week 0 and for PACK 2 at Week 1 (P < 0.05).The C20:4n-3 level was higher for PACK 2 at Week 18 than was found for PACK 1 at Week 0 and PACK 2 at Week 1 (P < 0.05). The C20:4n-3 level was lower for PACK 1 Week 0 than was found for PACK 1 and PACK 4 at Week 14; PACK 2 at Weeks 10-18; and PACK 2 at Week 2 and Week 14 (P < 0.05).

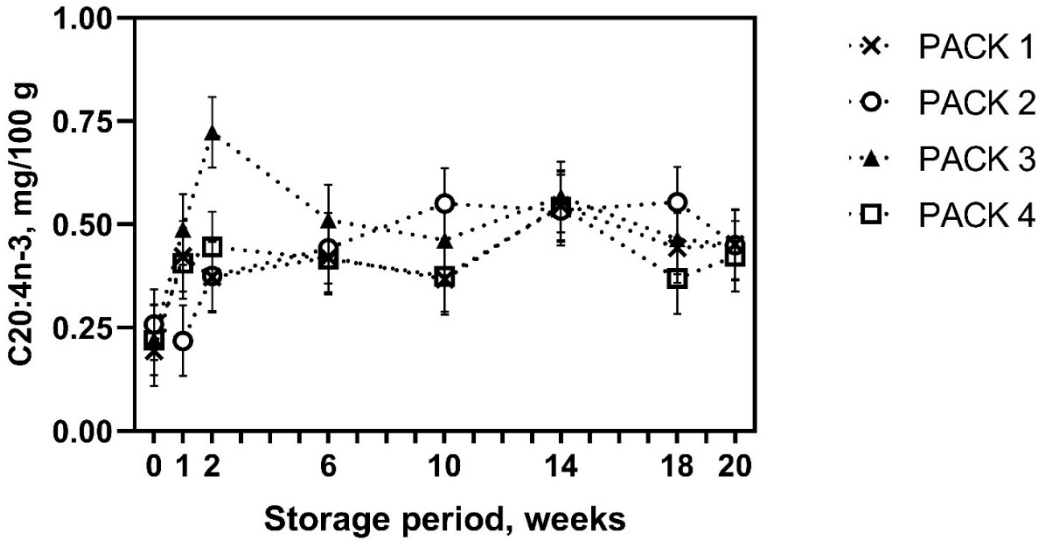

Figure 25. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* C20:4n-3 concentration (mg/100 g serve as-is). Means and standard error bars are plotted.

The C16:1n-7t level was higher for PACK 3 than was found for PACK 2 (P = 0.024; Table 10). The C18:1n-3 level was higher for PACK 2 than was found for PACK 3 (P = 0.035; Table 10). The C19:1n-8 level was higher for PACK 3 than was found for PACK 2 and PACK 4 (P = 0.004; Table 10).

The C16:1n-7t level was higher for Week 18 than was found for Week 20 (P = 0.006; Table 11). The C19:1n-12 level was higher for Week 2, Weeks 10-14, and Week 20 than was found for Weeks 0-1 (P = 0.008; Table 11).

Table 10. Predicted means and standard error (SEM) for the packaging type effects on lamb longissimus lumborum muscle concentrations (mg/100 g serve as-is) of monounsaturated (MUFA) fatty acids.[1]

Fatty acids	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
MUFA	1956	1852	1943	1948	97.1	0.672
C14:1n-5	4.24	3.95	3.91	4.02	0.26	0.604
C15:1n-6	8.31	7.26	8.15	7.70	0.42	0.057
C16:1n-7t	6.58^{ab}	5.56^{a}	6.85^{b}	6.35^{ab}	0.34	0.024
C16:1n-11	2.89	2.78	2.56	3.00	0.19	0.139
C16:1n-9	18.5	17.0	18.2	17.9	1.0	0.439
C16:1n-7	75.7	72.0	71.1	73.2	3.9	0.681
C16:1n-4	27.3	24.7	26.5	25.9	1.4	0.270
C17:1n-8	31.8	31.3	28.3	32.9	1.9	0.083
C17:1n-7 (coelute)	10.22^{b}	8.96^{a}	9.66^{ab}	9.62^{ab}	0.44	0.043
C18:1n-9t	22.7	20.7	22.9	24.4	1.9	0.261
C18:1n-7t	87.8^{ab}	68.5^{a}	92.5^{b}	82.7^{ab}	7.9	0.016
C18:1n-12	9.67	8.00	9.79	8.22	0.88	0.077
C18:1n-9	1605	1516	1538	1557	82.2	0.739
C18:1n-7	45.8	46.1	41.6	48.2	2.5	0.057
C18:1n-6	7.31	6.62	7.32	7.03	0.45	0.595
C18:1n-5	4.31	3.95	4.17	4.07	0.27	0.581
C18:1n-3	6.61^{ab}	5.75^{a}	7.15^{b}	6.30^{ab}	0.49	0.035
C19:1n-12	0.11	0.12	0.10	0.13	0.02	0.231
C19:1n-10	5.83	5.47	5.60	5.72	0.31	0.686
C19:1n-8	2.20^{ab}	2.23^{b}	1.84^{a}	2.34^{b}	0.14	0.004
C20:1n-9	4.02	4.02	4.03	4.24	0.23	0.712
C22:1n-9	0.49^{a}	0.69^{b}	0.47^{a}	0.61^{ab}	0.05	< 0.001
C24:1n-9	1.69	2.09	1.79	2.03	0.27	0.375

[1]Means within rows with different superscripts were significantly different ($P < 0.05$).

Table 11. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle concentrations (mg/100 g serve as-is) of monounsaturated (MUFA) fatty acids.[1]

Fatty acids	Storage period								SEM	*P*-value
	0	1	2	6	10	14	18	20		
MUFA	1934	1902	2011	1967	1917	2019	1945	1706	137.2	0.425
C14:1n-5	4.41	3.76	3.95	4.17	3.69	4.08	4.35	3.84	0.37	0.400
C15:1n-6	7.88	7.05	7.86	8.26	7.49	7.86	8.98	7.45	0.60	0.070
C16:1n-7t	5.91^{ab}	6.25^{ab}	6.76^{ab}	6.79^{ab}	5.57^{ab}	6.67^{ab}	7.35^{b}	5.70^{a}	0.47	0.006
C16:1n-11	2.75	2.88	3.07	2.74	2.92	2.96	2.93	2.30	0.28	0.211
C16:1n-9	18.2	16.8	18.5	18.9	16.6	18.4	19.5	16.3	1.4	0.178
C16:1n-7	76.3	70.6	73.3	75.7	68.4	76.6	75.0	68.2	5.5	0.599
C16:1n-4	26.1	24.6	26.6	27.7	24.6	26.6	28.8	23.7	1.9	0.150
C17:1n-8	30.7	30.8	32.6	30.4	30.7	33.1	31.5	28.9	2.6	0.843
C17:1n-7 (coelute)	8.24^{a}	8.26^{a}	8.94^{ab}	9.67^{abc}	9.35^{ab}	10.43^{bc}	11.58^{c}	10.44^{bc}	0.63	< 0.001
C18:1n-9t	17.2^{a}	17.7^{a}	19.3^{a}	19.2^{a}	37.4^{b}	30.3^{b}	21.8^{a}	18.2^{a}	2.7	< 0.001
C18:1n-7t	62.6^{a}	68.0^{a}	69.9^{a}	73.8^{ab}	141.1^{c}	107.9^{bc}	77.9^{ab}	61.9^{a}	11.1	< 0.001
C18:1n-12	7.82	9.35	7.74	8.69	8.95	9.03	10.80	8.99	1.25	0.315
C18:1n-9	1601	1553	1621	1619	1496	1581	1553	1408	116.2	0.626

Table 11. Continued.

Fatty acids	Storage period								SEM	*P*-value
	0	1	2	6	10	14	18	20		
C18:1n-7	47.1	46.2	47.2	44.5	44.8	47.1	44.4	42.0	3.5	0.808
C18:1n-6	7.50	6.98	7.36	7.22	6.52	7.39	7.15	6.43	0.63	0.595
C18:1n-5	4.36	4.06	4.60	4.30	3.79	4.06	4.05	3.76	0.38	0.365
C18:1n-3	6.42	6.83	6.75	6.21	6.10	6.63	6.97	5.14	0.69	0.631
C19:1n-12	0.08^{a}	0.08^{a}	0.12^{c}	0.11^{ab}	0.14^{c}	0.13^{c}	0.11^{ab}	0.15^{c}	0.02	0.008
C19:1n-10	5.70	5.40	5.85	5.95	5.29	5.89	6.02	5.16	0.44	0.355
C19:1n-8	2.13	2.21	2.35	2.07	2.19	2.25	2.03	1.98	0.20	0.668
C20:1n-9	4.13	4.05	4.13	4.34	4.11	3.91	4.22	3.74	0.33	0.730
C22:1n-9	1.07^{b}	0.54^{a}	0.53^{a}	0.53^{a}	0.50^{a}	0.36^{a}	0.53^{a}	0.46^{a}	0.07	< 0.001
C24:1n-9	1.73	1.74	2.08	2.20	1.71	1.74	2.22	1.79	0.38	0.645

[1]Means within rows with different superscripts were significantly different ($P < 0.05$).

There was a significant packaging type by storage period effect on C17:1n-7 (coelute) ($P = 0.041$; Figure 26). The C17:1n-7 (coelute) level was higher for PACK 4 at Week 18 than was found for PACK 2 at Week 0 and Week 2 and for PACK 3 at Week 1 ($P < 0.05$). The C17:1n-7 (coelute) level was lower for PACK 2 Week 0 than was found for PACK 1 at Weeks 14-18; PACK 2 at Weeks 18-20; and PACK 3 at Week 2 and Weeks 14-18 ($P < 0.05$).

