IDENTIFYING STORAGE THRESHOLDS IN FROZEN AND CHILLED RED MEAT

TABLE OF CONTENTS

1.0   EXECUTIVE SUMMARY

Frozen red meat (beef and lamb) represents a significant proportion of Australian exports to global markets, whose spread can make reaching them both expensive and time consuming. Therefore, understanding the effects of long duration frozen storage on red meat cut integrity and consumer safety is imperative, as failure poses considerable economic and health risks.

Red meat is often stored/held chilled (aged) to enhance its eating quality, and consequently is a preferred means to deliver high value product. There are, however, cases in the Australian industry of this product being 'accidentally frozen' during transit on occasion, with its implication on quality unknown.

Combined chilled-then-frozen storage could facilitate the quality improvements associated with chilled storage and then halt its continuation to guarantee long-term consumer appeal. This would be valuable in managing product distribution, better matching production gluts to instances of heightened demand; and allow cost effective transportation between geographically dispersed markets, in terms of shipping speeds and leveraging market access.

From two different commercial Australian abattoirs, a total of 360 lamb loins and 48 beef loins were randomly taken from the boning rooms; vacuum-packaged (the beef first being divided into 4 equal portions); and held on-site for the duration of their assigned chilled storage durations (lamb: 0, 2, 4, 6 and 8 weeks| beef: 0, 2, 3 and 5 weeks). These were then frozen (if dictated and again on-site) before being transported to Cowra Meat Laboratory where they were held for the duration of their assigned frozen storage periods (both: 0, 4, 8, 12, 24 and 52 weeks). Frozen storage holding temperatures were either $-12\,°C$ or $-18\,°C$ with freezers replicated.

At the completion of their allocated storage treatment, each sample was tested for instrumental measures of sensory quality characteristics; display and shelf-life; microbial loading of key spoilage and safety organisms; lipid oxidation and fatty acid profiles; and protein degradation and oxidation markers.

We found holding temperature effects to be negligible, which suggest $-12\,°C$ could deliver comparable quality loins to $-18\,°C$ across the storage periods examined in this study. That said, this observation should be applied using $-12\,°C$ as a 'maximum temperature threshold' as industry would be better to use a lower frozen storage holding temperature to allow for unforeseen interruptions.

Meat quality parameters varied significantly as a result of differing chilled-then-frozen storage treatments, but when compared to existing consumer thresholds these may not be perceptible. Colour stability did prove the exception as it became unacceptable earlier into retail display when either chilled or subsequent frozen storage periods were increased. This would not be an issue if the end product is destined for restaurants, food service or additional value-adding; likewise when frozen product is retailed as is, instead of thawed prior to sale.

Beef fatty acid profile variation was observed, with increasing frozen storage periods resulting in unsaturated FA levels declining as saturated fatty acid levels increased. Polyunsaturated and health claimable fatty acid levels also tended to decline with increasing chilled storage period, albeit

insignificant within the constraints of the experimental design. This result needs to be verified as it has important ramifications for marketing Australian grass fed beef as 'healthy'.

Lipid oxidation markers, including peroxidase activity, TBARS and oxidation-reduction potential, analyses reflected fatty acid profile variations. These, when compared against existing consumer thresholds, suggested a perceptible increase for red meat held under long-term frozen storage durations with the extent of the increase dependent on the preceding chilled storage period length.

Protein degradation, determined as particle size (PS) and protein solubility, increased over chilled and ensuing frozen storage periods, although at a lesser extent during the latter. Protein oxidation continued with storage durations, but generally following an inconsistent trend. Chilled-then-frozen storage also contributed to myoglobin (Mb) content and redox proportion variation across display to the detriment of retail appeal.

There was insufficient detection of key spoilage microbes in beef to allow for statistical analysis, potentially due to the hygienic and commercially representative loin source, although variation in water activity, glycogen content, pH and other moisture parameters conducive to microbial proliferation were influenced by chilled-then-frozen storage. Nevertheless, lamb loin Lactic acid bacteria, *Brochothrix thermosphacta* and *Enterobacteriaceae sp.* loads did increase with chilled storage, the latter two types then declining as ensuing frozen storage duration continued. It should be noted that these microbial types are associated with meat spoilage rather than product safety.

Supported by this project, a HDR student gained a Masters Qualification enrolled through CSU at Wagga Wagga and valuable experience in meat science. They remain based in Australia and have since expressed a wish to continue researching red meat so as to contribute to future advances for the industry.

This study has generated 30,000+ individual data points which were analysed to formulate 4 peer-reviewed scientific journal publications (further 2 submitted), a review paper, 7 conference papers presented at Australian and international conferences, and a Master's thesis – all of which are available to the Australian red meat industry. It is worth noting the value of peer review in assuring the scientific quality and validity of any conclusions based on the results. This acts as a *fact-checker* to strengthen the legitimacy of our recommendations to industry.

The finding that product can be held long term at − 12 °C instead of − 18 °C offers considerable energy saving potential to industry. This would reduce waste by improving the efficiencies of long term storage and transportation of lamb and beef – and consequently alone represents a significant return on investment from this project.

Lamb and beef managed within a good cold-chain and to have low microbial loads can be held over long term chilled and frozen storage and allow production and market demand variations to be stabilised without a reduction in tenderness, the development of rancidity or other adverse effects, and downgrade of consumer perceived value, as based on literature resources. It also counters claims of reduced quality as a result of chilled product moving to a frozen state (e.g. accidentally frozen, etc.) and held for extended periods.

Red meat display life or colour stability was found to deteriorate following either long term chilled or frozen storage. Although this is not a recommended practice, examples of this practice do exist in

some importing countries and it would be opportune to inform these markets of the likely negative effect on consumer acceptance and preferential purchase.

Several options for future research to complement the findings in this study were identified and included, exploring longer chilled and frozen storage durations to permit greater flexibility when managing product distribution and marketing; testing warmer frozen storage holding temperatures for better cost efficiency over long term duration; confirming health claimable fatty acid and microbial responses to storage using focused design and inoculation trials respectively; and broaden to include other red meat cuts and processed products so as to further our understand chilled-then-frozen storage implications on quality and safety.

2.0 INTRODUCTION

Red meat is an important global commodity and as such, industry must deliver safe and quality product to often distant end-users – to do otherwise would incur substantial market access and economic penalties. Chilled and frozen storage have each been applied to fulfil this need and have proven independently successful; evident from their universal use to preserve red meat and their broad scientific validation. Their combination, however, across long-term durations has not received comparable exploration and consequently the associated advantages may have been overlooked (Coombs, Holman, Friend, & Hopkins, 2017). These could directly impact red meat sensory and microbial loading; lipid and fatty acid composition; and protein degradation and oxidation characteristics.

Consumer satisfaction and potential to repurchase is a function of red meat organoleptic traits. These, in turn, vary depending on their storage method and duration. Using beef as an example, past research has reported that tenderness (20 weeks; (Hughes, McPhail, Kearney, Clarke, & Warner, 2015), flavour (24 weeks; (Jeremiah & Gibson, 2001), appearance (26 weeks; (Small, Jenson, Kiermeier, & Sumner, 2012) and other quality parameters to be maintained or improved across long term chilled storage durations. Likewise, frozen storage has resulted in increased tenderness (12 months; Farouk et al 2003); and flavour (90 d; (Vieira, Diaz, Martínez, & García-Cachán, 2009) and a stable spoilage microbial profile (12 months; Hinton et al 1998) across 'long term' periods. These same observations can be made for lamb with chilled storage shown to improve its eating qualities (De Brito, McGrath, Holman, Friend, Fowler, van de Ven, & Hopkins, 2016; Hopkins, Lamb, Kerr, van de Ven, & Ponnampalam, 2013; Kim, Frandsen, & Rosenvold, 2011) and frozen storage generally preserving these, again for long terms periods (9 month to 2 years) (Fernandes, Freire, Carrer, & Trindade, 2013; Muela, Monge, Sañudo, Campo, & Beltrán, 2015; Muela, Sañudo, Campo, Medel, & Beltrán, 2010, 2012; Winger, 1984). These studies and others demonstrate the impact on meat quality traits, but also highlight a general paucity in understanding of combined chilled and frozen storage practises across longer term durations that must be addressed.

Beef and lamb are both rich sources of dietary fats, including many essential and beneficial fatty acids (FA) important to a balanced diet (Williams, 2007; Wyness, Weichselbaum, O'Connor, Williams, Benelam, Riley, & Stanner, 2011). Production systems and genetic tools have been applied to optimise FA composition (Howes, Bekhit, Burritt, & Campbell, 2015; Ponnampalam, Plozza, Kerr, Linden, Mitchell, Bekhit, Jacobs, & Hopkins, 2017b) and in doing so, capitalise on consumer preference for healthy and nutritious food options (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017b). The level of polyunsaturated FA (PUFA) in red meat underpins its appeal (Aranceta & Pérez-Rodrigo, 2012), especially the health claimable long-chain omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which are associated with improved cardiovascular function and cognitive welfare (Swanson, Block, & Mousa, 2012). PUFAs are susceptible to oxidation because of the multiple double bonds between the carbon atoms that characterise their chemical structures and their resultant highly reactive hydrogen atoms that, if uninhibited, will oxidise, infer rancidity and compromise the sensory and nutritive acceptability of beef (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998) and lamb (Morrissey et al., 1998; Rousset-Akrim, Young, & Berdague, 1997).

Chilled and frozen storage each offer a strategic means to impede lipid oxidation, their individual capacities to preserve these long term being the topic of much previous review (Coombs et al., 2017; Leygonie, Britz, & Hoffman, 2012). These included several studies which reported an increase in beef lipid oxidation levels across chilled storage, measured as thiobarbituric acid reactive substances (TBARS), often without detracting from the sensory quality traits – even for periods up to 20 weeks (Hughes et al., 2015). Likewise, TBARS levels have been reported to increase over frozen storage durations (Awad, Powrie, & Fennema, 1968; Coombs et al., 2017), albeit to a lesser extent than chilled, but unlike the chilled studies, those which also analysed changes to FA profiles with continued frozen storage found a proportional increase in saturated FA (SFA) and a decrease in PUFA levels (Alonso, Muela, Tenas, Calanche, Roncalés, & Beltrán, 2016). Based on this and the paucity, to the best of our knowledge, in understanding the effects of a combination of chilled-then-frozen storage on beef lipid oxidation and FA profiles, it is necessary to explore their implications on lipid stability, nutritional value and health claimable benefits associated with beef consumption.

Lipid and protein oxidation could be considered co-promoting reactions because their biochemical mechanisms are both either 'slowed' or 'driven' by the availability of antioxidants and reactive oxidative species (ROS), respectively – albeit other catalysing compounds also contribute to the rate and extent of oxidation – and the products released from the oxidation of one can promote the oxidation of the other (Xiong, 2000; Zhang, Xiao, & Ahn, 2013). For instance, lipid oxidation releases ROS, such as malondialdehyde, hydroxynonenal, and other examples, which prompts protein oxidation (Estévez, 2011; Lund, Heinonen, Baron, & Estévez, 2011; Utrera, Morcuende, & Estévez, 2014b). It is worth noting that co-promoting does not infer initiation, as base ROS levels may be sourced from various biological systems, occurring in normal metabolism and enzymatic pathways, and exogenous agents, such as ultraviolet light, irradiation, and other environmental factors (Zhang et al., 2013). Lipid oxidation too has been linked to protein degradation, with instances of protein structure deterioration often mirrored by changes in TBARS and other markers of lipid oxidation proliferation (De Brito, Holman, McGrath, Friend, van de Ven, & Hopkins, 2017; Utrera, Parra, & Estévez, 2014c).

The extent of protein oxidation and degradation can impact meat sensory characteristics and ultimately influence product value (Zhang et al., 2013). For example, L. J. Rowe, R. J. Maddock, S. M. Lonergan, & E. J. Huff-Lonergan (2004b) reported beef carbonyl content, one measure of protein oxidation, increased correspondingly to shear force, indicating toughness and undesirability. The protein degradation marker 'particle size' (PS) had instead been found to decrease corresponding with shear force (Karumendu, van de Ven, Kerr, Lanza, & Hopkins, 2009), but this degradation is often to the detriment of meat water-holding capacity and drip loss (Huff-Lonergan & Lonergan, 2005). And, haem-iron oxidation within myoglobin (Mb) protein structures has an intrinsic relationship to beef colour development, stability and acceptability (Suman & Joseph, 2013). From these, we can identify an apparent advantage to inhibiting protein oxidation and managing protein degradation so as to optimise beef's consumer and retail appeal. There are many examples in literature which have independently compared chilled and frozen storage to achieve this goal, but none, to the best of our knowledge, have explored long term chilled-then-frozen storage combination (Coombs et al., 2017).

Based on these observations and scrutiny of existing literature, it was hypothesised that chilled-then-frozen storage could facilitate improvements to red meat quality that are associated with chilled storage (or ageing) from proteolysis etc., and then halt its continuation upon freezing to prevent excessive degradation and ensure future 'long-term' consumer appeal. In practise, this would be valuable in managing product distribution, better managing production gluts to instances of

heightened demand; and allowing cost effective transportation between geographically dispersed markets, in terms of shipping speeds and leveraging market access.

Also evident were the comparatively few studies exploring frozen storage holding temperature effects on these same red meat quality, oxidative stability, and microbial status traits (Coombs et al., 2017). Possibly as a consequence, and to the best of our knowledge, industry in Australia have not defined frozen storage holding temperature guidelines (Anonymous, 2007); although − 18 °C has been widely adopted as the standard operational temperature and is therefore within the requirement of − 12 ° C, being the minimum threshold for frozen beef as per European Union definitions (Anonymous, 2013). Clarification of this ambiguity while understanding its flow-on effects on beef and lamb acceptability could infer significant reductions in operating and transportation costs and as such, merited investigation.

3.0 PROJECT OBJECTIVES

The objectives of this project, outlined in the research agreement, were to:

// determine the effects of long-term freezing on red meat integrity and safety.