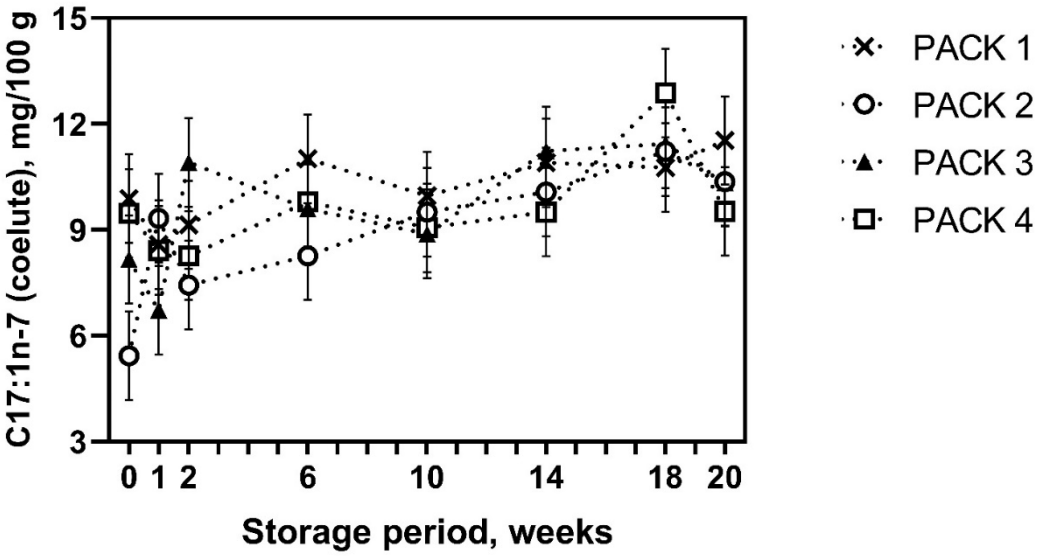

Figure 26. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* C17:1n-7 (coelute) concentrations (mg/100g service as-is). Means and standard error bars are plotted.

There was a significant packaging type by storage period effect on C18:1n-9t ($P < 0.001$; Figure 27). C18:1n-9t levels were higher for PACK 1 and PACK 3 at Week 10 than was found for all other packaging type by storage period combinations, except for PACK 2 at Week 14 and PACK 4 at Weeks 10-14 ($P < 0.05$). The C18:1n-9t level was higher for PACK 4 at Week 14 than was found for PACK 1 at Weeks 0-6 and Weeks 14-20; PACK 2 at Week 0-10 and Weeks 18-20; PACK 3 at Weeks 0-6, Week 14, and Week 20; and PACK 4 at Weeks 0-6 and Week 20 ($P < 0.05$). The C18:1n-9t level was higher for PACK 2 at Week 14 than was found for PACK 1 at Weeks 0-2; PACK 2 at Week 0, Week 6 and Week 20; PACK 3 at Week 0; and PACK 4 at Weeks 0-1 and Week 20 ($P < 0.05$).

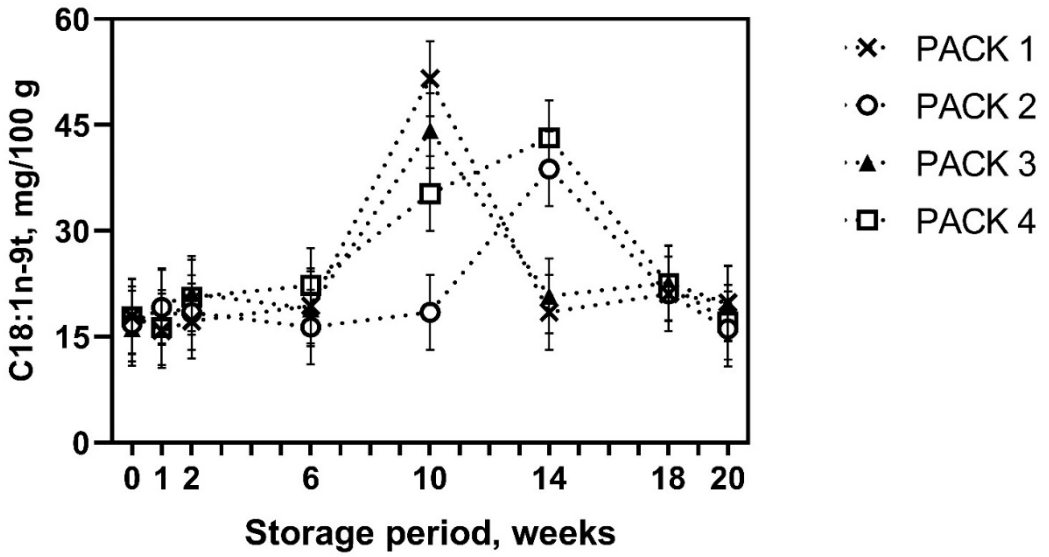

Figure 27. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* C18:1n-9t concentration (mg/100g service as-is). Means and standard error bars are plotted.

There was a significant packaging type by storage period effect on C18:1n-7t ($P < 0.001$; Figure 28). C18:1n-7t levels were higher for PACK 1 and PACK 3 at Week 10 than was found for all other packaging type by storage period combinations, except for PACK 2 and PACK 4 at Week 14 ($P < 0.05$). The C18:1n-7t level was higher for PACK 4 at Week 14 than was found for PACK 1 and PACK 3 at Week 0 and Week 20; PACK 2 at Weeks 0-10 and Week 20; and PACK 4 at Weeks 1-2 and Week 20 ($P < 0.05$).

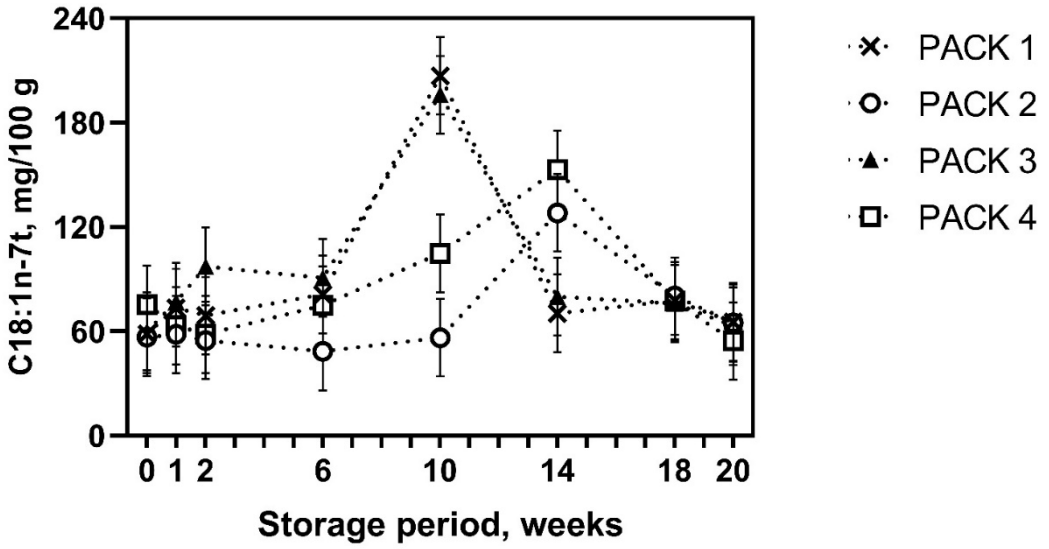

Figure 28. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* C18:1n-7t concentrations (mg/100g service as-is). Means and standard error bars are plotted.

There was a significant packaging type by storage period effect on C22:1n-9 (P = 0.016; Figure 29). The C22:1n-9 level was higher for PACK 2 at Week 0 than was found for all other packaging type by storage period combinations, except for PACK 1 and PACK 4 at Week 0 (P < 0.05). The C22:1n-9 level was lower for PACK 1 and PACK 3 at Week 14 than was found for PACK 1, 2, and 4 at Week 0 (P < 0.05).

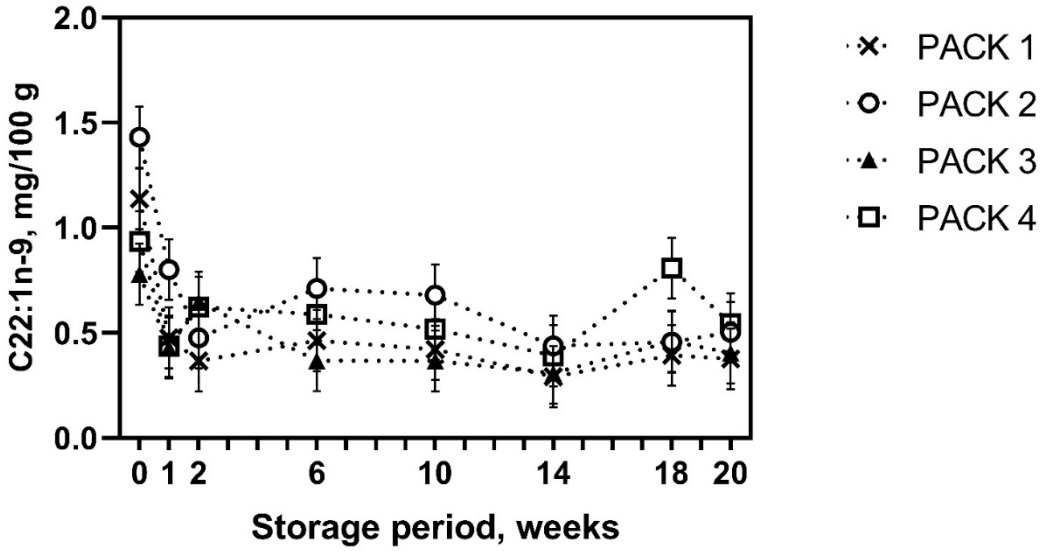

Figure 29. The effect of storage period by packaging type interactions on lamb *longissimus lumborum* C22:1n-9 concentration (mg/100g service as-is). Means and standard error bars are plotted.

LA:ALA were higher for PACK 2 than was found for PACK 1 and PACK 4; and higher for PACK 4 than was found for PACK 3 ($P < 0.001$; Table 12).

Table 12. Predicted means and standard error (SEM) for the packaging type effects on lamb *longissimus lumborum* muscle fatty acid indices and oxidation biomarkers.[1]

Fatty acids	Packaging type				SEM	*P*-value
	PACK 1	PACK 2	PACK 3	PACK 4		
Carbonyl, nmol/mg protein	4.13	3.86	4.17	4.13	0.29	0.700
Fatty acid indices						
Atherogenic index	0.67	0.70	0.69	0.67	0.04	0.922
Peroxidability index	23.8	25.1	25.8	25.0	0.8	0.085
Thrombogenic index	1.36	1.36	1.34	1.35	0.07	0.987
Fatty acid ratios						
ARA:EPA	2.17	2.57	1.86	2.15	0.27	0.075
LA:ALA	3.18^{ab}	4.03^{c}	2.92^{bc}	3.76^{a}	0.26	< 0.001
n-6:n-3	2.03	2.25	1.89	2.12	0.15	0.099
PUFA:SFA	0.21	0.22	0.21	0.21	0.01	0.401
FRAP, Fe^{2+} reduction potential mM/g	0.57	0.57	0.57	0.59	0.02	0.759
TBARS, MDA mg/kg	0.58	0.58	0.60	0.53	0.07	0.751
Alpha-tocopherol, mg/kg	0.71	0.66	0.68	0.63	0.05	0.498

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include the ratio of arachidonic acid to eicosapentaenoic acid (ARA:EPA), ratio of linoleic acid to alpha-linolenic acid (LA:ALA), ratio of omega-6 to omega-3 fatty acids (n-6:n-3), ratio of polyunsaturated to saturated fatty acids (PUFA:SFA), ferric reducing antioxidant potential (FRAP), and thiobarbituric reactive substances (TBARS).

The protein carbonyl levels was higher for Week 2 than was found for Weeks 10-14, and higher for Week 0 than was found for Week 10 ($P = 0.001$, Table 13). Alpha-tocopherol levels were higher for Week 20 than was found for Weeks 0-2 and Week 10; higher for Week 14 than was found for Weeks 1-2 and Week 10; and higher for Week 6 and Week 18 than was found for Week 2 and Week 10 ($P < 0.001$; Table 13). The LA:ALA was higher for Week 1 and Week 10 than was found for Weeks 6 and 18 ($P = 0.040$; Table 13).

Table 13. Predicted means and standard error (SEM) for the storage period effects on lamb *longissimus lumborum* muscle fatty acid indices and oxidation biomarkers.[1]

Fatty acids	Storage period								SEM	*P*-value
	0	1	2	6	10	14	18	20		
Carbonyl, nmol/mg protein	4.69^{bc}	3.99^{abc}	4.86^{c}	3.94^{abc}	3.31^{a}	3.44^{ab}	4.02^{abc}	4.29^{abc}	0.41	0.001
Fatty acid indices										
Atherogenic index	0.68	0.66	0.70	0.70	0.66	0.67	0.71	0.73	0.05	0.884
Peroxidability index	21.8	25.6	24.4	24.5	24.3	24.9	24.5	26.3	1.1	0.568
Thrombogenic index	1.34	1.31	1.35	1.39	1.33	1.32	1.38	1.40	0.09	0.980
Fatty acid ratios										
ARA:EPA	2.22	2.93	2.12	1.76	2.48	2.18	1.93	1.91	0.39	0.070
LA:ALA	3.67^{ab}	3.98^{b}	3.49^{ab}	3.05^{a}	3.93^{b}	3.31^{ab}	2.97^{a}	3.37^{ab}	0.36	0.040
n-6:n-3	2.23	2.44	2.00	1.85	2.21	2.03	1.82	2.00	0.21	0.052
PUFA:SFA	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.23	0.01	0.184
FRAP, Fe^{2+} reduction potential mM/g	0.58	0.62	0.56	0.56	0.55	0.58	0.58	0.56	0.03	0.386
TBARS, MDA mg/kg	0.57	0.55	0.65	0.57	0.51	0.55	0.54	0.66	0.10	0.796
Alpha-tocopherol, mg/kg	0.63^{abc}	0.57^{ab}	0.44^{a}	0.76^{bcd}	0.49^{a}	0.84^{cd}	0.75^{cd}	0.88^{d}	0.08	< 0.001

[1]Means within rows with different superscripts were significantly different ($P < 0.05$). Other abbreviations include the ratio of arachidonic acid to eicosapentaenoic acid (ARA:EPA), ratio of linoleic acid to alpha-linolenic acid (LA:ALA), ratio of omega-6 to omega-3 fatty acids (n-6:n-3), ratio of polyunsaturated to saturated fatty acids (PUFA:SFA), ferric reducing antioxidant potential (FRAP), and thiobarbituric reactive substances (TBARS).

5.4 Spectroscopic prediction of in-pack microorganism populations

The raw spectra and baseline corrected spectra have been included (Figure 30; Figure 31).

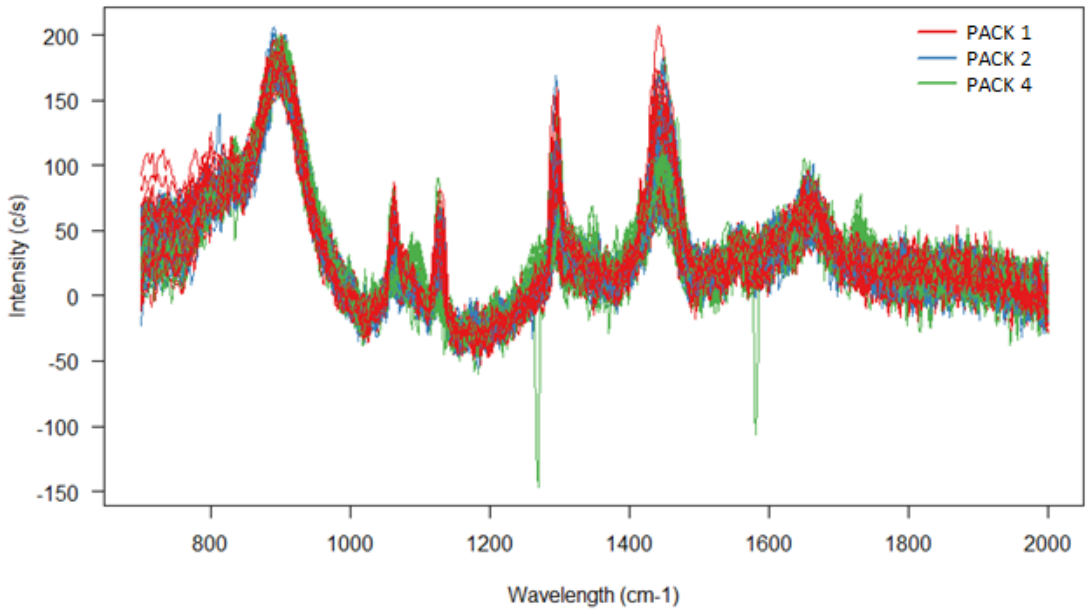

Figure 30. The raw spectra averaged by lamb *longissimus lumborum* muscle (sample).

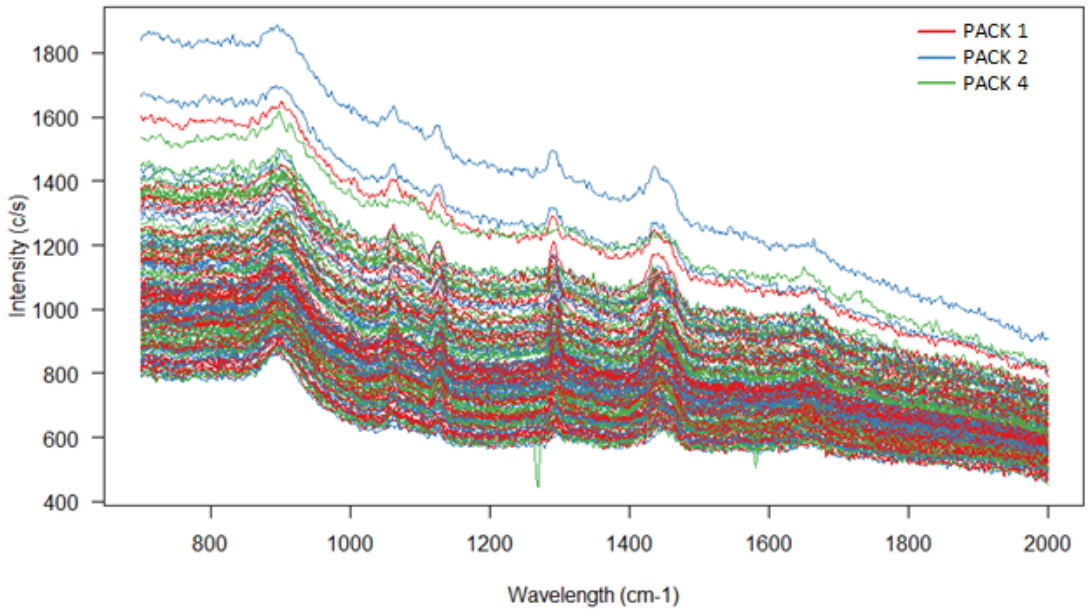

Figure 31. The spectra for each lamb *longissimus lumborum* muscle (sample), corrected for background.

The PLS-R model to predict absolute TVC values had a modest R^2 = 0.29 and RMSE of 1.34 as high TVC values were under predicted (Figure 32).