// identify the effects of chill period duration prior to freezing on meat quality and value.

// develop thresholds indicative of freeze duration and product quality.

4.0 METHODOLOGY

4.1 Experimental Design and Sampling

In an effort to improve readability, chilled storage period and following frozen storage period (chilled-then-frozen) will be reported hereafter as c*[chilled period]*:f*[frozen period]* – for example, 3 weeks chilled storage followed by 8 weeks frozen storage would be c3:f8, etc.

4.1.1 Beef

On a single day, a total of 48 beef *M. longissimus lumborum* (LL) which met Japanese market specifications (Mulley, Lean, & Wright, 2014) were randomly selected from the boning room of a commercial Australian abattoir. These were divided into four equal portions (*n* = 192) which were then assigned to each of four chilled storage periods x six ensuing frozen storage periods (0, 4, 8, 12, 24 and 52 weeks) x two freezer temperature (– 12 °C and – 18 °C; observed mean ± standard deviations: – 11.49 ± 0.73 and – 17.98 ± 0.39 °C respectively) combinations. It should be noted that LL portions assigned to 0 weeks frozen storage were not frozen (chilled-only) and therefore not subject to freezer temperature treatments. LL portions assignment was balanced within LL, as near as possible, over the frozen storage periods and the remaining combinations fitted with randomly allotted LL portions.

All LL portions were individually vacuum-packaged, processed and stored for the duration of their assigned chilled storage period (mean ± standard deviation: 0.10 ± 0.35 °C) onsite and as per routine commercial practice for Australian abattoirs. LL portions allocated for frozen storage, having frozen storage periods > 0 weeks, were frozen onsite using an industrial plate freezer and then transported to the Cowra Meat Laboratory (Centre for Red Meat and Sheep Development, New South Wales, AUS) where they were assigned as per their frozen storage temperature to one of two replicate freezers set at each temperature (total: four freezers). Once storage treatment combinations were complete samples were sectioned, with care taken to maintain experimental status (i.e. frozen LL were sectioned still frozen and not allowed to thaw) and aseptic conditions. Sections not immediately tested were stored at – 80 °C until analysis.

4.1.2 Lamb

On a single day, at 24 h *post-mortem*, a total of 360 lamb *M. longissimus lumborum* (LL) were randomly sampled from the boning room of a commercial Australian abattoir. These were assigned to five chilled storage periods (0, 2, 4, 6 and 8 weeks) and six ensuing frozen storage periods (0, 4, 8, 12, 24 and 52 weeks), set at two frozen storage temperatures (– 12 and – 18 °C). All LL were individually weighed, vacuum-packaged and stored onsite as per commercial practice for the duration of their assigned chilled storage period (mean temperature: 0.6 ± 1.8 °C). LL assigned to frozen storage were frozen onsite using a commercial plate freezer at Australian industry settings and then transported to the Centre for Red Meat and Sheep Development (NSW Department of Primary Industries, Cowra, New South Wales, Australia) where they were allocated to four freezers set as two replicates per frozen storage temperature. Chilled-then-frozen temperature profiles were recorded by temperature loggers and are presented per treatment combination in Table 4.1.2.

Table 4.1.2. Observed mean (± standard deviations) temperature (°C) of each storage treatment.

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	(unfrozen)	1.9	1.3 ± 2.4	0.7 ± 1.9	0.3 ± 1.9	0.6 ± 1.8
4	-12	-12.0 ± 3.6	-12.4 ± 3.0	-12.0 ± 2.6	-11.8 ± 2.8	-12.8 ± 3.7
	-18	-15.9 ± 3.7	-15.2 ±3.6	-15.1 ± 3.6	-15.0 ± 3.9	-16.0 ± 4.0
8	-12	-11.4 ± 0.1	-11.3 ± 0.2	-11.2 ± 0.3	-11.4 ± 0.4	-11.5 ± 0.4
	-18	-17.6 ± 0.1	-17.6 ± 0.2	-17.8 ± 0.3	-18.0 ± 0.2	-18.0 ± 0.2
12	-12	-11.2 ± 0.3	-11.4 ± 0.3	-10.9 ± 0.7	-10.7 ± 0.6	-10.5 ± 0.6
	-18	-17.8 ± 0.3	-18.0 ± 0.2	-17.9 ± 0.5	-17.9 ± 0.5	-17.8 ± 0.5
24	-12	-10.7 ± 0.6	-18.0 ± 0.2	-17.9 ± 0.5	-17.9 ± 0.5	-17.8 ± 0.5
	-18	-17.7 ± 0.4	-17.8 ± 0.4	-17.9 ± 0.4	-17.9 ± 0.4	-17.9 ± 0.4
52	-12	-12.0 ± 0.3	-12.1 ± 0.2	-12.1 ± 0.2	-12.1 ± 0.2	-12.1 ± 0.2
	-18	-18.2 ± 0.3	-18.2 ± 0.3	-18.1 ± 0.3	-18.4 ± 0.1	-18.2 ± 0.3

Chilled storage preceded frozen storage. Each frozen storage treatment is a continuation from the previous treatment.

Upon completion of each chilled-then-frozen storage treatment, each corresponding LL was removed from its vacuum packaging; excess moisture removed with a paper towel; weighed; and the change in weight between pre- and post-treatment used to calculate purge loss as a percentage (Honikel, 1998). These LL were then sub-sectioned, with care taken to maintain status (i.e. frozen LL were not allowed to thaw) to eliminate the effect of thawing and re-freezing and aseptic conditions to prevent microbial contamination. Portions assigned for colour stability and shear force testing were immediately tested and all other portions stored frozen at – 80 °C until evaluation.

4.2   Carbonyl Content

Approximately 25.0 mg of frozen sample was homogenised using micro-tube pestles in 200.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals Ltd., Michigan, USA); centrifuged and the supernatant tested using the protein carbonyl assay kit (MAK094, Sigma-Aldrich Pty. Ltd., Missouri, USA) colorimetric protocol (Sigma-Aldrich, 2015e). This used the same micro-plate reader instead set to measure absorbance at 375 nm, and BCA1 protocol (Sigma-Aldrich, 2015a) to determine sample protein content described previously so as to allow carbonyl content to be expressed as nmole per mg protein.

4.3   Colour Stability

From each sample, colour stability was assessed using a slice (thickness: 3.0-4.0 cm) arranged on individual black Styrofoam trays (13.5 cm x 13.5 cm) so that muscle fibres on the exposed surface were orientated perpendicular; over-wrapped with PVC food film (thickness: 15.0 µM); and, allowed to bloom for 30-40 min (post-thawed, if necessary) and at 1-2 °C. A spectrophotometer (No. 7237, Model 45/0 MiniScan, HunterLab™ Associates Laboratory Inc., Hong Kong, PRC) with 25.0 mm aperture size (Holman, Ponnampalam, van de Ven, Kerr, & Hopkins, 2015) and using Illuminant D-65 and 10° observer setting, calibrated on black and white tile standards (X = 80.4, Y = 85.3, Z = 91.5), was then used to measure reflectance at 400 nm to 700 nm and CIE colorimetrics (L*, a*, b*(CIE, 1978). Duplicate measures were made for each sample, so that the aperture window avoided connective and fatty tissue deposits, with the over-wrapping remaining *in situ* and the spectrophotometer rotated 90° between measures. This was repeated across each 1 d interval of the display period (total: 4 measurements, 0-3 d) during which samples were kept under continuous fluorescent lighting (58 W NEC tubes delivering 865 lx (mean) to the sample surfaces, as measured using a handheld lux and foot-candles light meter) and refrigeration (mean ± standard deviation: 2.9 ± 0.1 °C). Hue, chroma and the ratio of light reflectance at 630 nm and 580 nm (R630/580) were then calculated (AMSA, 2012).

4.4   Cooking Loss

Cooking loss (CL) was the percentage difference in weight between vacuum-packaged sample blocks (mean: 67.6 g) pre- and post-cooking *sous vide* in a 71 °C water bath (90 L water bath, Ratek Instruments™ Ltd. Melbourne, AUS) for 35 min before their immediate emersion in cold water for 30 min, to halt the cooking process. Cooked samples were dried with paper towel before final weighing. It should be noted that cooked sample block status was unchanged prior to cooking – that is, blocks were cooked from frozen and unfrozen dependent on their assigned storage treatment combination (Holman, Van de Ven, Coombs, & Hopkins, 2017c).

4.5   Fatty Acid Profile

A protocol adapted from Ponnampalam, Butler, Pearce, Mortimer, Pethick, Ball, & Hopkins (2014a) was then used to determine FA profiles in a commercial laboratory, wherein approximately 5.0 g freeze-dried sample (*see 4.21 Total Moisture*) was combined with 500.0 µg tridecanoic acid (dissolved in methanol) to act as an internal standard (C13:0; Sigma-Aldrich Ltd., New South Wales, AUS). This was then hydrolysed with 700.0 µg 10 N potassium hydroxide (KOH; Sigma-Aldrich Ltd., New South Wales, AUS) and 5.3 mL methanol by incubation for 90 min at 55 °C and regular agitation (every 20 min). Samples were then cooled to below room temperature before 600.0 µL 24 N sulphuric acid (H_2SO_4 in water; Sigma-Aldrich Pty. Ltd., New South Wales, AUS) was added and the previous incubation step was repeated. Again, samples were cooled to below room temperature following incubation; combined with 3.0 mL hexane and 1.0 mL saturated sodium chloride (NaCl; Sigma-Aldrich Ltd., New South Wales, AUS); vortex mixed for 5 min; and then aliquot into auto-sampler vials to be analysed by gas chromatography (GC).

CG column settings were: 60 m x 0.25 mm, 70% cyanopropyl polysilphenylene-siloxane with 0.25 um of BPX-70(SGE). CG oven settings were: 30 s at 100 °C before 20 °C temperature increases per min to 130 °C at which it is isothermally held for 2 min, then 1 °C temperature increases per min until 150 °C and a 3 min hold, after which temperature increased at 3 °C per min until 220 °C is achieved and followed by 6 min isothermal holding. FA profiles were quantified against included reference standards and reported as g per 100 g extracted lipid (g/100 g) and used to calculate summative FA terms and the ratio of omega-3 to omega-6 (Rn3n6).

4.6   Glycogen Content

Glycogen content was quantified using 1.0 g sample incubated with 10.0 mL MilliQ water in a boiling water bath (100 °C) for 5 min; homogenised at 22,000 rpm (Series X10/25, Ystral™, Ballrechten-Dottingen, GER); and then centrifuged (Model CPR, Beckman Instruments™, California, USA) at 3,500 rpm and 4 °C for 15 min. The supernatant was then compared against a glycogen standard using the colorimetric protocol outlined in the glycogen assay kit (No. MAK016, Sigma-Aldrich Ltd., Missouri, USA) technical bulletin (Sigma-Aldrich, 2015b) and a microplate reader set to measure absorbance at 570 nm (Model FLUOstar OPTIMA™, BMG Labtechnologies, Victoria, AUS). Technical duplicate samples were measured and these readings averaged so that mean glycogen content was expressed as mmole per kg fresh (wet) meat.

4.7   Intramuscular Fat Content

The freeze-dried samples (*see 4.21 Total Moisture*) were ground in a sample mill (Model 1095, Knifetech™, FOSS Pacific, New South Wales, AUS) and then used for intramuscular fat (IMF) analysis. An adapted Soxhlet method was applied (Smith, Bush, van de Ven, Hall, Greenwood, & Hopkins, 2017) wherein 3.0 g of each freeze-dried sample was extracted with 85.0 mL hexane for 80 min

within a Soxtec machine, and the residual was then dried for 30 min at 105 °C. The difference in weight before and after extraction was used to calculate IMF as % fresh (wet) sample weight.

4.8   Microbial Loading

The analysis of microbial loading was undertaken at a commercial laboratory. Here, under aseptic conditions, 10.0 g of each sample was individually homogenised with 90.0 mL 0.1% peptone salt solution for 60 s using a laboratory paddle blender. From this homogenate, microbial load was quantified as colony forming units per g fresh (wet) meat:

// *Brochothrix thermosphacta*: Homogenised samples were first serially diluted using 0.1% peptone salt solution. The appropriate dilution was spread in duplicate STAA plates which were the incubated for 48 ± 4 h at 22-25 °C. Suspect colonies were verified using an oxidase test (*B. thermospacta* is oxidase negative) before being counted. Duplicate replicates were analysed and the results averaged.

// *Clostridium perfringens*: Homogenised sample (1.0 mL) was combined with 10.0-15.0 mL SC agar with added supplement, mixed well and incubated at 44-47 °C in a water bath until it solidified. This was then overlaid with 10.0 mL of the same SC agar which was then streaked with a reference culture. Control blank plates were also made and evaluated. All plates were then incubated under anaerobic conditions at 37 ± 2 °C for 20 ± 2 h before the number of black colonies were counted, with success dilutions employed when necessary.

// *E. coli*: Homogenised samples (1.0 mL) were placed onto the centre of the top 3M™ Petrifilm™ of *E. coli* count plates. Each petrifilm base was immediately replaced, with care taken to avoid air entrapment, and the sample was spread across the plate. These were allowed to solidify before incubation at 37 ± 2 °C for 24-48 h. Colonies were verified as blue with bubbles formations evident on the petrifilm, and then counted.

// *Enterobacteriaceae sp.*: Diluted homogenised samples (1.0 mL) were used with VRBG agar as the medium to pour plates which, once set and overlaid with an additional VRBG, were incubated for 21 ± 3 h at 25-27 °C. Presumptive colonies were verified (*Enterobacteriaceae sp.* colonies are typically small (0.5-2.0 mm diameters) and dark red or purple) and then counted.

// *Lactic acid bacteria* (LAB): Homogenised samples were diluted (decimally) and these were spread on MRS agar to be then incubated for 72 ± 2 h at 30 ± 1 °C under anaerobic conditions – which included a Campygen modified atmosphere gas generator. Typical colonies were counted once verified using both catalase and Gram stain testing (LAB are catalase negative and have Gram positive cocci, coccobacilli or rod morphology).