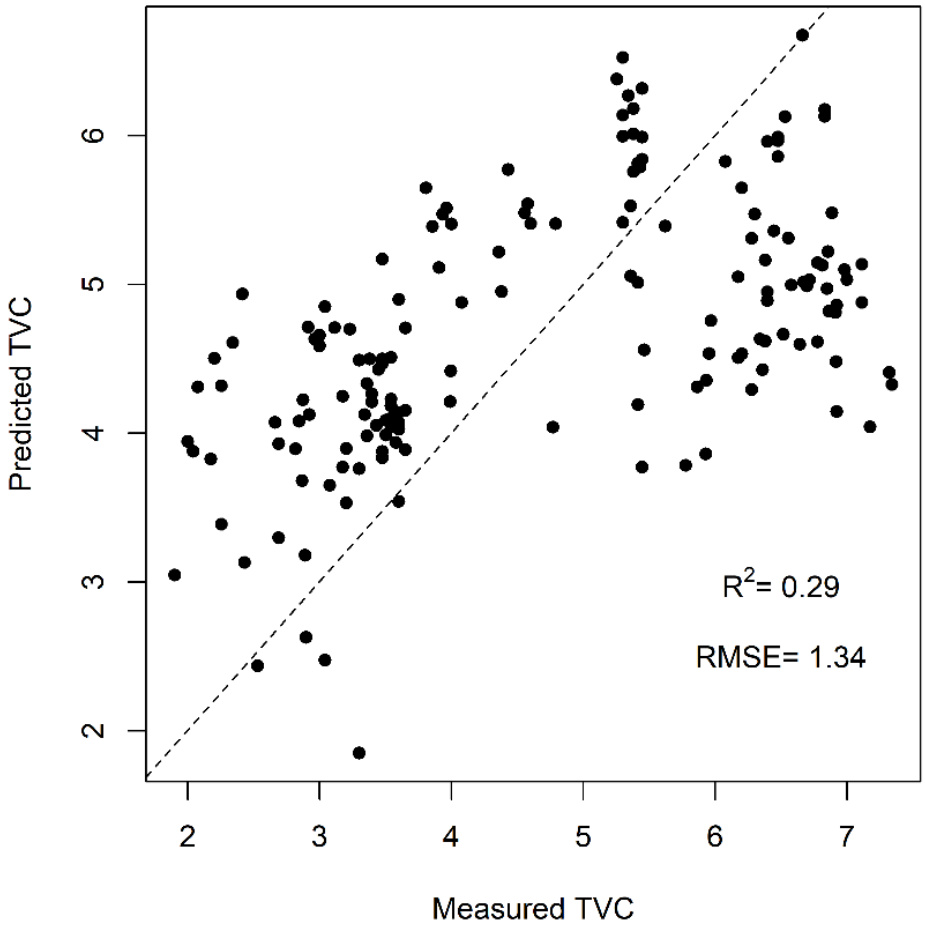

Figure 32. The partial least square regression model prediction of absolute aerobic plate count (TVC) value using baseline corrected spectra, where the dashed line represents a 1:1 correlation between predicted and observed values and RMSE is the root mean squared error).

A total of 75 samples had TVC values > log 5 CFU/g (high TVC) and PLS-DA models were found to predict these with a 92.5% and 88.0% accuracy and sensitivity, respectively, using 5 latent variables (Table 14).

Table 14. The PLSA-DA model prediction of lamb *longissimus lumborum* muscle (samples; *n* = 159) based on absolute aerobic plate count (TVC) groups classified by the log 5 CFU/g threshold of high (TVC > log 5 CFU/g) and low (TVC < log 5 CFU/g).

True positive	False positive	True negative	False negative	Sensitivity, %	Accuracy, %
66	3	81	9	88.0	92.5

6.0 Discussion

6.1 Microbiology and freshness biomarkers

While TVC increased with storage period, it did not exceed the upper limits defined for microbial spoilage (log 7 CFU/g) in lamb meat held under any combination of storage period by packaging type.

The delivery of lamb meat that adheres to microbiological safety standards is a base requirement, as it helps prevent foodborne disease, maintains quality, and reinforces consumer trust and their willingness to purchase. The monitoring of microbiological safety often involves TVC quantification and comparison to regulatory guidelines, these sometimes differing between authorities, countries, and types of meat (Kim et al., 2018). The upper microbiological limits for meat to be classified as 'fresh' have been proposed to be log 5-7 CFU/g (Kim et al., 2018). TVC < log 7 CFU/g is the limit applied red meat products in Australia and is considered to be the standard benchmark for the current study (CSIRO, 1995). This means that all of the lamb meat, regardless of storage period or packaging type, was within microbiological limits and thereby could be categorised as being 'fresh'. This outcome could be indicative of hygienic practices when processing the lamb carcasses and fabricating the LL cuts, with difference abattoirs (Alonso-Calleja et al., 2017) and different processing interventions (Han et al., 2024) reported to affect initial TVC and microbiological shelf life. Initial TVC are important because microorganism growth rates, under ideal conditions, are exponential, following an initial lag phase and provided that nutrients/waste products are not limited (Monod, 1949). However, it is noted that packaging type and storage conditions used in the current study did not halt the proliferation of microorganisms as TVC was observed to increase with storage period.

It has been proposed that initial TVC < log 3 CFU/g, storage temperatures controlled at -1.5 ± 0.5 °C, and packaging material with low gas permeability are necessary to achieve a 10-12 week shelf-life for lamb meat (Mills et al., 2014). The current study met these conditions and is the first to have demonstrated acceptable microbiological status in lamb meat held chilled for 20 weeks. Past research likewise shows TVC to increase with chilled storage duration, but at rates slowed by the use of low storage temperatures and investigated over shorter storage periods. Berruga et al. (2005), for example, reported a significant increase in TVC for lamb meat held for 28 days at 2 °C, although no changes were observed between 14-28 days with levels stable at ~ log 8 CFU/cm^2. Kiermeier et al. (2013) observed the TVC for vacuum packaged lamb shoulder cuts (bone-in and

-out) to exceed log 7 CFU/cm^2 after 9 weeks at -0.3 °C, but stabilised thereafter at < log 8 CFU/cm^2 until Week 12 (the final storage period). Liang et al. (2021) reported no significant change to the TVC of lamb meat held for 70 days at -1.5 °C, with the TVC consistently < log 5 CFU/g and proposed to be the result of the cold storage temperature. In addition, Gribble et al. (2014) reported TVC to be < 6 CFU/cm^2 for vacuum packaged lamb loins stored at -1.5 °C for 12 weeks, counts lower than were found for lamb stored at 0 °C, the latter had TVC < 6 CFU/cm^2 between Week 9-12 of the total storage period. Variation to the current study findings may be the result of the effects of storage temperature, meat pH, in-pack exposure to oxygen, and the initial microorganism populations (Gill and Penney, 1985). Furthermore, some phylum and/or genus of microorganisms are better suited to cold storage conditions, meaning it is necessary to understanding the changes to the microorganism populations with long-term storage is necessary to confirm spoilage potential of meat (Liang et al., 2021, Rood et al., 2022).

TVB-N concentrations increased between Week 1 and Week 14 but remained within defined limits for fresh lamb meat.

TVB-N is often used to define meat as fresh or spoilt, based on its positive association with protease activities, of either endogenous enzymes or microorganism (Bekhit et al., 2021a). This means that meat with high TVB-N is likely to be spoilt and *vice versa* for fresh meat. There is some variation in the upper limits defined for fresh meat, depending on the statutory body, type of meat, and criteria for spoilage (Bekhit et al., 2021b). The general consensus is that fresh meat has TVB-N of < 25 mg/100 g (Bekhit et al., 2021b) – although it has recently been proposed that 5.1 mg/100 g is a more robust threshold for spoilage in vacuum packaged beef (Holman et al., 2021). TVB-N concentrations were observed to increase with storage period, in the current study, but this did not result in any of the lamb meat exceeding the consensus threshold of 25 mg/100 g. Few studies have investigated TVB-N for lamb meat. Hu et al. (2011) investigated the aerobic shelf-life of mutton that had been dipped into different concentrations of M. officinalis extract, finding levels of ~ 10 mg/100 g after 12 days at 7 °C. Alizadeh-Sani et al. (2020) compared the shelf-life of lamb mince held under active packaging with antimicrobial and antioxidant properties, finding these to inhibit proteolysis and TVB-N accumulation. This study was based on pseudo-replicates of minced lamb, but did find TVB-N to be > 25 mg/100 g after 15 days at 4 °C. The comparatively colder storage temperature used in the current study is thought to have contributed to the lower TVB-N observed, with proteolysis and microorganism activity effectively suppressed at subzero temperatures (Mills et al., 2014). This is confirmed by Liang et al. (2021), who reported increases

in TVB-N concentration of ~ 2 mg/100 g for lamb meat held for 70 days at -1.5 °C, a slower rate of accumulation than was observed at warmer storage temperatures. In addition, research with beef has shown no relationship between TVB-N and lipid and oxidative capacity parameters (Holman et al., 2021) – parameters likely to be impacted by in-pack oxygen exposure. Consequently, while there was a difference in the oxygen transfer rate between the packaging types, there was no packaging type effect found on TVB-N concentrations of lamb meat.

Packaging type affected pH and TVC, although with little practical difference, and did not affect diversity indices.

The packaging types, investigated in the current study, each held lamb meat under anerobic conditions, an environment that slows the growth of aerobic microorganisms and reduces TVC. PACK 1-2 were shrink bags and PACK 3-4 were vacuum pouches, meaning there was a greater potential for oxygen to be trapped within the latter packaging types. This could have impacted on initial TVC and the average across the total storage period. Alternatively, the oxygen transfer rate was higher for the shrink bags (PACK 1-2) than the other packaging types, which would have affected the total oxygen exposure for the lamb meat. The barrier properties of PACK 4 were lesser than PACK 3, potentially the result of its biopolymer packaging film (Kaewprachu et al., 2016, McMillin, 2017), and this would affect the in-pack environment and could facilitate the proliferation of aerobic microorganisms. The anaerobic status within the packaging environment would affect the growth of lactic acid bacterium (LAB), a gram positive order of microorganisms that increase the acidity of packaged meat via the production of organic acids *viz*. lactic acid (Pothakos et al., 2015). These could be the basis for the observed variation in pHu between packaging types. However, microorganism diversity indices were not impacted by packaging type. This outcome may help explain the little ‘real’ difference observed in pH and TVC for lamb meat held under different packaging types.