4.9   Myoglobin Content

Myoglobin content was tested using 2.0 g frozen sample homogenised (model 5810R, Eppendorf™ Pty. Ltd., Hamburg, GER) at 22,000 rpm with 10.0 mL ice-cold phosphate buffer (AMSA, 2012). This was then centrifuged for 30 min at 2,465 g and 4.0 °C (model 5810R, Eppendorf™ Pty. Ltd., Hamburg, GER) before the supernatant was measured in triplicate by the same micro-plate reader set to 525 nm. Data was then transformed so that myoglobin content was expressed as mg per g of fresh (wet) sample (AMSA, 2012) and averaged across technical replicates.

4.10   Myoglobin Redox Fractions

From the spectral data previously collected (4.3 Colour Stability) sample oxymyoglobin (OMb), deoxymyoglobin (DMb) and metmyoglobin (MMb) fractions (%) were estimated (AMSA, 2012) across display periods (0-3 d). Akin to the colorimetric analyses, these data were the average of technical duplicates.

4.11   Nitrate & Nitrite Contents

With 25.0 mL ice-cold Milli-Q water, approximately 1.0 g frozen sample was homogenised at 22,000 rpm (series X10/25, Ystral™, Ballrechten-Dottingen, GER). These were centrifuged for 15 min at 2,465 g and 4.0 °C (model 5810R, Eppendorf™ Pty. Ltd., Hamburg, GER) and the supernatant analysed against the nitrate and nitrate plus nitrite standards using the nitrite/nitrate assay kit (no. 23479, Sigma-Aldrich Pty. Ltd., Missouri, USA) colorimetric protocol (Sigma-Aldrich, 2015c). This entailed measuring absorbance at 540 nm, using the same micro-plate reader as previous and subtracting nitrate results from nitrate plus nitrite results to determine nitrite content. All samples were tested as technical duplicates which were averaged and expressed as ppm.

4.12   Oxidation-Reduction Potential

To determine oxidation-reduction potential (ORP), approximately 1.0 g of LL was combined with 25.0 mL Milli-Q water; homogenised at 22,000 rpm (series X10/25, Ystral™, Ballrechten-Dottingen, GER); and centrifuged at 3,500 rpm and 4 °C for 15 min (model CPR, Beckman Instruments™ Ltd., California, USA). A calibrated ORP probe attached to a benchtop monitor (models ORP110-GS and HQ440d respectively, HACH Pacific™ Pty. Ltd., Victoria, AUS) was then used to quantify supernatant ORP and temperature data. Technical duplicates were assessed with the average ORP expressed as millivolts (mV) per g LL.

4.13   Particle Size Analysis

Particle size (PS) was measuring using approximately 1.0 g frozen sample, homogenised at 16,000 rpm (series X10/25, Ystral™, Ballrechten-Dottingen, GER) and measured using a laser diffraction PS analyser (model LS-13/320, Beckman Coulter Pty. Ltd., Miami, USA) connected to a water unit with samples added in a drop-wise fashion (Karumendu et al., 2009). Sample duplicates were averaged to calculate PS.

4.14   Peroxidase Activity

Peroxidase activity (PA) was measured using approximately 25.0 mg of LL homogenised using micro-tube pestles with 200.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals™ Ltd., Michigan, USA). These were centrifuged and the supernatant was then analysed against peroxide (H_2O_2) standards using the colorimetric protocol outlined by the Peroxidase Activity Assay Kit (no. MAK092, Sigma-Aldrich Ltd., Missouri, USA) technical bulletin (Sigma-Aldrich, 2015d) and a micro-plate reader set to measure absorbance at 570 nm (FLUOstar OPTIMA, BMG Labtechnologies™ Ltd., Victoria, AUS). Regular measurements were made over 10-15 min so as to allow the catalysis of H_2O_2 from PA to be charted. PA was expressed as the amount of peroxidase (mmole) that reduces 1.0 µmole H_2O_2 per minute per g LL at 37 °C (U). Each sample was tested in duplicate with the average recorded.

4.15   Protein Solubility

An adapted method was used (Farouk & Swan, 1998), where 25.0 mg frozen sample was placed into twin tubes that contained either total or sarcoplasmic protein buffers. Total protein buffer (pH 7.2) contained 1.1 M potassium iodine and 0.1 M potassium phosphate monobasic; and the sarcoplasmic

buffer (pH 7.2) contained 0.025 M potassium phosphate monobasic – these reagents were all sourced from Sigma-Aldrich Pty. Ltd (Missouri, USA). Samples were homogenised using microtube pestles; incubated under refrigeration (4.0-5.0 °C) overnight; and centrifuged prior to analysing the supernatant as per the bicinchoninic acid kit for protein determination (BCA1, Sigma-Aldrich Pty. Ltd, Missouri, USA) technical bulletin (Sigma-Aldrich, 2015a) and a micro-plate reader (FLUOstar OPTIMA™, BMG Labtechnologies, Victoria, AUS) set to measure absorbance at 540 nm. Myofibrillar protein content was determined as the difference between total and sarcoplasmic protein content, and technical duplicates were averaged so that all protein solubilities were expressed as mg protein per fresh (wet) weight in g.

4.16   Purge

Once storage treatment combinations were complete, each corresponding LL portion was weighed, prior to subsection, and the percentage difference pre- and post-storage was used to calculate *purge loss*.

4.17   Sarcomere Length

Thin slices (thickness: < 1.0 mm) were removed parallel to the muscle fibre orientation from each sample. These were then analysed using laser-diffraction, as per Bouton, Carrol, Harris, & Shorthose (1973), with the average from five technical replicates used to derive sarcomere length (SL).

4.18   Shear Force

All cooked samples (*see 4.4 Cooking Loss*) were kept refrigerated overnight (at 4-5 °C) and then sectioned so that six cuboidal strips (with 1.0 cm x 1.0 cm cross-sectional area) were removed parallel to muscle fibre direction. These were then cut perpendicular to the fibre direction using a Warner-Bratzler blade with 200.0 mm/min crosshead speed, attached to a texture analyser (Model LRX, Lloyd Instruments™ Ltd., Hampshire, UK) that measured the peak force in newtons. Care was taken so that the cutting line avoided connective and fatty tissue, and shear force (SF) was calculated as the average of these six technical replicates (Holman, Alvarenga, van de Ven, & Hopkins, 2015).

4.19   Thiobarbituric Acid Reactive Substances

Sample TBARS quantification method was adapted from Hopkins, Clayton, Lamb, Van de Ven, Refshauge, Kerr, Bailes, Lewandowski, & Ponnampalam (2014) and used 500.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals™ Ltd., Michigan, USA) added to 100.0 mg of LL, then homogenised using micro-tube pestles. These were centrifuged and the supernatant analysis as per the OXI-tek TBARS Assay Kit (no. ALX-850-287-KI01, Enzo® Life Sciences Inc., New York, USA) technical bulletin (Zeptometrix, 2006). Absorbance was measured at 532 nm using a benchtop spectrophotometer. TBARS data was expressed as mg malondialdehyde (MDA) per kg LL.

4.20   Total Losses

Total loss was calculated as per Farouk & Swan (1998) as the summation of purge (*see 4.16 Purge*), thaw loss (*see 4.22 Thaw Loss*) and cooking loss (*see 4.4 Cooking Loss*) results.

4.21   Total Moisture Content

Moisture content was the percentage weight difference between pre- and post-freeze drying of samples at – 50 °C (ScanVac CoolSafe™, LaboGene ApS., Lynge, DEN). Duplicates (~ 25.0 g each) were evaluated and averaged.

4.22 Thaw Loss

The colour stability portions (*see 4.3 Colour Stability*) were used to measure *thaw losses*, as the percentage differences in weight between frozen (measured on 0 d, prior to display) and thawed (measured on 1 d). Weights were recorded after samples were dried with paper towel for each interval.

4.23 Ultimate pH (final)

Ultimate pH (pHu), sometimes termed final pH, was quantified using a pH meter (Model smartCHEMC-CP, TPS Ltd., Queensland, AUS) fitted with a polypropylene spear-type gel electrode (Model IJ-44, Ionode™ Ltd., Queensland, AUS) calibrated at pH 4.0 and pH 6.8 (at 20 °C) to evaluate samples, following the method described by De Brito et al. (2016). Duplicate measures were averaged to determine pHu.

4.24 Water Activity

Water activity (a_w) was measured using a specialised handheld hygrometer (Model HC2-AW: HygroPalm23-AW, Rotronic Instruments™ Corp., New York, USA) that capped a 14.0 mm deep sample cup containing approximately 1.0 g of frozen and minced sample. The probe remained *in situ* until a_w values stabilised (~ 30 min) and both a_w and temperature (°C) data were recorded. Each sample was measured twice (technical duplicates), and the results were averaged.

5.0 STATISTICAL ANALYSES

5.1 Beef

The response of the measured parameters to the experimental factors freeze time, chill time and temperature was estimated by linear models. A response surface modelling framework was attempted given the quantitative nature of the treatment matrix but exploration of the data revealed no systematic responses to the treatments. Therefore the experimental factor levels were taken as qualitative and the linear model was formed to estimate mean response at every combination of freeze time, chill time and temperature. The nonsensical contrast of -12 °C vs -18 °C for unfrozen samples (0 weeks freezing) was excluded from the model by construction of a 2 level control factor classifying the samples as frozen or unfrozen. Terms in the linear model were then formed by nesting the main effects and interactions between freeze time, chill time and freeze temperature within the control factor levels. In the commonly accepted shorthand for linear statistical models, the treatment model was:

Control / (Frozen Storage Period × Chilled Storage Period × Frozen Storage Holding Temperature)

Null hypothesis significance tests for the experimental factors were conducted by analysis of variance derived from the model. Mean responses under each treatment combination and standard errors of the means were also calculated. Pairwise comparison of means after rejection of the relevant null hypothesis was conducted by least significant difference calculated at 5% critical value. All data analysis was conducted in the R environment (R Core Team, 2016).

5.2 Lamb

5.2.1 Sensory Quality and Microbial Load

To fit smooth non-linear effects of chilled and frozen storage periods, the linear mixed model smoothing spline framework was used as described by Verbyla, Cullis, Kenward, & Welham (1999)

and was implemented in the statistical package *asreml* (Butler, 2009) under R (R Core Team, 2016). The full model for each response incorporated all the terms required to fit a two dimensional smoothing spline for chilled storage period by frozen storage period, as well as accounting for the experimental design.

Fixed effects included were linear chilling period, linear freezing period, temperature and all interactions; random effects were spline chilled and frozen storage periods, as well as their interactions with linear chilled and frozen storage periods, temperature and each other. Remaining terms in the model accounted for design effects and included non-smooth (factor) effects of chilled and frozen storage period, freezer effects and interactions of freezer with chilled and frozen storage periods. Of the measured traits only data for purge required transformation (square root). Additional covariates were added to the above model as fixed linear effects and a random spline effect; these included pHu on all traits, SL on SF, and glycogen content on microbial loading.

For microbial data, random data was imputed as data above or below the upper or lower limits of detection, which was generated as random uniform data on a logarithmic ($\log_{10}$) scale. In this case, the imputed data was given a reduced weight of 0.5 in the analysis.

Following the approach in (Verbyla et al., 1999), the statistical significance of fixed and random effects were examined separately in *asreml* (Butler, 2009) in R (R Core Team, 2016), with a Wald-type test with Kenward-Roger adjustment (Kenward & Roger, 1997) and likelihood ratio comparisons used to examine significance of temperature-related and spline components, respectively. The level of significance was set at $P < 0.05$.

5.2.2   Fatty Acid and Lipid oxidation Markers

The response of the measured parameters to the experimental factors (chilled storage period, frozen storage period and frozen storage temperature) was estimated by linear models. A response surface modelling framework was attempted given the quantitative nature of the treatment matrix, but exploration of the data revealed no systematic responses to the treatments. Therefore, the experimental factor levels were taken as qualitative and a linear model was formed to estimate mean responses at every combination of chilled storage period, frozen storage period and frozen storage temperature. The nonsensical contrast of frozen storage temperature (-12 vs -18 °C) for chilled-only (unfrozen) samples was excluded from the model by construction of a 2 level control factor classifying the samples as frozen or unfrozen. Terms in the linear model were then formed by nesting the main effects and interactions between chilled storage period, frozen storage period and frozen storage temperature within the frozen group of samples.

Null hypothesis significance tests for the experimental factors were conducted by analysis of variance derived from the model. Mean responses under each treatment combination and standard errors of the means were also calculated. Pairwise comparison of means after rejection of the relevant null hypothesis was conducted by least significant difference calculated at 5% critical value. All data analysis was conducted in the R environment (R Core Team, 2016). The level of significance in this study was set at $P < 0.05$.

Because of the large number of individual FA evaluated in this study, these were simplified as summative values and ratios (Table 5.2.2) to be analysed, along with the major individual contributors for each FA type.

Table 5.2.2. Individual fatty acid (FA) added together to calculate summative (total) FA profile terms. FA in bold italics were the two most significant contributors to the summative terms.

Summative FA Profile Terms	Individual FA Profile Contributors
Saturated fatty acids (SFA)	C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0
Unsaturated fatty acids (UFA)	C14:1, C15:1, C16:1, C17:1, C18:1iso, C18:1ω9, C18:2ω6, C18:2ω6t, C18:3t, C18:3ω3, C18:3ω6, C20:1, C20:2ω6, C20:3ω3, C20:3ω6, C20:4ω3, C20:4ω6, C20:5ω3, C22:1, C22:2ω6, C22:5ω3, C22:6ω3, C24:1
Monounsaturated fatty acids (MUFA)	C14:1, C15:1, C16:1, C17:1, C18:1iso, C18:1ω9, C20:1, C22:1, C24:1
Polyunsaturated fatty acids (PUFA)	C18:2ω6, C18:2ω6t, C18:3t, C18:3ω3, C18:3ω6, C20:2ω6, C20:3ω3, C20:3ω6, C20:4ω3, C20:4ω6, C20:5ω3, C22:2ω6, C22:5ω3, C22:6ω3
Omega-6s (n-6)	C18:2ω6, C18:2ω6t, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:2ω6
Omega-3s (n-3)	C18:3ω3, C20:3ω3, C20:4ω3, C20:5ω3, C22:5ω3, C22:6ω3
Health claimable fatty acids (EPA+DHA)	C20:5ω3, C22:6ω3

5.2.3 Indicators of Protein Oxidation and Indicators of Protein Degradation

To fit smooth non-linear effects of chilled and frozen storage periods, the linear mixed model smoothing spline framework was used as described by Verbyla et al. (1999) and was implemented in the statistical package *asreml* (Butler, 2009) under R (R Core Team, 2016). The full model for each response incorporated all the terms required to fit a two dimensional smoothing spline for chilled storage period by frozen storage period, as employed previously (5.2.1. Sensory Quality and Microbial Load).

Fixed effects included in the model included temperature, linear freezing period, linear chilling period and all interactions; random effects were spline chilled and frozen storage periods, as well as their interactions with linear chilled and frozen storage periods, temperature and each other. Remaining terms in the model accounted for design effects and included non-smooth (factor) effects of chilled and frozen storage period, freezer effects and interactions of freezer with chilled and frozen storage periods.

For the SF and predictive traits model, fixed linear and random spline components for each trait (carbonyl, total protein solubility, myofibrillar protein solubility, sarcoplasmic protein solubility, nitrate, nitrite, PS, DMb, MMb and OMb) were included in turn. The relationship between the mean values of SF for each chilled-only storage period against each of the first seven covariates was also examined, for chilled-only storage periods only, and summarised using coefficients of determination (r^2).

Following the approach in Verbyla et al. (1999), the statistical significance of fixed and random effects were examined separately in *asreml* (Butler, 2009) in R (R Core Team, 2016) with a Wald-type test with Kenward-Roger adjustment (Kenward & Roger, 1997) and likelihood ratio comparisons used to examine significance of temperature-related and spline components, respectively. The level of significance was set at $P < 0.05$.

6.0 PROJECT OUTCOMES

6.1 Beef

6.1.1 Sensory Qualities

Colour stability

Chilled-only storage effects were observed as linear increases to L* (lightness) values between 0 to 3 weeks storage and to similar extents over all display periods (0-3 d), although L* levels tended to be, in general, higher at the latter display periods. L* was also influenced by frozen-only storage with slight linear increases evident with increased storage period ($P < 0.05$). The degree of these increases was different between chilled- and frozen-only samples ($P < 0.05$), but when combined as chilled-then-frozen, L* increases over chilled storage periods were most when followed by 52 weeks frozen storage comparative to shorter subsequent frozen storage durations and regardless of display period ($P < 0.05$).

Sample a* (redness) values were shown to decline as chilled-only storage period increased, the degree of the decline was more apparent in the latter display periods and following 2 weeks storage ($P < 0.05$). A more linear decline in a* was observed with increased frozen-only storage period, provided the display period exceeded 0 d, and this divergence resulted in the a* difference between chilled- and frozen-only samples ($P < 0.05$). The chilled-then-frozen storage period effect also proved significant with a decline in a* values more obvious between c0:f52 to c5:f52 than other combinations where a* was somewhat consistent until this decline. These plateaued a* values over chilled storage periods seemed to endure into subsequent frozen storage periods to a greater degree as their display period increased.

Generally, b* (yellowness) values were consistent across chilled-only storage periods except at 1-2 d display where a duality was apparent as c0:f0 and c2:f2 had higher b* than the subsequent chilled storage periods ($P < 0.05$). Frozen-only storage effects were instead evident from 1 d display forward where b* seemed to decline at 12 weeks and remained comparatively unchanged at these levels for the remaining frozen storage periods ($P < 0.05$). Somewhat similar chilled-then-frozen storage effects resulted in b* levels tending to increase from c0:f4 to c5:f4 and c0:f8 to c5:f8 beyond which, 12 weeks subsequent frozen storage, b* levels were relatively unchanged ($P < 0.05$). It was also worth noting that chilled-only sample b* results were, for the most part and with the exception of at 0 d display, higher than their frozen-only counterparts ($P < 0.05$).

Chroma was shown to decrease across the chilled-only storage periods, but this was only observed after 1 d display and between 2 and 3 weeks storage ($P < 0.05$). Frozen-only storage also resulted in lower chroma levels, again following 1 d display, especially when periods exceeded 8 weeks ($P < 0.05$). Combined, chilled-then-frozen was found to influence chroma with levels tending to increase or remain stable between 0 and 5 weeks chilled storage when these were followed by 4 and 8 weeks subsequent frozen storage periods, unlike at longer frozen storage periods where chroma seemed to decline ($P < 0.05$). The extent of these changes to chroma were different across the display periods evaluated ($P < 0.05$).

Compared to their frozen-only counterparts, hue was found to be lower when samples were kept under chilled-only storage ($P < 0.05$). Furthermore, hue peaked at 5 weeks chilled-only storage when displayed for 2-3 d, but earlier when displayed for shorter periods ($P < 0.05$). Like b* and Chroma, hue also tended to increase across the first frozen-only storage periods (4 and 8 weeks) before

stabilising into the longer durations ($P < 0.05$) – yet, at display periods 2-3 d the samples kept at -12 °C were shown to have more dramatic adherence to this trend than those kept at -18 °C throughout the frozen storage ($P < 0.05$). Chilled-then-frozen storage effects were also evident, for example at 2 d display there were differences in the level of hue increase from 0 to 5 weeks preceding chilled storage periods that developed relative to increases to the following frozen storage periods ($P < 0.05$).

Chilled-then-frozen storage resulted in variations to the R630/580 ratio across all, but 3 d display, wherein the decline across chilled storage period was shown to increase in magnitude as the subsequent frozen storage period surpassed 12 weeks ($P < 0.05$). Individually, frozen-only storage resulted in a slight linear decline across increasing periods provided samples were displayed for more than 1 d ($P < 0.05$). Chilled-only storage also resulted in R630/580 declines as period increased, but instead of a gradual decrease this was most obvious between 2 and 3 weeks chilled-only storage ($P < 0.05$). Comparatively, chilled-only R630/580 values were higher than frozen-only samples for all display periods ($P < 0.05$).

Unless otherwise listed, all other storage treatment combinations and temperature effects were found to be insignificant in their influence on beef colorimetrics. All colour results are presented in Figs. 6.1.1.1 and 6.1.1.2.
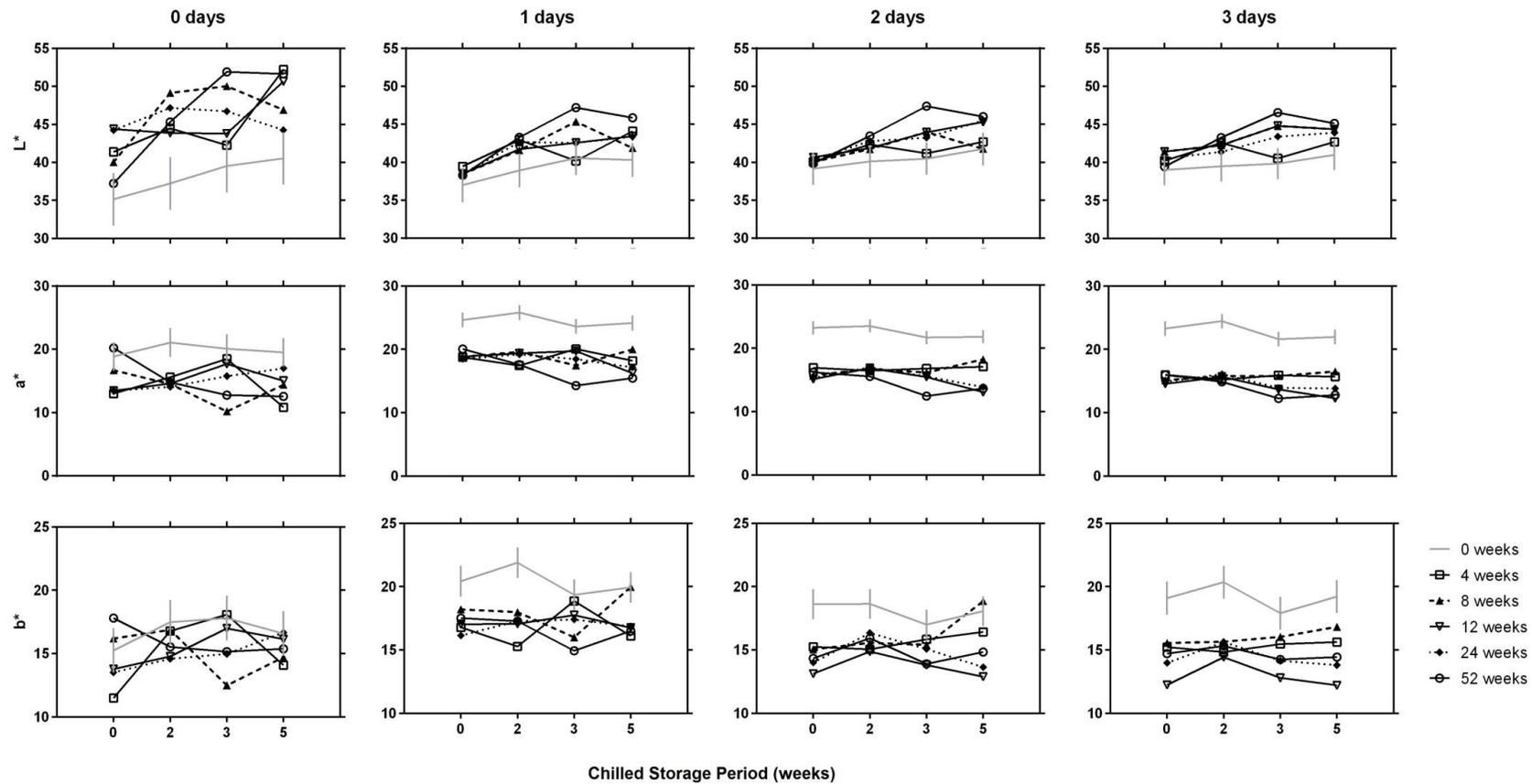
Fig. 6.1.1.1. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* CIE colorimetric (a*, b* and L*) stability across 3 d display. Predicted means from samples frozen at – 18 °C and – 12 °C were averaged because of the absence of significant difference, these were plotted so frozen storage period (unique identifiers are depicted in the key) could be compared against chilled-only samples (grey lines with no markers), across preceding chilled storage and within display periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.
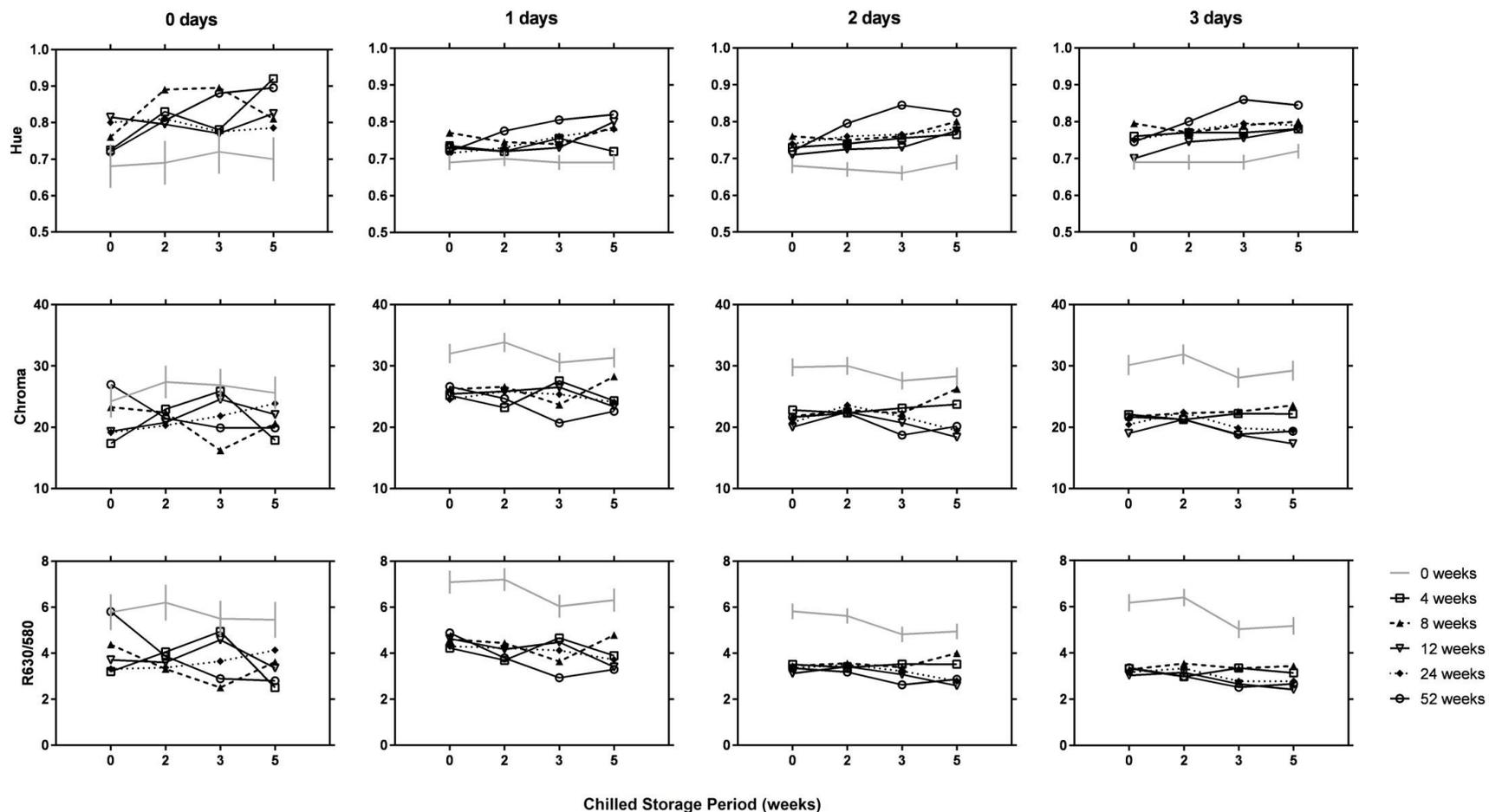
Fig. 6.1.1.2. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* chroma, hue and the ratio of reflectance measured at 630 nm and 580 nm (R630/580) stability across 3 d display Predicted means from samples frozen at − 18 °C and − 12 °C were averaged because of the absence of significant difference, these were plotted so frozen storage period (unique identifiers are depicted in the key) could be compared against chilled-only samples (grey lines with no markers), across preceding chilled storage and within display periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

25

Intramuscular fat content

No significant effects were observed from storage treatment combinations on IMF content (mean ± SE: 6.1 ± 1.6 %).