Microbial diversity tended to decline as storage period increased. This was confirmed in changes to the in-pack microorganism populations, particularly after 6 weeks of chilled storage.

Alpha diversity indices are calculated to assess the richness and diversity of the in-pack microorganism populations; whereas CHAOS1 is calculated to evaluate the richness/diversity of microorganisms and Shannon and Simpson indices were calculated to evaluate the diversity of microorganism (Xu et al., 2024). The changes to these indices with storage period therefore

demonstrated a decline in microorganism population diversity, potentially the result of some microorganisms being more suited for the in-pack conditions. This outcome aligns with previous research, with a decline in CHAOS1 found in vacuum packaged lamb shoulder cuts held chilled for 0 or 12 weeks (Kiermeier et al., 2013). It is further confirmed in the relative abundance (proportion) of microorganisms identified within the total in-pack population. Specifically, after 6 weeks of storage there was a decline in the relative abundance of *Bacteriodaceae*, *Campylobacteraceae*, and *Streptococceae*; and an increase in the relative abundance of *Carobacteriaceae*, *Chlostridiaceae*, and *Lactobacillaceae*. This change is thought to be due to the changing in-pack environment, in terms of oxygen availability, pH, storage temperature, and competition between microorganisms. For example, beef loins stored for 80 days at -1 °C exhibited microbial stability with little change in LAB and *Enterobacteriaceae* observed, although *Brochothrix thermosphacta* levels increased to unacceptable levels by Day 20 under these same conditions and thereafter decreased (Imazaki et al., 2019). The same authors suggested that -1 °C favoured B. *thermosphacta* growth, compared to warmer chilled storage temperature of 4 °C (Imazaki et al., 2019). *Pseudomonadaceae* (*Pseudomonas* spp.) were reported to dominant the bacterial communities in beef stored under aerobic packaging and LAB (*Lactobacillus* sp.) beef stored under vacuum packaging (Mansur et al., 2019). After 21 days of vacuum packaged storage, there were substantial increases observed in the population of *Lactococcus* sp., *Carnobacterium* sp., and *Enterobacteriaceae* sp. (Mansur et al., 2019). *Lactococcus* sp. and *Carnobacterium* sp. are reportedly able to grow at low temperatures and are often the dominant microorganism within populations found on chilled beef (Sakala et al., 2002). These same authors found that vacuum packaged lamb shoulders microorganism populations tended to change after 6 weeks of storage, from aerobic bacteria, such as *Pseudomonas* spp., to facultative anaerobes such as *Carnobacterium* spp. and *Serratia* spp. (Kiermeier et al., 2013). Some of these microorganisms may have direct effects on the relative abundance of others, with LAB reported to inhibit the proliferation of other microorganisms, meaning higher initial levels could have preservative outcomes (Pothakos et al., 2015). This could be a contributing factor to the observed changes with storage period.

It is noted that there were no significant packaging type effects on the indices of microorganism diversity, the high-throughput sequencing data was reflected this outcome with relative consistency between the relative abundance of major microorganisms. The population of microorganisms did align with past research of vacuum packaged lamb meat (Bellés et al., 2017, Berruga et al., 2005,

Kiermeier et al., 2013, Kaur et al., 2017, Kaur et al., 2021b) and thereby demonstrates the representativeness of the experimental samples to the population. This supports the transferability of the findings from the current study. They also suggest that the packaging types maintained anaerobic conditions sufficient to alter the relative abundance of in-pack microorganism populations. Gill and Penney (1985), for example, reported variation in the LAB, *Enterobacteria*, and B. *thermosphacta* populations of lamb meat held for up to 12 weeks under nylon-polyethylene or polyester-foil-polyethylene packaging films. These same authors proposed that the differences in gas permeability was likely the causal factor for these results (Gill and Penney, 1985). Comparing packaging types with different in-pack oxygen concentrations, it was reported that LAB, B. *thermosphacta*, and *Enterobacteriaceae* counts were affected in lamb meat stored at 2 °C for a total of 28 days. For B. *thermosphacta*, the inhibitory effect under anaerobic conditions also depends on the combination of several intrinsic (pH, l-lactate, water activity, fat content) and extrinsic (residual oxygen, temperature) factors (Berruga et al., 2005). A comparison of microorganism populations on lamb held under high-oxygen MAP and vacuum packaging found the latter resulted in less diversity as storage period increased and as *Carnobacterium* spp. and other LAB became more dominant (Kiermeier et al., 2013). Similarly, *Pseudomonas* sp. were observed as dominant within the population of microorganism on lamb chops held under aerobic packaging conditions; and L. *cellobiosus* was dominant when lamb chops were held under anaerobic vacuum packaging (Henry et al., 1983).

6.2 Meat quality and colour stability

There were few storage period effects on lamb meat quality, with all the lamb found to be within thresholds defined for consumer acceptance.

IMF was consistent across the total storage period and > 4 %, thereby exceeding the lower limit defined for consumer satisfaction with lamb meat tenderness and overall liking (Hopkins et al., 2013, Lambe et al., 2017). These results confirm the stability of IMF in aged lamb meat, although it has been observed that IMF may increase as a result of decreases to the water content of meat (Coombs et al., 2017a). It is also observed that the IMF (4.4-4.9%) was comparatively higher than has been reported for Australian lamb (Hopkins et al., 2006, Pannier et al., 2014). This could have implications on the proteolytic (tenderisation) potential of these samples *viz*. high IMF can enhance proteolytic enzyme activity (Liu et al., 2009) by weaken the protein microstructures of meat via reducing the total number of myofibrils (Zhang et al., 2022). These relationships should be considered when assessing the effect of storage period on lamb meat tenderness.

Improvements to lamb meat tenderness were most apparent between Weeks 0-1, although shear force was observed to be lowest at Week 14. These changes are likely due to the tenderising activities of endogenous proteolytic enzymes, such as calpain, calpastatin, and caspase (Geesink and Koohmaraie, 1999, Bhat et al., 2018), which are slowed but not inhibited by cold storage temperatures (Kaur et al., 2021a). The magnitude of change to shear force between Week 0 and Week 1 could result from apoptosis being increased during the initial periods of cold storage (Kemp and Parr, 2012, Zhao et al., 2024). Specifically, cellular breakdown releases additional proteases into the extracellular substrate that, consequently, accelerate the rate of myofibril protein degradation (Kemp and Parr, 2012, Longo et al., 2015, Herrera-Mendez et al., 2006). Other studies have reported the rate of tenderisation to be highest during the initial periods of a long-term storage period (Starkey et al., 2015) and reported comparable shear force values for lamb stored chilled for long-term periods (Ponnampalam et al., 2017, Coombs et al., 2017a, De Brito et al., 2016). It is noted that the shear force for all the lamb was < 49 N and therefore within limits defined for consumer acceptance (Hopkins et al., 2006, Shorthose et al., 1986). In addition, for changes in shear force to be detectable by consumers they must exceed 10 N (Destefanis et al., 2008). The only change in shear force observed to be > 10 N was between Week 0 and Week 1, meaning that consumers would likely identify a variance in tenderness between Week 0 and meat held for any of the subsequent storage periods. Moyes et al. (2024) supports this observation, as a sensory panel was found to detect improvements to the eating quality of lamb meat aged between 5-14

days *post-mortem* (Moyes et al., 2024). These same authors reported that longer ageing periods did improve eating quality, but only when pH decline parameters were not met (Moyes et al., 2024).

The level of myofibrillar protein solubility increased with storage period, being higher at Weeks 6-10 than was observed at Week 0. Myofibrillar proteins include actin and myosin, and the denaturation or degradation of these will increase myofibrillar protein solubility (Farouk and Swan, 1998). Protein denaturation is the result of cross-linkage formation and/or oxidation, biochemical changes associated within increases to myofibrillar protein solubility and that may occur gradually over a storage period i.e., the staged unfolding of myofibrillar proteins and exposure of hydrophobic domains (Zhang et al., 2017, Feng et al., 2020). These conformational changes are likely affected by pH, namely because of the relationship between ionic strength and myofibrillar solubility (Xiong, 2014). Protein degradation is the result of activities of endogenous proteolytic enzymes (Hopkins and Geesink, 2009, Geesink and Koohmaraie, 1999) as well as the weakening of bonds between myofibrillar proteins (Xiong, 2018). The observed variation between shear force and myofibrillar protein results suggests a combination of denaturation and degradation to have occurred as lamb meat was stored. Nonetheless, changes to myofibrillar protein solubility are also indicative of changes to the water-holding capacity of meat, whereby immobile water is converted into free water and free water mobility is increased.

There were no changes observed in sarcoplasmic protein solubility for lamb meat stored up to 20 weeks. When denatured, sarcoplasmic proteins have been proposed to improve promote the expulsion of water via its binding to myofibrils and compression/shrinkage of lattice spacing between the myofilaments (Liu et al., 2016, Farouk and Swan, 1998). It has been proposed that these effects on water-holding capacity may be countered by the formation of a coagulated sarcoplasmic protein network that captures the expelled water between the myofibrils and the extracellular space (Liu et al., 2016). This latter mechanism offers insight into the observed disparity between sarcoplasmic protein solubility and water-holding capacity response to the storage periods. Myoglobin is a prominent sarcoplasmic protein found in red meat (Suman and Joseph, 2013b, Faustman and Cassens, 1990) The consistent levels of sarcoplasmic protein and the losses in free water content observed as storage period increased suggest that sarcoplasmic proteins were concentrated in the lamb meat. This could be the basis for the total myoglobin content being higher at Week 14 than was observed at the preceding storage periods. It should be noted, however, that in real terms, total myoglobin content was consistent across the total storage period.