Fluid losses (including, purge, cook, thaw and total losses)

Purge differed between chilled-only storage periods, as levels at 0 weeks were significantly lower than at 3 weeks ($P < 0.05$; Fig. 6.1.1.3a). Likewise at chilled-only storage periods > 3 weeks, purge was found to be higher than the frozen-only equivalents ($P < 0.05$) where levels seemed consistent over frozen-only storage periods except for a few uncharacteristic and significant variations ($P < 0.05$) that could account for the observed chilled-then-frozen storage effect on purge ($P < 0.05$). No significant effects were observed from storage treatment combinations on thaw loss (mean ± SE: 5.3 ± 1.2 %).

Cooking loss was higher when samples were kept under chilled-only storage compared to frozen-only ($P < 0.05$; Fig. 6.1.1.3b). This is reflected by the increases in CL level observed as chilled-only storage period increased before levelling out after 3 weeks ($P < 0.05$) and the CL decreases across increasing frozen-only storage periods ($P < 0.05$). When these were considered together as chilled-then-frozen, CL was found to decrease over chilled storage duration when subsequent frozen storage durations were 12 weeks or more, albeit at c5:f24 a recovery was apparent. This differed from the preceding chilled-then-frozen combinations where CL proved consistent between c0:f8 to c5:f8 and increased between c0:f4 to c3:f4 ($P < 0.05$). No other storage treatment combination effect was significant.

Only chilled-then-frozen storage effects were significant ($P < 0.05$; Fig. 6.1.1.3c) with total losses shown to increase within chilled storage and across the subsequent frozen storage before peaking at different periods – which, for the most part, was following 12 weeks frozen storage.
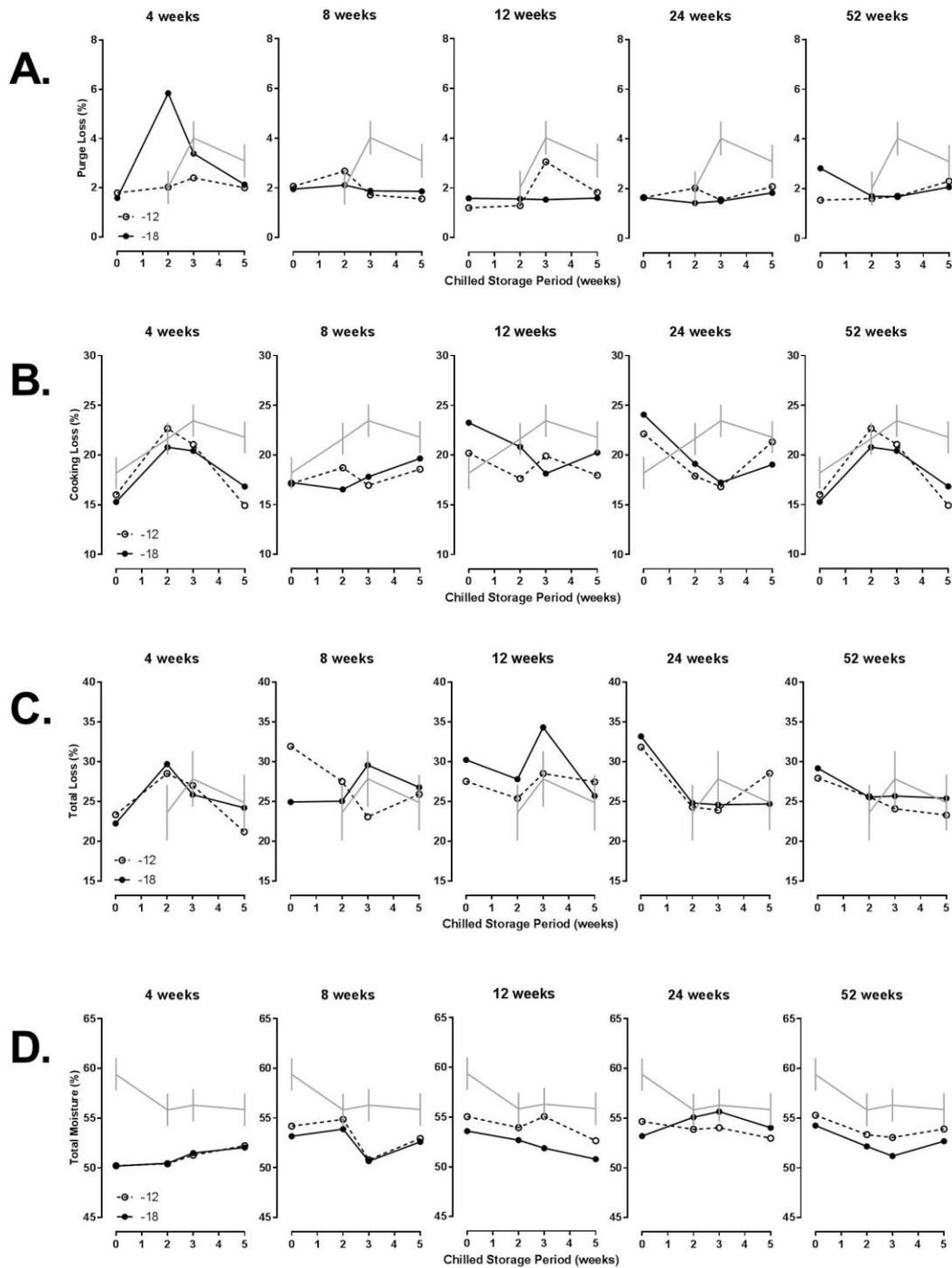
Fig. 6.1.1.3. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* percentage; a) Purge; b) Cook loss; c) Total loss; and d) Moisture content Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

27

Glycogen content

Frozen-only sample glycogen contents were shown to increase across the storage periods ($P < 0.05$) although, as apparent in Fig. 6.1.1.4, this was not a linear change. As a result, chilled-then-frozen storage effects were found ($P < 0.05$) as the linear increase only occurred across chilled storage periods followed by 4 and 8 weeks frozen storage and the other periods were static in terms of any variation. No other differences from storage treatment combinations were identified.

Fig. 6.1.1.4. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* glycogen content in mmol per kg. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Moisture content

Sample moisture content was found to decrease with increasing chilled-only storage periods, especially between 0 and 2 weeks ($P = 0.05$; Fig. 6.1.1.3d). Moisture content also increased as frozen-only storage periods increased ($P < 0.05$), but when comparing the two, moisture was proportionally higher in chilled-only samples compared with frozen-only samples ($P < 0.05$). Chilled-then-frozen storage was shown to broadly result in moisture content declines at differing severities ($P = 0.05$), and even the opposite direction, instead increasing between c0:f4 to c5:f4. No other storage treatment combination proved significant.

Particle size

PS was shown to decline with increasing chilled-only storage periods ($P < 0.05$), and this trend was also observed for chilled-then-frozen samples ($P < 0.05$) albeit the extent of this decline was broadly reduced as ensuing frozen storage periods increased – for example, there was a 41.5% decrease in PS between c0:f52 and c5:f52 compared to 33.1% between c0:f4 and c5:f4 (Fig. 6.1.1.4). Increasing frozen-only storage period instead resulted in increased PS ($P < 0.05$) suggesting it as the likely basis for the observed chilled-then-frozen storage effect and the significant difference between chilled-only and frozen-only sample PS. No other storage treatment effects were significant.

Fig. 6.1.1.5. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* particle size. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Protein solubility

Fig. 6.1.1.6a illustrates the significant decline in sarcoplasmic protein solubility across chilled-only storage which was then preserved over the ensuing frozen storage periods, evident from the absence of any chilled-then-frozen storage effect. Frozen-only storage also resulted in declining sarcoplasmic protein solubility between 4 and 12 weeks before levelling across the following periods. Total protein solubility increased between 0 and 2 weeks before it plateaued and declined for samples held in chilled-only storage. This was influenced by chilled-then-frozen storage ($P < 0.05$) whereby some variation in this initial 'peak' observed with increasing subsequent frozen storage periods. Alone, frozen-only storage resulted in total protein solubility peaking at 12 weeks, followed by a significant decline – although some inconsistencies with this trend did occur and maybe resulted from the chilled-then-frozen storage effect (Fig. 6.1.1.6b). Myofibrillar protein solubility, similarly to total protein solubility, also increased from 0 to 2 weeks chilled-only storage, but by contrast plateaued across the remaining periods (Fig. 6.1.1.6c). This outcome was reflected by chilled-then-frozen storage effects as the extent of myofibrillar protein solubility increase beyond 2 weeks differed dependent on the ensuing frozen storage period ($P < 0.05$). Myofibrillar protein solubility was broadly shown to increase with increasing frozen-only storage periods ($P < 0.05$), but as per total protein solubility, this was not a uniform trend. All other storage treatments and their combination effects were insignificant.
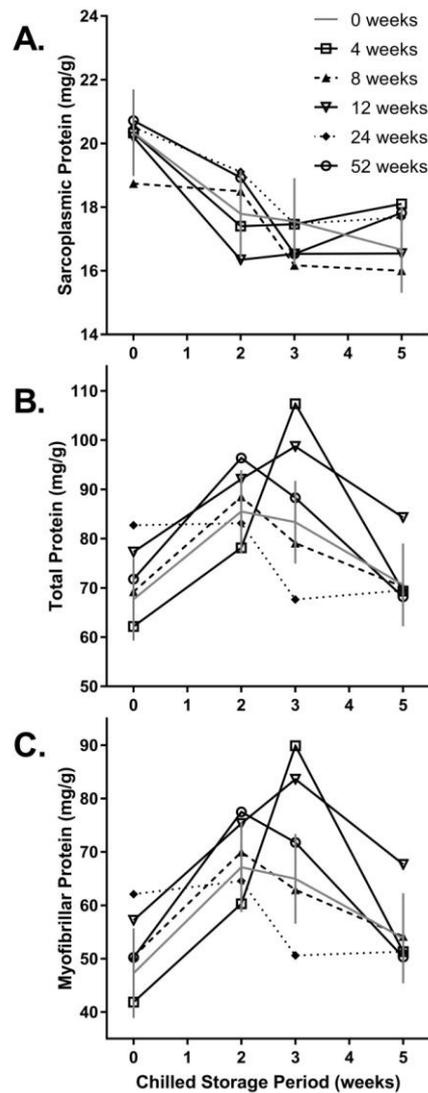
Fig. 6.1.1.6. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* protein solubility of A) sarcoplasmic, B) total, and C) myofibrillar proteins. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Shear force determination

Chilled-only storage resulted in declining SF as period increased, albeit this was only significant when 0 and 5 weeks were compared ($P < 0.05$; Fig. 6.1.1.7). Frozen-only sample SF instead increased with storage period ($P < 0.05$). From chilled-then-frozen storage, SF was seen to decline as chilled storage periods increased, with this decline beginning to plateau at earlier chilled storage periods when the

following frozen storage periods increased ($P < 0.05$) – for example, SF plateaued at c3:f8 compared with c2:f24. All other storage treatment combination effects on SF were insignificant.
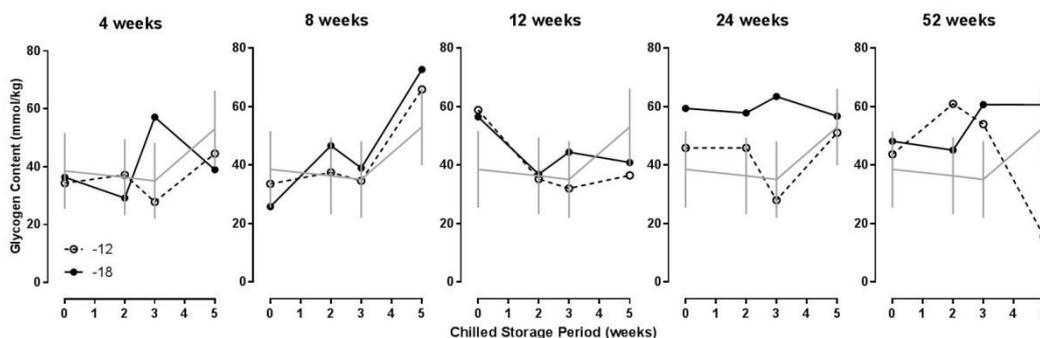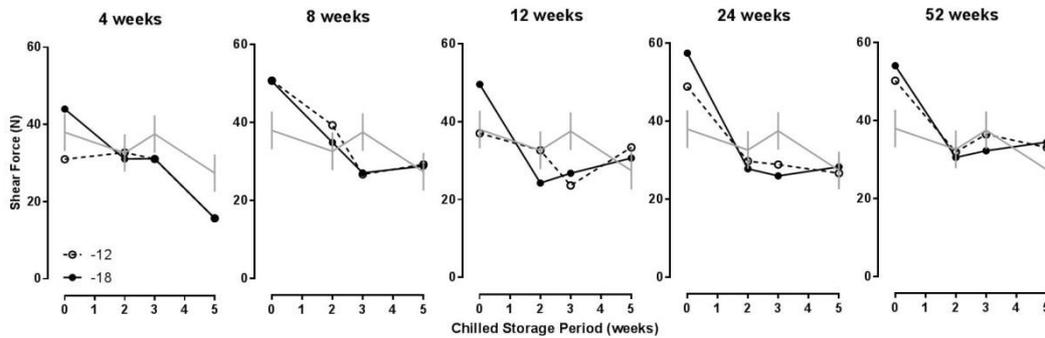

Fig. 6.1.1.7. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* shear force (SF) in newtons. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Sarcomere length

No significant effects on SL were found for any storage treatment combination (mean ± SE: 1.85 ± 0.09 µm).

Ultimate (Final) pH

pHu increased with increased chilled-only storage periods (Fig. 6.1.1.8; $P < 0.05$) and the opposite for frozen-only storage periods up until 12 to 24 weeks when pHu then increased again ($P < 0.05$). The point of this trajectory change was dependent on chilled-then-frozen storage combination ($P < 0.05$) and as a result of this divergence, chilled- and frozen-only samples were found to significantly differ ($P < 0.05$). No other storage treatment combinations proved to significantly influence pHu.
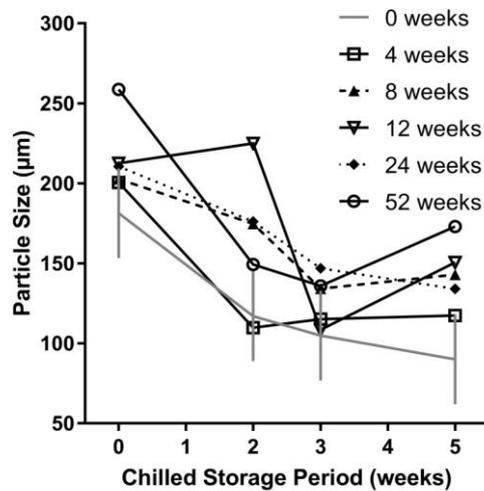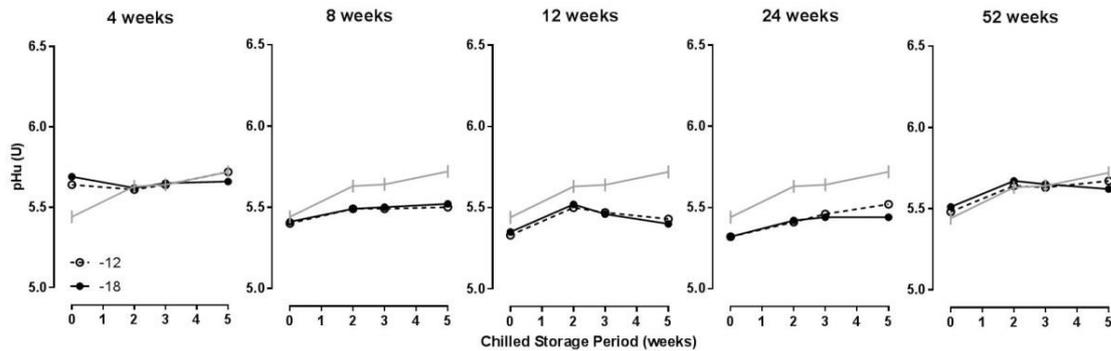
Fig. 6.1.1.8. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* final or ultimate pH (pHu). Predicted means were plotted so that samples frozen at – 18 °C (solid lines and solid markers) and – 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Water activity

Between 0 and 5 weeks chilled-only storage periods a_w was observed to increase ($P < 0.05$; Table 6.1.1.1), however the opposite was found across frozen-only storage periods where a_w values were stable before declining at 24 and 52 weeks ($P < 0.05$). Consequently, it was no surprise that chilled- and frozen-only sample a_w were found to differ ($P < 0.05$). Chilled-then-frozen storage effects were also significant ($P < 0.05$) if not as consistent, with a_w levels shown to increase between c0:f8 to c5:f8 and c0:f24 to c5:f24, but decrease from c0:f12 to c5:f12 and c0:f52 to c5:f52. Neither frozen storage temperature nor any other storage treatment combination was found to otherwise influence a_w.

Table 6.1.1.1. Predicted means for beef *M. longissimus lumborum* water activity (a_w) levels for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp).

Frozen Storage (weeks)	Temp (°C)	Chilled Storage (weeks)				
		0	2	3	5	SEM
0	(unfrozen)	0.95	0.95	0.95	0.96	<0.01
4	-12	0.95	0.96	0.95	0.95	
	-18	0.96	0.96	0.95	0.95	
8	-12	0.96	0.91	0.96	0.97	
	-18	0.96	0.91	0.96	0.97	
12	-12	0.96	0.91	0.96	0.91	<0.01
	-18	0.96	0.91	0.96	0.91	
24	-12	0.91	0.92	0.91	0.96	
	-18	0.92	0.91	0.91	0.96	
52	-12	0.93	0.92	0.91	0.91	
	-18	0.93	0.92	0.91	0.91	

6.1.2 Microbial Status

For each microbial species tested, there were insufficient instances of detection to merit analysis and as such, *E. coli* (detection level: < 10), *Enterobacteriaceae sp.* (< 10), *C. perfingens* (< 10), LAB (< 100) and *B. thermospacta* (< 100) failed to significantly vary across storage treatment combinations.

6.1.3 Fatty Acid Profile

SFA levels were observed to be higher in frozen samples compared to their unfrozen counterparts (P < 0.05; Table 6.1.3.1), with 16:0 and 18:0 shown as the major contributors to SFA values (28% and 20% respectively). Frozen sample unsaturated FA (UFA) was instead lower than their unfrozen equivalents (P < 0.05) with 18:1ω9 the key constituent (35%). This trend permeated to monounsaturated FA (MUFA) levels which were found to increase with chilled-only storage periods (P < 0.05) and marginally decrease with frozen-only storage periods (P < 0.05); but not PUFA where no significant storage treatment effects were shown. That said, PUFAs did seem to decline as chilled-only storage period increased and instead remain consistent over frozen-only storage periods (Table 6.1.3.1). This insignificant trend was also reflected by total omega-3, total omega-6 and Rn3n6 results (Fig. 6.1.3.1), but is noteworthy for health claimable FA data as EPA+DHA levels reduced by approximately 43% between 0 and 5 weeks chilled-only storage (Fig. 6.1.3.2) – manifesting as a 100% and 46% decline in DHA and EPA, respectively, over this same interval. This, however, and all other storage treatment effects on FA summative terms not otherwise listed were not significant.
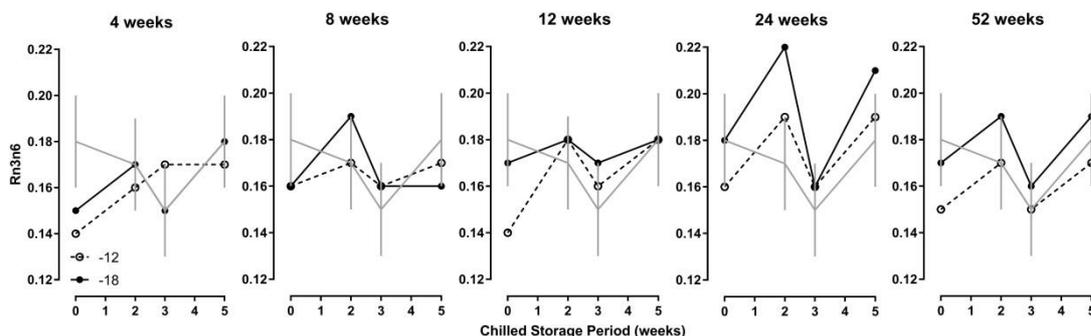
Fig. 6.1.3.1. Effect of chilled-then-frozen storage duration on the ratio of omega-3 and omega-6 fatty acids (Rn3n6) for beef *M. longissimus lumborum*. Predicted means were plotted so that samples frozen at – 18 °C (solid lines and solid markers) and – 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.
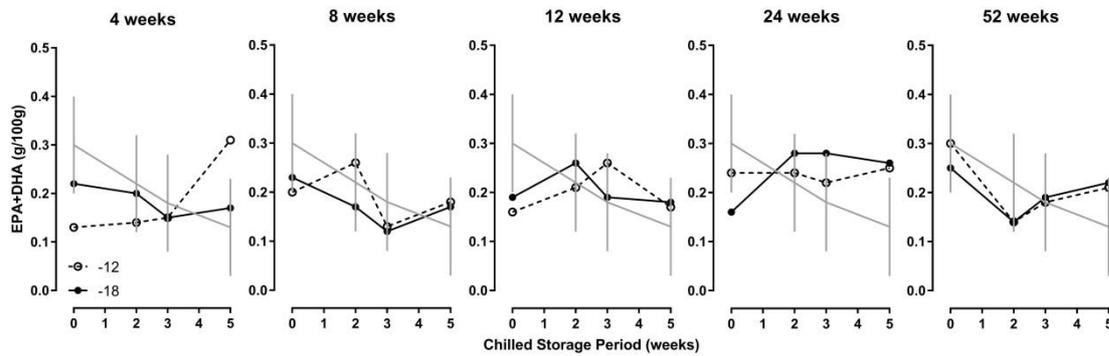
Fig. 6.1.3.2. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) content in g per 100 g lipid. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Table 6.1.3.1. Predicted means and their standard error (SEM) for beef M. *longissimus lumborum* summative fatty acid (FA) terms including total saturated fatty acid (SFA); monounsaturated fatty acid (MUFA); polyunsaturated fatty acid (PUFA); and unsaturated fatty acid (UFA) values (g per 100 g lipid) for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp).

FA Summative Term	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
	0	(unfrozen)	47.58	48.88	48.43	47.44	0.78
	4	-12	48.45	48.44	50.35	48.03	
		-18	49.16	48.55	49.23	50.27	
	8	-12	48.47	49.36	51.59	49.45	
		-18	48.25	48.87	49.71	48.95	
SFA	12	-12	49.09	48.51	48.74	49.47	
		-18	50.18	48.27	49.02	48.28	1.05
	24	-12	48.70	49.92	49.18	50.41	
		-18	50.15	50.18	48.04	49.47	
	52	-12	47.10	49.85	49.03	49.89	
		-18	48.37	50.12	48.34	47.78	
	0	(unfrozen)	45.06	44.72	45.59	48.01	0.85
	4	-12	46.01	46.49	44.58	44.70	
		-18	44.88	45.46	45.30	44.71	
	8	-12	45.00	44.11	43.65	44.34	
		-18	45.69	45.82	45.47	46.04	
MUFA	12	-12	44.47	45.12	43.82	44.95	
		-18	44.51	45.25	45.34	46.24	1.07
	24	-12	43.40	43.14	44.70	42.50	
		-18	44.72	43.70	44.24	44.87	
	52	-12	44.42	44.50	44.87	43.45	
		-18	45.29	45.13	45.39	46.42	
	0	(unfrozen)	7.31	6.51	5.99	4.66	0.62
	4	-12	5.27	5.08	5.17	7.34	
		-18	6.32	5.96	5.33	5.35	
	8	-12	6.36	6.80	4.79	5.99	
		-18	6.38	5.51	4.81	5.04	
PUFA	12	-12	6.10	6.10	7.41	5.34	
		-18	5.53	6.54	5.68	5.43	0.85
	24	-12	7.58	6.70	6.20	6.91	
		-18	5.24	6.17	7.77	5.99	
	52	-12	8.37	5.46	6.20	6.29	
		-18	6.65	4.71	6.01	5.76	
	0	(unfrozen)	52.42	51.12	51.57	52.56	0.78
	4	-12	51.55	51.56	49.65	51.97	
		-18	50.84	51.45	50.77	49.73	
	8	-12	51.53	50.64	48.41	50.55	
		-18	51.75	51.13	50.29	51.05	
UFA	12	-12	50.91	51.49	51.26	50.53	
		-18	49.82	51.73	50.98	51.72	1.05
	24	-12	51.30	50.08	50.82	49.59	
		-18	49.85	49.82	51.96	50.53	
	52	-12	52.90	50.15	50.97	50.11	
		-18	51.63	49.88	51.66	52.22	

Table 6.1.3.1. (cont.) Predicted means and their standard error (SEM) for beef M. *longissimus lumborum* summative fatty acid (FA) terms including omega-3 (n-3); and omega-6 (n-6) values (g per 100 g lipid); and, ratio of n-3 to n-6 (Rn3n6) for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp).