Water-holding capacity declined with storage period, with purge loss tending to increase with storage period, cooking loss tending to decline with increased storage period, and drip loss declining until Week 14, and thereafter increasing between Weeks 14-20. These measures describe the amount of free water within a meat microstructure, a fraction that, unlike bound water, can be released unimpeded when conditions are conducive (Warner, 2014). Storage period can impact on the conversion of bound water to free water via several pathways, including; 1) increases to the longitudinal and transverse shrinkage of myofibrils, actions that increase the spacing between myofilaments; 2) cellular membrane disintegration (apoptosis) to result in increased permeability; 3) reductions to intercellular cytoskeletal integrity, inferring muscle cell shrinkage; and 4) alterations to extracellular spacing (channels) to better facilitate water accumulation and flow (Hughes et al., 2014, Huff-Lonergan and Lonergan, 2005, Warner, 2014). The changes in water-holding capacity confirm those reported in the literature for purge loss, cooking loss, and drip loss in aged lamb meat (Coombs et al., 2017a, De Brito et al., 2016, Warner, 2023). These findings demonstrate a need to consider the effects of storage period on yield or the amount of saleable lamb meat preserved until the point of consumption. In addition, they suggest that the juiciness of lamb meat is reduced as it is held stored over long-term storage periods – although there is little consensus on the relationship between these measures for water-holding capacity and sensory scores of juiciness (Xu and Falsafi, 2024).

The packaging effects on lamb meat quality were somewhat limited to PACK 3, relative to the other packaging types. These excluded measures of water holding capacity, with PACK 4 demonstrating higher amounts of water loss as storage period increased.

The lamb meat held under PACK 3 was generally tougher, contained less myoglobin, and had less drip loss and total moisture to the other packaging types. These may be the result of the low oxygen transfer rate for PACK 3, being < 1 CC/m^2/24 h, and a correspondingly lower exposure to oxygen across the total storage period. Alternatively, variance to meat quality between packaging types may be the result of PACK 3-4 being vacuum pouches and PACK 1-2 being shrink bags – the latter having a lower potential for air to become trapped within the packaging during processing. Furthermore, it has been observed that the chemical, physical, and barrier properties of packaging films made with biopolymers, such as used in PACK 4, are less desirable than conventional plastic materials (Kaewprachu et al., 2016, McMillin, 2017).

Lamb meat stored for > 6 weeks became unacceptably discoloured increasingly sooner into the retail display period, with few practical differences apparent from the significant storage period by packaging type interactions on colour parameters.

Colour is often the first point of reference by which a consumer will gauge the freshness and value of retailed lamb meat (Holman et al., 2016). The prolongment of satisfactory colour stability is therefore a preferable outcome for producers, processors, and retailers of lamb meat. Intrinsic and extrinsic factors have been shown to affect the colour stability of lamb meat, including animal genotypes, feeding systems, methods for slaughter and carcass handling, storage conditions, and packaging types (Ramanathan et al., 2020, Priolo et al., 2001, Mortimer et al., 2010). The broad basis for these effects is their impact on lamb meat biochemistry – specifically, the 'redox shifts in myoglobin forms that infer redness (bloom) with the accumulation of oxymyoglobin (OMb) and the gradual browning or discolouration with the accumulation of metmyoglobin (MMb)' (Suman and Joseph, 2013a, Mancini and Hunt, 2005). In effect, it is influence of prooxidants that accelerates colour change and reduces the retail appeal of lamb meat. The observed changes to lamb meat colour as it was displayed is therefore likely to be due to associated aerobic storage conditions. Furthermore, the increasingly rapid changes to the colour of lamb meat that is held for longer storage periods is likely due to in-pack exposure to oxygen, the subsequent accumulation of MMb, and lower potential for MMb to be reduced. The in-pack exposure to oxygen would depend on initial vacuum efficacy and the oxygen permeability of the packaging material (Penney and Bell, 1993, McMillin, 2017, Savell et al., 1986), with variance in these contributing to the packaging type interactions observed in the current study. Past research confirms the effect of storage period and oxygen exposure on the colour stability of lamb meat. Coombs et al. (2017a), for example, reported the rate of discolouration across a 3-day display period was higher for lamb meat held chilled for 8 weeks than was found for lamb meat held for < 6 weeks. Ponnampalam et al. (2017) likewise reported that lamb meat held chilled for 60 days discoloured quicker than was observed for lamb meat held chilled for 5 days, by assessing *a** and R630/580 change over a 4 day display period. These and the results of the current study show that colour stability is affected by chilled storage, but it is important to consider the practical implications from these changes.

Consumers will preference red meat that has a bright cherry-red colour, which is affected by display and storage periods (Corlett et al., 2021). Objective thresholds have been defined to enable colour parameters to be interpreted against consumer satisfaction with the colouration of lamb meat

(Holman and Hopkins, 2021). It has been proposed that lamb meat with *a** values > 14.5 are of unacceptable colour (Khliji et al., 2010). When applied to the current study, it seems that samples held for 20 weeks would be discoloured after 1 day of retail display, sampled held for 14-18 weeks would be discoloured after 2 days, and all other samples remained acceptable across the total 3 day display period. Further, when averaged across display period, it could be observed that samples held under PACK 1 and PACK 3 for 20 weeks retained *a** values > 14.5 and those held under PACK 2 and PACK 4 were unacceptably discoloured. This change in *a** aligned which changes to the proportion of MMb in the samples. Alternatively, it has been proposed that lamb meat with R630/580 values < 3.5 (Morrissey et al., 2008), < 3.3 (Khliji et al., 2010), or < 3.0 (Jacob et al., 2007) are of unacceptable colour. The variation in these thresholds may be due to the differences in instruments, samples, and the demographics of the panellists used in their calculation (Holman and Hopkins, 2021). These considered, it was found that samples held for 1-6 weeks maintained acceptable colouration across the total display period; samples held for 10-14 weeks discoloured after 2 days of retail display; samples held for 18 weeks discoloured after 1 day of retail display; and samples held for 20 weeks were discoloured at Day 1 of retail display. Looking at the interactions with packaging type, at Week 18 PACK 2 and PACK 4 were of unacceptable colour; and on average lamb meat held under PACK 4 was discoloured after 2 days of retail display. These observations confirm the need to understand the end use or customer for the chilled lamb meat as this should inform storage period and packaging type selections.

6.3 Fatty acids and oxidation biomarkers

Changes to the fatty acid profile of lamb meat were most evident between Weeks 6-18 of long-term chilled storage.

Changes to the fatty acid profile of lamb meat, as it was stored, were observed to be most apparent between Weeks 6-18, noting that these changes were limited to trans-MUFA, omega-3 PUFA, and conjugated linoleic acids. The primary cause for changes to meat fatty acid profile is exposure to oxygen and the associated interactions between lipids and free radical species. These reactions can oxidise fatty acids, leading to the generation of hydroperoxides, that can breakdown into aldehydes, ketones, and other volatile compounds associated with spoilage or rancidity (Ponnampalam et al., 2021b, Ponnampalam et al., 2022, Johnson and Decker, 2015). It is probable, therefore, that in-pack oxygen content and exposure were the driving factors for the observed changes to lamb meat fatty acid profiles and that the cold storage temperature was sufficient to inhibit peroxidation until Week 6. Alternatively, low pH conditions have been reported to promote the rate of propagation and the peroxidation of fatty acids (Mozuraityte et al., 2016) – although pH was found to be relatively consistent for all the samples across the total storage period. High amounts of free water, measurable as water activity or as water-holding capacity, can increase the permeability of meat microstructures, support lipid and oxygen interactions, and thereby increase the rate of peroxidation (Karel, 1980). In addition, the activities of microorganisms, especially those that can release lipases, have been shown to alter the fatty acid profile of meat (Shao et al., 2021). All things considered, these alternate pathways by which fatty acids may change with storage period are likely to have had only a minor contribution to the observed variance, when compared to the in-pack oxygen and the rate of oxygen transmission for the different packaging types. This is somewhat supported by the fatty acid changes being limited to unsaturated fatty acids and the interaction between packaging type and storage period on C17-C18 trans-MUFA concentrations.

The rate of peroxidation depends on the ‘strength’ of the carbon-hydrogen bonds potentially autoxidised by free radicals (Cosgrove et al., 1987). This means that unsaturated fatty acids are highly susceptible to peroxidation, and increasingly so as their chain length increases and the number of double bonds increases *viz*. long chain PUFA are more likely to be oxidised that their shorter chain counterparts or equivalent MUFA (Porter et al., 1995). This was observed in the current study, with changes limited to trans-MUFA, omega-3 PUFA, and conjugated linoleic acid. Previous research affirms these findings, with Adeyemi et al. (2016) reporting omega-3 and

omega-6 PUFA concentrations reduced as chevon was held chilled at 4 °C for up to 12 days. Coombs et al. (2018b) instead observed omega-6 PUFA concentrations to increase and EPA+DHA concentrations to maintain as lamb meat was held chilled at ~ 1 °C for up to 8 weeks. This latter study is confirmed by the current results, with a consistent amount of EPA and DHA found in lamb meat across the storage period. This merits consideration in terms of oxidative stability of these specific long-chain PUFA – although it is observed that docosapentaenoic acid (DPA) concentrations did change with storage period. It has been proposed that EPA and DHA are found in the phospholipid fraction of meat and that increases to the calcium ion activation of PLA2 (apoptosis) can contribute to sarcoplasmic membrane instability and an increase in phospholipid products in aged meat (Chao et al., 2020, Ji and Takahashi, 2006). It is unclear as to whether this could have 'compensated' for the EPA and DHA oxidised across the total storage period or if the loss of water during storage resulted in a concentration of phospholipids in the lamb meat. The mechanism behind these results therefore requires additional investigation.

Biomarkers for oxidative stability were inconsistent or unaffected by storage period.

Lipid and protein oxidation occurs when there are insufficient endogenous antioxidants to counter the effects of free radicals, generated by metabolism and other oxidative reactions. Unchecked, the reactive oxygen intermediates (free radicals) generated from lipid oxidation will oxidise proteins and *vice versa*, to the detriment of total oxidative (redox) stability. The variation to protein carbonyl concentrations across the total storage period indicated some shift in the oxidative stability of the lamb meat and the insufficiency of antioxidants that can slow the oxidative reactions. These changes were, however, inconsistent. Some key antioxidants and prooxidants were not quantified in the current study (e.g., selenium, iron, and copper) and these could provide an alternative insight into the oxidative potential of the lamb meat (Kanner, 1994). Nonetheless, it was observed that α-tocopherol concentrations increased with storage period. This was unexpected, given the peroxy-radical scavenging functions of tocopherol that result in lower α-tocopherol concentrations in meat that is exposed to prooxidant factors (Faustman et al., 1999, McDowell et al., 1996, Chauhan et al., 2014). The result of the current study is thought to reflect the increases in water loss from lamb meat as it was stored, combined with the analysis of surface and deep samples – the latter reported to have a less dramatic change in α-tocopherol during storage (Faustman et al., 1999). The rate by which α-tocopherol was concentrated is proposed to have exceeded the rate of α-tocopherol utilisation and thereby addressed the observed divergence between α-tocopherol and fatty acid profiles in aged lamb meat. Furthermore, Ponnampalam et al. (2014) concluded that dietary

interventions which enhance muscle vitamin E concentrations to > 3.45 mg/kg are sufficient to inhibit lipid oxidation to acceptable levels. Arnold et al. (1993) similarly concluded that muscle α-tocopherol concentrations of > 3.3 mg/kg are sufficient to inhibit lipid and protein oxidation in beef. The α-tocopherol concentrations in the current study were lower with those presented in the literature for chilled lamb (Holman et al., 2022b, De Brito et al., 2017, Chauhan et al., 2016, Ponnampalam et al., 2016, Salvatori et al., 2004); and were found to be below these thresholds. These suggest the oxidative stability of the lamb meat from the current study to be outside of acceptable limits.

There was no storage period effect on lamb meat TBARS, FRAP, and peroxidability index values. These biomarkers suggest oxidation to be effectively halted for the 20 week storage period; with found concentrations of TBARS and FRAP comparable to those presented in the literature for lamb meat held chilled for 14 days (Radzik-Rant et al., 2024), 40 days (De Brito et al., 2017), 70 days (Liang et al., 2021), and 84 days (Kim et al., 2013). In addition, comparison to consumer thresholds based on TBARS, it is observed that all the lamb meat would have been considered to be of acceptable quality (McKenna et al., 2005, Campo et al., 2006, Zhang et al., 2019). The cold and anaerobic storage conditions would have acted to reduce oxidative processes within the lamb meat (Johnson and Decker, 2015, Kanner, 1994, Domínguez et al., 2019) and may have, therefore, contribute to the observed low levels of oxidation. Alternatively, it has been proposed that the lipolysis of certain lipolytic enzymes can inhibit lipid oxidation and lipolysis has been reported to increase when meat is aged (Tatiyaborworntham et al., 2022). Additional investigation is consequently required to comprehensively understand the biochemistry of lamb meat that is held chilled for long-term periods.

Fatty acid indices and EPA+DHA confirmed that lamb meat supported health outcomes in consumers irrespective to storage period and/or packaging type.

The EPA+DHA concentrations were sufficient for all the lamb meat to be classified as a ‘source of omega-3s’, as they contained > 30 mg of EPA+DHA per serve (FSANZ, 2012); and/or ‘high in omega-3 fatty acids’, as they contain > 60 mg of ALA and > 40 mg of EPA+DHA per 100 g serve (EU, 2010). This is advantageous, with these N-3 PUFA associated with cardiovascular, cognitive, and optical health as well as having anti-inflammatory properties (Aranceta and Pérez-Rodrigo, 2012, Swanson et al., 2012, Simopoulos, 1991). Furthermore, it has been reported that many people fail to achieve their daily recommended intakes for these fatty acids (Meyer, 2016); with

approximately 1.5 million deaths each year that may be attributed to low intakes of N-3 PUFA and deficiency in these fatty acids contributes to nearly 10% of all diet-related health burdens (Afshin et al., 2019). Within this context, the current study demonstrated that lamb meat is a candidate food to address this dietary shortfall. It is noted, however, that there is variation in the EPA+DHA concentration of lamb meat with studies reporting higher (Hopkins et al., 2014, Kashani et al., 2015) and lower (Clayton et al., 2024, Uushona et al., 2023) concentrations dependent on the production system under which the lambs were reared (Ponnampalam et al., 2024). This must be considered when extrapolating these findings to other lamb meat products.

The atherogenic and thrombogenic indices account for the different fatty acids that may affect atherosclerotic and thrombus formation in humans (Chen and Liu, 2020). Effectively, they provide insight into the likelihood of developing cardiovascular disease; with atherogenic indices values of < 0.5 and thrombogenic indices values of < 1.0 indicative of a lower risk to consumer health and are therefore preferable (Dal Bosco et al., 2022). The lamb meat in the current study were slightly above these recommendations, being within acceptable but not ideal levels (Chen and Liu, 2020). The observed values for these indices do, however, reflect those previously reported for meat from lambs reared under different production systems (Salvatori et al., 2004, Ghafari et al., 2016, Majdoub-Mathlouthi et al., 2015). This acts to demonstrate the representativeness of the lamb meat included within the current study. LA and ALA are considered to be essential fatty acids as they are not synthesised in the human body and must, therefore, be obtained from food consumed (Russo, 2009). Through a series of desaturation and elongation reactions, LA is transformed into arachidonic acid and ALA into EPA and DHA (Ponnampalam et al., 2021b). It is recommended for LA:ALA to be 5:1 or 10:1 depending on the advisory group (Ponnampalam et al., 2021b, FAO, 1994). While there was variation across the storage period, all the lamb meat had LA:ALA of < 5 and were therefore with the target range for a balanced diet (FAO, 1994). Foods with a low n-6:n-3 have been recommended as a means to promote consumer health (Ponnampalam et al., 2021b) with a value closer to 1 considered to be preferable (Russo, 2009). The n-6:n-3 for lamb meat from the current study was found to range from 1.9 to 2.5 and therefore in excess of the defined target range of 1-2 (Simopoulos, 2010) but within the defined range of < 5 (Moreira et al., 2001). Scrutiny of these health indices demonstrates that lamb meat had comparable appeal to health conscious consumers, irrespective of the storage period and/or packaging type.

Lamb held under PACK 3 had generally higher concentrations of omega-3 and omega-6 fatty acids. MUFA concentrations were less impacted by packaging type.

The exposure of fatty acids to oxygen will impact on their rate of oxidisation and their relative abundance in a meat product, especially those with a higher degree of unsaturation. It is apparent that PACK 3 had a negligible oxygen transmission rate (< 1 CC/m^2/ 24 h) when compared to the other packaging types. This difference would have compounded across the 20 weeks of chilled storage and resulted in lamb meat, held under PACK 3, having less exposure to oxygen, its free radicals, and being less affected by peroxidation. This is thought to be the basis for PACK 3 preserving lamb meat with, generally, higher concentrations of N-3, N-6, and CLA. The differences between the other packaging types, in terms of oxygen transmission rate, is likewise proposed to be the cause of the variation in C17-C18 trans-MUFA concentrations in lamb meat across the storage period. The effects of oxygen exposure on meat oxidative status is evident from the literature, albeit, to the best of our knowledge no study has compared vacuum packaging films or packaging system effects on fatty acid concentrations. Nonetheless, research comparing meat held under high oxygen modified atmosphere packaging and anaerobic vacuum packaging has confirmed the acceleratory effects of oxygen exposure on the rate of peroxidation (McMillin, 2008, Kandeepan and Tahseen, 2022, Warner et al., 2017).

6.4 Spectroscopic prediction of in-pack microorganism populations

Raman spectroscopy provided modest predictions of in-pack TVC, however, it could differentiate between lamb meat with high or low TVC (based on log 5 CFU/g) with relatively high accuracy and sensitivity.

Raman spectroscopy illuminates a sample with a laser beam and measures the shifts in wavelength, that result from light scattering interactions with molecular vibration. This means that Raman spectroscopy can deliver a unique spectral fingerprint for identifying molecules and the non-destructive analyse of samples of interest. Therefore, Raman spectroscopy could be used to unobtrusively detect microorganisms on meat products. The results of the current study and those presented in the literature support this application. Argyri et al. (2013), for example, collected Raman spectra from compressed and unpackaged minced beef, finding that the PLS model provided moderate predictions of TVC (R^2 = 0.63) and PLS models could offer differential predictions for specific microorganism. These included PLS model predictions of LAB (R^2 = 0.55), *Enterobacteriaceae* (R^2 = 0.53), and *Pseudomonaceae* (R^2 = 0.81) counts (Argyri et al., 2013). Liu et al. (2023) collected Raman spectra from unpackaged beef, after it had been held chilled for up to 80 days, to find that the PLSR model provided moderate predictions of TVC (R^2 = 0.54; RMSE = 1.15). Yang et al. (2020) likewise collected Raman spectra from unpackaged beef that had been held chilled for up to 21 days and found the PLSR model provided a strong prediction of TVC (R^2 = 0.90-99). These same authors also found that prediction accuracy varied between the packaging types used to store the beef (Yang et al., 2020). It was proposed that Raman spectra can detect TVC from associated changes in beef protein structures and the free amino acid content of the ultrastructure (i.e., tyrosine, cadaverine, putrescine, and other diamines), biochemical and physiological changes associated with proteolysis and increases in microorganism populations (Jay and Shelef, 1976, Zhang et al., 2013, Yang et al., 2020). These and the results of the current study suggest there to be merit but variation in Raman predictions of TVC. This may be the result of sample variation, representation, spot size, or number of replicates used to establish and test the PLS models.