FA Summative Term	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
	0	(unfrozen)	1.10	0.96	0.79	0.68	0.11
	4	-12	0.63	0.73	0.72	0.99	
		-18	0.87	0.90	0.73	0.84	
	8	-12	0.84	1.07	0.66	0.83	
		-18	0.91	0.85	0.68	0.74	
n-3	12	-12	0.76	0.91	1.01	0.74	0.16
		-18	0.80	0.95	0.81	0.82	
	24	-12	0.96	1.04	0.85	1.18	
		-18	0.78	1.11	1.06	1.05	
	52	-12	1.03	0.75	0.77	0.87	
		-18	0.96	0.75	0.83	0.93	
	0	(unfrozen)	6.21	5.70	5.20	4.06	0.54
	4	-12	4.23	4.42	4.38	6.45	
		-18	5.70	5.07	4.45	4.75	
	8	-12	5.62	6.10	4.29	5.07	
		-18	5.63	4.57	4.24	4.30	
n-6	12	-12	4.90	4.82	6.41	4.34	0.76
		-18	4.89	5.79	4.87	4.56	
	24	-12	6.40	5.43	5.46	5.75	
		-18	4.20	5.02	6.71	5.18	
	52	-12	7.48	4.47	5.43	5.13	
		-18	6.21	3.98	5.01	4.75	
	0	(unfrozen)	0.18	0.17	0.15	0.18	0.01
	4	-12	0.14	0.16	0.17	0.17	
		-18	0.15	0.17	0.15	0.18	
	8	-12	0.16	0.17	0.16	0.17	
		-18	0.16	0.19	0.16	0.16	
Rn3n6	12	-12	0.14	0.18	0.16	0.18	0.02
		-18	0.17	0.18	0.17	0.18	
	24	-12	0.16	0.19	0.16	0.19	
		-18	0.18	0.22	0.16	0.21	
	52	-12	0.15	0.17	0.15	0.17	
		-18	0.17	0.19	0.16	0.19	

6.1.4 Oxidative Markers

Thiobarbituric acid reactive substances

Fig. 6.1.4.1 shows TBARS levels tended to increase with frozen storage period ($P < 0.05$) and that this increase was greater when the preceding chilled storage period was longer (chilled-then-frozen; $P < 0.05$). Frozen storage holding temperature was found to contribute to these differences ($P < 0.05$) so as, for example, samples held at -18 °C had a greater disparity in TBARS between c0:f52 and c5:f52 than c0:f4 and c5:f4 and these differences were less so for samples held at – 12 °C. Interestingly, the effect of frozen storage temperature was not observed when comparing frozen storage periods independent to chilled storage period ($P = 0.66$). Chilled-only storage period did not prove a significant influence on TBARS although these samples did result in significantly lower TBARS than their frozen-only equivalents.
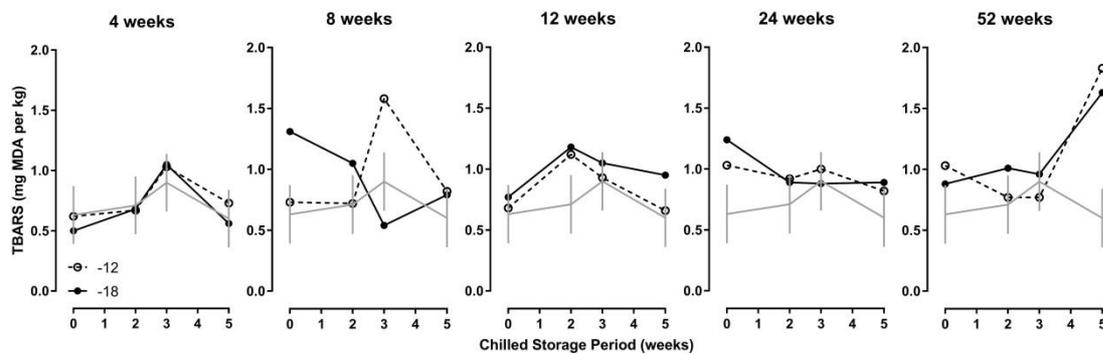
Fig. 6.1.4.1. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* (LL) thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA) per kg LL. Predicted means were plotted so that samples frozen at – 18 °C (solid lines and solid markers) and – 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Peroxidase activity

Peroxidase activity (PA) was shown to increase with chilled-only storage, especially when 0 and 5 weeks were compared ($P < 0.05$; Fig. 6.1.4.2). The opposite was observed for frozen-only storage when comparing 4 weeks with the longer periods ($P < 0.05$), although this was better reflected in terms of chilled-then-frozen storage where PA declined after 4 weeks frozen storage were found to be less severe as the preceding chilled storage period increased ($P < 0.05$). For the most part, PA was found to be lower if an LL portion was frozen compared to their corresponding chilled-only counterparts. No other storage treatment combination or effect proved significant.
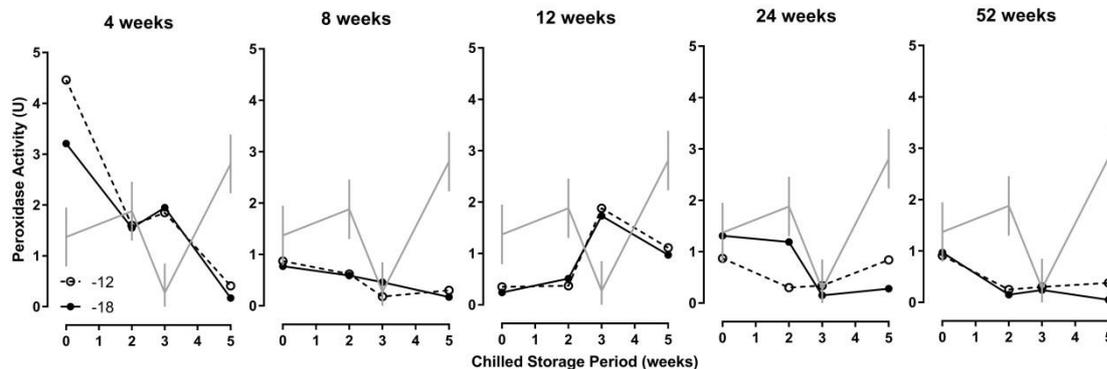
Fig. 6.1.4.2. Effect of chilled-then-frozen storage duration on beef *M. longissimus lumborum* (LL) peroxidase activity expressed as the amount of peroxidase (nmole) that reduces 1.0 µmole H_2O_2 per minute per g LL at 37 °C (U). Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Oxidation-reduction potential

Oxidation-reduction potential (ORP) levels increased across chilled storage periods before plateauing between 3 and 5 weeks ($P < 0.05$; Fig. 6.1.4.3). Trends across frozen storage periods were less linear, with a decrease and then recovery of ORP levels evident ($P < 0.05$). This same observation across frozen storage periods was shared over each precursory chilled storage period, albeit the point of ORP decline was at an earlier frozen period for the latter chilled storage periods ($P < 0.05$). For example, minimum ORP was observed at c5:f8 and c2:f12 respectively. No other treatment combination effect was significant, nor did the temperature at ORP measurement act as a covariate.
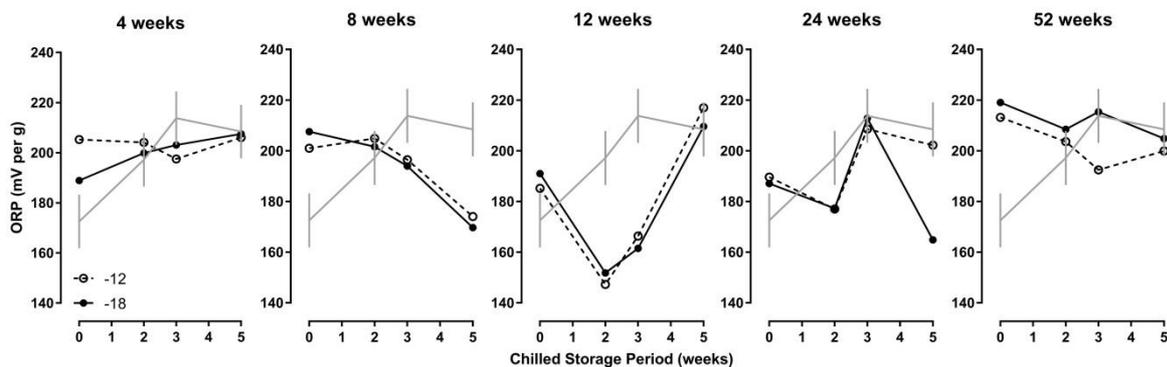
Fig. 6.1.4.3. Effect of chilled-then-frozen storage duration on beef M. *longissimus lumborum* (LL) oxidation-reduction potential (ORP) expressed in millivolts (mV) per g LL. Predicted means were plotted so that samples frozen at − 18 °C (solid lines and solid markers) and − 12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Carbonyl content

Carbonyl content was observed to increase between 0 and 3 weeks chilled-only storage ($P < 0.05$) before then returning to initial levels. Frozen-only storage also resulted in increasing carbonyl contents which generally peaked at 8 weeks before declining ($P < 0.05$; Fig. 6.1.4.4). When considered together there was a significant difference between chilled-only (unfrozen) and frozen muscle carbonyl contents as the latter levels consistently proved higher when samples were first aged (chilled period) 5 weeks before freezing. A chilled-then-frozen storage period effect was evident from the different carbonyl content peaks within various chilled-then-frozen storage combinations ($P < 0.05$), albeit this occurred without adherence to a uniform trend. No other treatment effects were found to be significant.
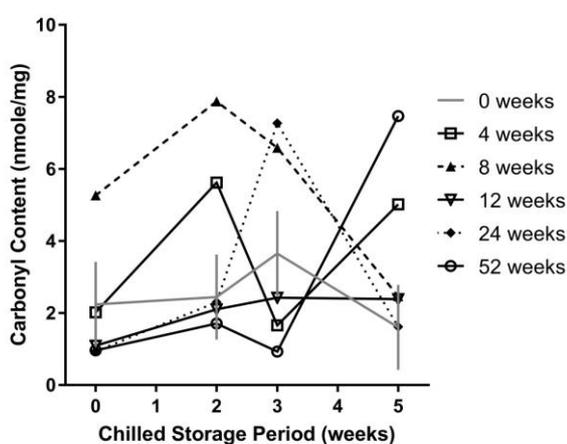
Fig. 6.1.4.4. Effect of chilled-then-frozen storage duration on beef *m. longissimus lumborum* carbonyl content. Predicted means were plotted so that samples frozen at -18 °C (solid lines and solid markers) and -12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold).

Nitrate and nitrite content

Frozen-only storage periods ≥ 12 weeks had higher nitrate content than observed in the previous durations ($P < 0.05$) with the exception of c0:f4 compared to c5:f4 which is the probably basis of the chilled-then-frozen storage effect ($P < 0.05$). Frozen storage holding temperature had a significant impact on nitrate content as samples held at − 18 °C for 24 and 52 weeks frozen-only storage had higher levels than their equivalents held at − 12 °C (Fig. 6.1.4.5a.). No holding temperature effect was observed for nitrite content and yet frozen-only storage resulted in inconsistent increases of nitrate levels as periods increased (Fig. 6.1.4.5b). Nitrite content also increased with chilled-only storage although only between 0 and 2 weeks before returning to initial levels across the remaining periods ($P < 0.05$) – a difference in trend highlighted by the significant chilled-then-frozen storage effect and instances of frozen-only storage resulting in sample nitrite levels being higher than their chilled-only (unfrozen) counterparts ($P < 0.05$).
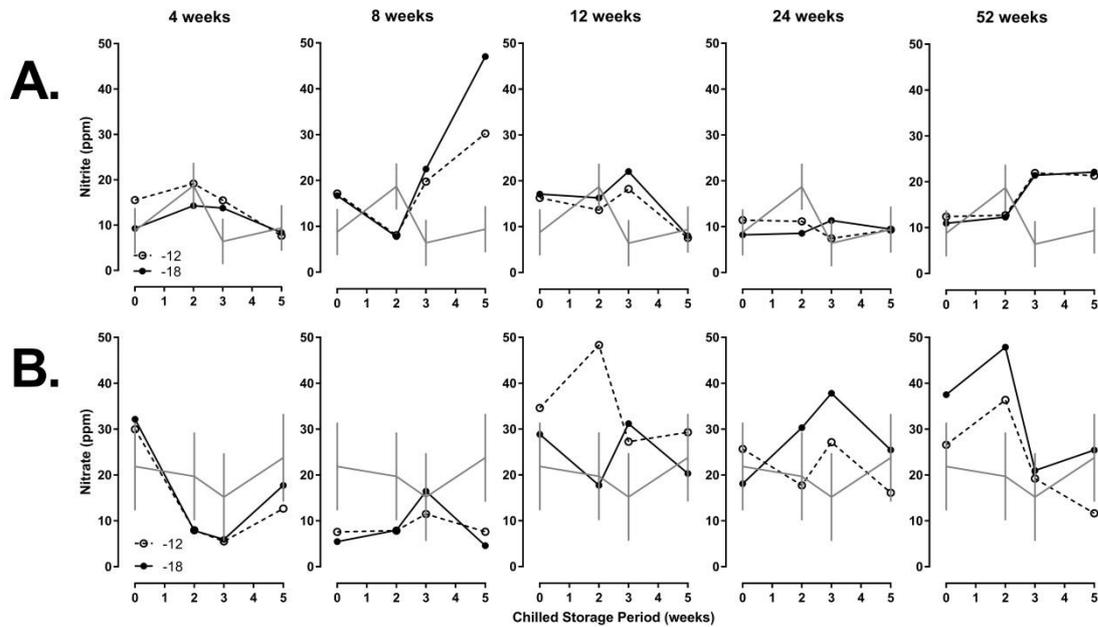
Fig. 6.1.4.5. Effect of chilled-then-frozen storage duration on beef *m. longissimus lumborum* A) nitrite, and B) nitrate content. Predicted means were plotted so that samples frozen at -18 °C (solid lines and solid markers) and -12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

Myoglobin content and redox status

Myoglobin (Mb) content decreased across increasing frozen-only storage periods ($P < 0.05$; Table 6.1.4.1), although the point of this decline was delayed as the preceding chilled storage period increased (chilled-then-frozen; $P < 0.05$). All other storage treatment combinations were insignificant in their influence on Mb content.

Table 6.1.4.1. Predicted means (mg/g) and their standard error (SEM) for beef m. *longissimus lumborum* total myoglobin content for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp).