TVC limits will be different, depending on the regulatory authority, product, and meat under consideration (Kim et al., 2018). Meat with TVC > log 7 CFU/g are considered to have an unacceptable level of microorganisms and to be ‘spoilt’ by food safety authorities in Australia, Japan, South Korea, and the International Commission on Microbiological Specifications for Food (CSIRO, 1995, Kim et al., 2018). This threshold, or a close proximate to this upper limit, is often

the criteria against which Raman spectra have been used to differentiate meat as being spoilt or fresh. Sowoidnich et al. (2012), for example, collected Raman spectrum from unpackaged pork, that had been held refrigerated for up to 7 days, and used principal component analysis to demonstrate its alignment with TVC data – specifically its potential to differentiate between pork at a threshold of log 6 CFU/cm^2. Raman spectrum was found to correctly classify beef as spoilt and fresh with 90.5% and 80.8% accuracy, respectively (Argyri et al., 2013). Liu et al. (2023) reported differences in wavelength shifts at peaks 1267, 1315, and 1650 cm^{-1} for beef categorised as fresh and spoilt using TVC = log 7 CFU/g as the threshold. These peaks have been associated with the products generated from myofibril degradation and proteolysis. These findings also suggest that Raman spectroscopy is suited to classify meat as being not spoilt or spoilt and that its predictions are not necessary based on microorganism-affected changes to the meat microstructures. This practical application would likely serve industry, as a means to identify meat products which have or are 'at risk' of becoming microbial spoilt. This application is supported by the current study, with it observed that lamb could be classified on its potential to become spoilt (< log 5 CFU/g). It is further noted that there were no packaging type or storage period effects to these predictions, with predictions based on Raman spectra found to be equally effective for samples held under PACK 1, PACK 2 and PACK 4. Estimations were limited to these packaging types, as PACK 3 packaging materials (i.e., foil layer) could not be penetrated by the laser/light wavelengths applied by Raman spectroscopy.

7.0 Conclusions / recommendations

The general objective of this project was to provide evidence as to the practical advantages to using 'sustainable' packaging in place of conventional packaging systems when holding lamb meat chilled over a 20 week storage period. The physiochemical changes across this extensive storage period was to provide industry with a benchmark, against which their processes and product shelf-life could be gauged. There was a secondary objective to use spectroscopic technology to predict the safety of lamb meat, in-pack and non-destructively. To address these objectives, the project aimed to answer five associated questions.

Research Question 1: Can 'sustainable' packaging preserve microorganism populations to acceptable levels in lamb meat held chilled for up to 20 weeks?

It was apparent that TVC increased with storage period, but not to levels that exceed the upper limits defined for microbial spoilage in domestic and export markets (log 7 CFU/g) (Kim et al., 2018) and regardless of the storage period by packaging type effects on lamb meat. The diversity of in-pack microorganism populations tended to decline as storage period increased, particularly after 6 weeks of chilled storage. Packaging type was found to affect the pH and TVC of lamb meat, although the differences were of little practical importance. Packaging type did not substantively affect microorganism populations. TVB-N concentrations increased between Week 1 and Week 14, but again remained within defined limits for fresh lamb meat (Bekhit et al., 2021b). These results show all the packaging types to be viable means to preserve microbial stability across a 20 week chilled storage period. It is acknowledged that initial microbial status, in terms of count and diversity, would have been a significant factor in these outcomes. The current results are indicative of good processor hygiene and temperature controls across the total chilled storage period. Alternative hygiene, storage conditions, and handling of the product could, therefore, have resulted in different microorganism populations and associated differences in the freshness/spoilage of lamb meat. Likewise, the application of processing interventions designed to limit microbial populations on the meat, prior to packaging, would have impacted on the final microbial populations and counts (Han et al., 2024). It is further noted that TVC and microorganism population analyses may overlook or misrepresent microorganisms of importance for food safety, i.e., *E. coli*, *Listeria monocytogenes*, *Salmonella*, etc. For example, lamb meat with TVC within defined limits could still be unacceptable or spoilt if it has high counts of these microorganisms. It

would be advisable for future study to quantify the microbial load of microorganisms particularly associated with foodborne illnesses.

Research Question 2: Can 'sustainable' packaging preserve the quality and consumer appeal of lamb meat held chilled for up to 20 weeks?

There were few storage period effects observed to impact on lamb meat quality, with all the lamb meat found to be within thresholds defined for consumer acceptance (Holman and Hopkins, 2021). It is noted that variation exists when using objective measures to estimate consumer sensory scores, with variation contributed by instrument settings, methods, as well as the consumer demographics for which it is being tasked to represent (Holman and Hopkins, 2021). Efforts to minimise the effect of these external factors does, however, provide confidence in the within study comparisons and conclusions made herein. The packaging effects on lamb meat were somewhat limited to PACK 3, relative to the other packaging types. This was thought to be due to its low oxygen transfer rate and/or the efficiency by which vacuum pouches eliminate in-pack air content, comparative to shrink packaging; both properties affecting the accumulative in-pack exposure of the lamb meat to oxygen. The lamb meat stored for > 6 weeks became unacceptably discoloured increasingly sooner into the retail display period, with few practical differences apparent from the storage period by packaging type interactions on colour parameters. Collectively, these outcomes demonstrate little advantage to holding lamb meat chilled for long-term periods – given that enhancements to meat quality occurred within the initial 1-2 weeks of storage. They do, however, demonstrate that quality does not significantly deteriorate when lamb meat is stored chilled for 20 weeks. This finding advocates a longer potential shelf-life for chilled lamb meat, beyond the 10-12 weeks previously recommended in the literature (Mills et al., 2014, Rood et al., 2022, Coombs et al., 2017b). It is acknowledged that objective measures for aroma and taste (flavour) were not directly investigated in the current study, and these organoleptic properties are important to consumer satisfaction with lamb meat. It would be advisable for future studies to quantify these attributes to ensure the market appeal of lamb meat held chilled for extended periods.

Research Question 3: Can 'sustainable' packaging preserve the fatty acid and oxidative status of lamb meat held chilled for up to 20 weeks?

The concentration of some fatty acids changed with long-term chilled storage, mostly between Weeks 6-18. The fatty acid indices and EPA+DHA confirmed that lamb meat supported health outcomes, associated with fatty acid consumption, irrespective to storage period or packaging

type. Lamb held under PACK 3 generally had higher concentrations of omega-3 and omega-6 fatty acids. MUFA concentrations were affected, but to a lesser extent, by packaging type. In addition, it was found that biomarkers for oxidative stability were inconsistent or unaffected by storage period. Consumers are willing to pay more for red meat that promotes their health and well-being (Zhang et al., 2024), outcomes that are supported by the fatty acid composition. The results of this project show the health claimable fatty acids (EPA+DHA) are retained within lamb meat that is chilled for up to 20 weeks. Lipid oxidation has also been associated with the generation of malodourous compounds and rancidification. The results of this project show little change in lipid oxidation and therefore suggest a satisfactory sensorial experience for the consumers. Other factors influence the healthiness of lamb meat, including mineral composition and content of bioactive compounds (vitamins, antioxidants, etc.). Future studies should investigate the effect of storage period and packaging type on these nutrients, especially considering the efforts of producers to enhance lamb meat with higher amounts these compounds. In addition, consideration should be given to the end-user of the chilled lamb meat *viz*. confinement odour is characteristic of vacuum packaged meats, being malodours that develop and disappear within a short period of time after the pack is opened (Reis et al., 2016). Confinement odour of vacuum packaged lamb meat can result in its misclassification as being spoilt. Future studies should investigate the changes in smell and aroma of lamb meat held under different packaging types for long-term chilled storage periods.

Research Question 4: Is there a storage period by packaging type interaction that could impact on the lamb meat?

There were few storage period by packaging type interactions found to impact on lamb meat and the effect of those that did were nugatory on practical terms, i.e., unperceivable to consumers or inferring minimal ‘real’ change to the lamb meat. A possible exception was PACK 4, as the lamb meat held under this packaging type discoloured rapidly after long-term chilled storage periods and to a greater extent that was observed in PACK 1-3. This outcome withstanding, this project demonstrated that lamb meat can be preserved for 20 weeks using all of the packaging types investigated. The transference of these findings to alternative cuts of lamb, including bone-in product, as well as to alternative storage temperatures merits further investigation.

Research Question 5: Can Raman spectroscopy provide real-time information of the microbial load present on vacuum packaged lamb meat?

Raman spectroscopy provided modest predictions of in-pack TVC, however, it could differentiate between lamb meat with high or low TVC (based on log 5 CFU/g) with relatively high accuracy and sensitivity. This means that Raman spectra can be applied to non-destructively identify lamb meat that is 'at risk' of microbial spoilage and thereby, provides industry with an opportunity to accordingly manage its supply. Only the samples held under transparent packaging types could be assessed using Raman spectroscopy and therefore, packaging design and films must be considered when seeking to adopt this technology. Few samples from the current project exceeded log 7 CFU/g – resulting in a dataset that did not represent these TVC. It would be advisable for future studies to include more samples numbers and samples representative of the TVC range for lamb meat so as to improve upon the modest predictions.

By answering these questions, this project has demonstrated that new and sustainable plastic packaging can deliver the shelf-life of conventional practice for long-term chilled lamb meat products. It demonstrated that vacuum packaged lamb meat can be held chilled for up to 20 weeks and therefore 10-15% longer than has been previously recommended in the literature. This project also demonstrated that Raman spectroscopy can provide non-destructive information of the in-pack microbial status of lamb meat. These findings will help to leverage market access for Australian lamb meat, particularly into markets that preference sustainable practices or that have adopted stringent requirements for meat packaging materials. These findings will help protect Australia's clean and green brand, by helping to reduce meat spoilage and food waste in supply chains for highly valued chilled lamb meat products. These findings will also help to ensure consumer health and wellbeing, by ensuring that lamb meat retains its content of health claimable fatty acids across long-term storage periods and by providing an objective and non-destructive means to identify lamb meat at risk of microbial spoilage. Furthermore, by answering these research questions, this project has identified several areas necessitating future research and investment.

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