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	3	5	SEM
0	(unfrozen)	4.7	4.8	4.9	4.9	0.4
4	-12	6.3	5.1	4.7	4.9	
	-18	5.5	4.9	4.6	4.5	
8	-12	6.7	5.2	4.6	4.9	
	-18	5.2	6.4	4.7	5.6	
12	-12	3.9	5.6	5.8	6.8	0.6
	-18	4.9	4.8	6.4	5.9	
24	-12	4.4	5.2	5.1	4.6	
	-18	4.7	4.3	5.4	4.9	
52	-12	4.0	4.1	4.0	4.6	
	-18	3.9	4.4	5.3	6.4	

MMb fractions were significantly higher in frozen-only samples compared to their chilled-only (unfrozen) counterparts across each display period (Fig. 6.1.4.6). Likewise, MMb were observed to increase corresponding to increasing chilled-only ($P < 0.05$) and frozen-only storage periods ($P < 0.05$) within these same display periods. The extent of this change in MMb was found to depend on the chilled-then-frozen storage treatment ($P < 0.05$) with levels seemingly compounded when samples had longer preceding chilled and subsequent frozen storage periods – for example, MMb increased 2.2 units (%) between c0:f4 and c0:f52 compared to 1.3 units (%) c3:f4 and c3:f52 at 2 d display. Fig. 6.1.4.6 also shows that OMb fractions increased with chilled-only storage periods, but only at 0 and 1 d display after which this response was revised and OMb declined across these same chilled-only periods ($P < 0.05$). This response was reflected in the frozen-only storage periods with the direction of significant OMb fraction differences being a function of display period. Chilled-then-frozen storage was also a significant effect on sample OMb however there were inconsistencies observed in their influence. DMb was found to instead mirror OMb results across chilled-only ($P < 0.05$) and frozen-only ($P < 0.05$) storage periods with the relative variation observed within this latter treatment further reflected by the significant chilled-then-frozen storage treatment effect (Fig. 6.1.4.6). Frozen storage holding temperature and other treatment combinations not listed here had no significant effect on MMb, OMb or DMb.
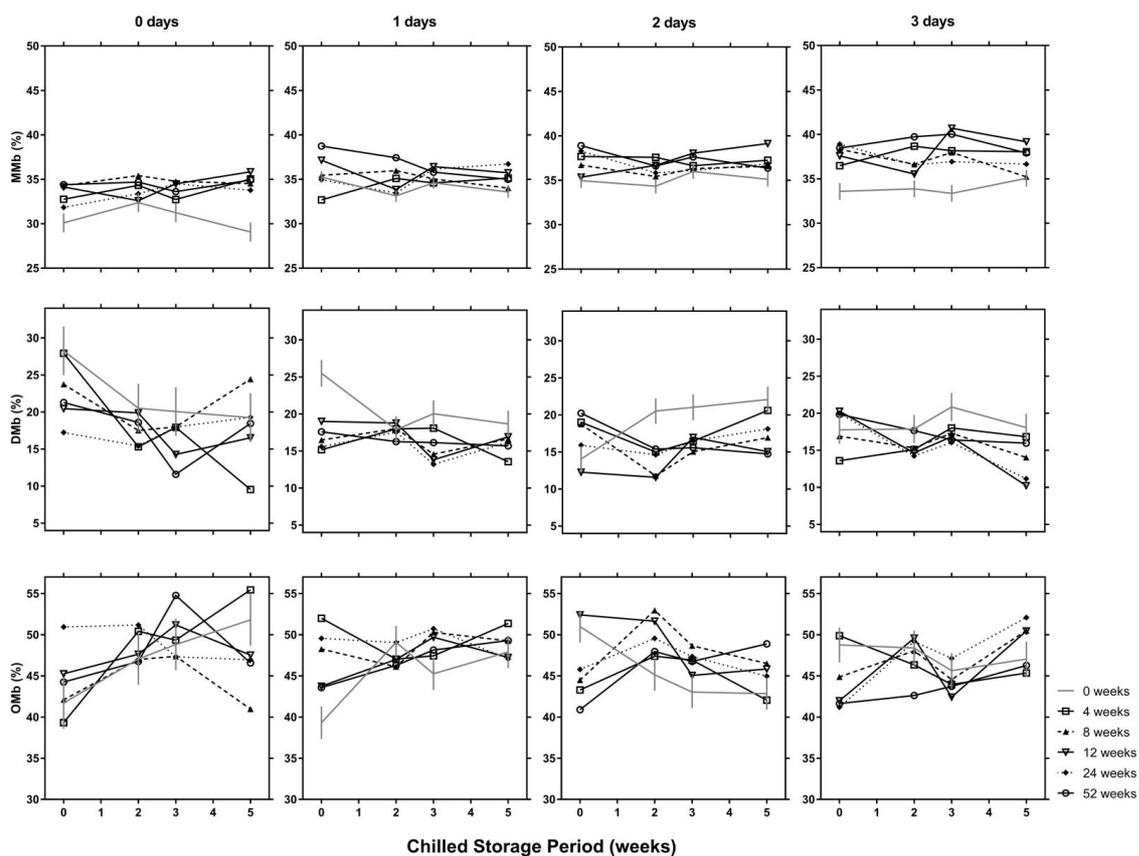
Fig. 6.1.4.6. Effect of chilled-then-frozen storage duration on beef *m. longissimus lumborum* myoglobin redox state proportions, including metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb). Predicted means were plotted so that samples frozen at -18 °C (solid lines and solid markers) and -12 °C (dotted lines and hollow markers) could be compared against chilled-only samples (grey lines with no markers) across preceding chilled storage and within subsequent frozen storage periods (above each graph, in bold). Vertical bars illustrate ± 2 standard error estimates.

6.2 Lamb

6.2.1 Sensory Qualities

Colour stability

Chilled-only storage resulted in decreased colour stability over the display period (3 d) for all CIE traits except b* ($P < 0.05$; Fig. 6.2.1.3). The variation and the difference due to chilled-only storage (frozen = 0 weeks) between L* ($P = 0.046$; Fig. 6.2.1.1) and hue angle ($P = 0.006$; Fig. 6.1.2.6) on day 0 and L* ($P < 0.001$; Fig. 6.2.1.1), a* ($P < 0.001$; Fig. 6.2.1.2), R630/580 ($P < 0.001$; Fig. 6.2.1.4), chroma ($P = 0.003$; Fig. 6.2.1.5 and hue angle ($P < 0.001$; Fig. 6.1.2.6) on day 3 are illustrated. L* was not influenced by frozen storage period, whether frozen-only or in combination with chilled (chilled-then-frozen; $P > 0.05$; Fig. 6.2.1.1), unlike a*, b*, R630/580 and chroma ($P < 0.05$), which all decreased as frozen storage period increased (Figs. 3-6), or hue angle which increased ($P < 0.01$; Fig. 6.1.2.6). This shift was especially obvious following 52 weeks frozen storage (whether frozen-only or chilled-then-frozen). The longer periods of frozen storage (24 and 52 weeks) led to the greatest decline in colour stability and for traits like a* and R630/580 there was a notable effect of freezing compared to chilled meat (Figs. 6.2.1.2 & 6.2.1.4). No chilled-then-frozen storage period combination or frozen storage temperature effects were observed ($P > 0.05$).

Fig. 6.2.1.1. Predicted mean (± standard error) lightness score (CIE L*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
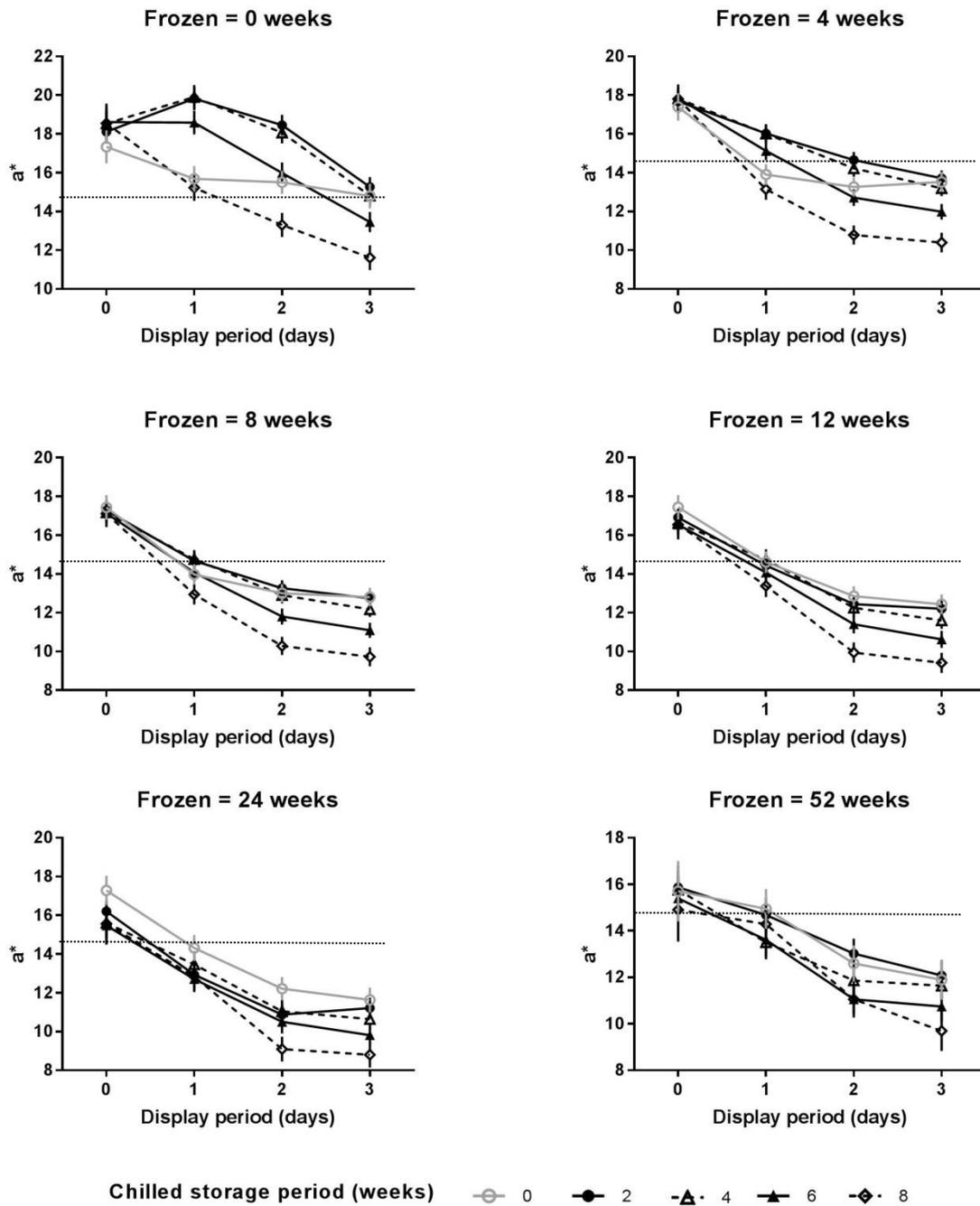
Fig. 6.2.1.2. Predicted mean (± standard error) redness score (CIE a*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line. The dotted horizontal lines indicate the a* threshold for 50% consumer acceptability (Khliji, van de Ven, Lamb, Lanza, & Hopkins, 2010), with any data below the line indicative of unacceptability.
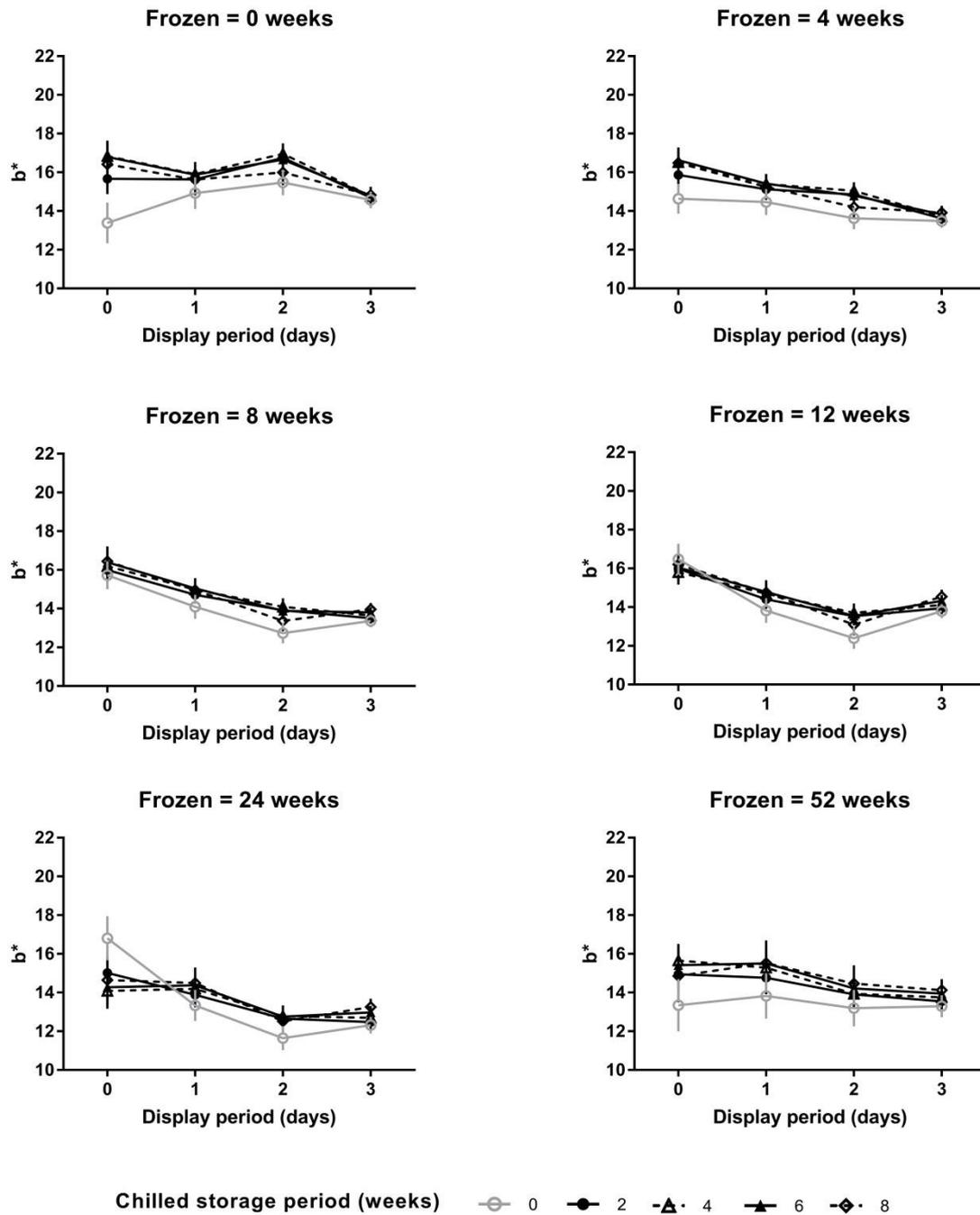
Fig. 6.2.1.3. Predicted mean (± standard error) yellowness score (CIE b*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
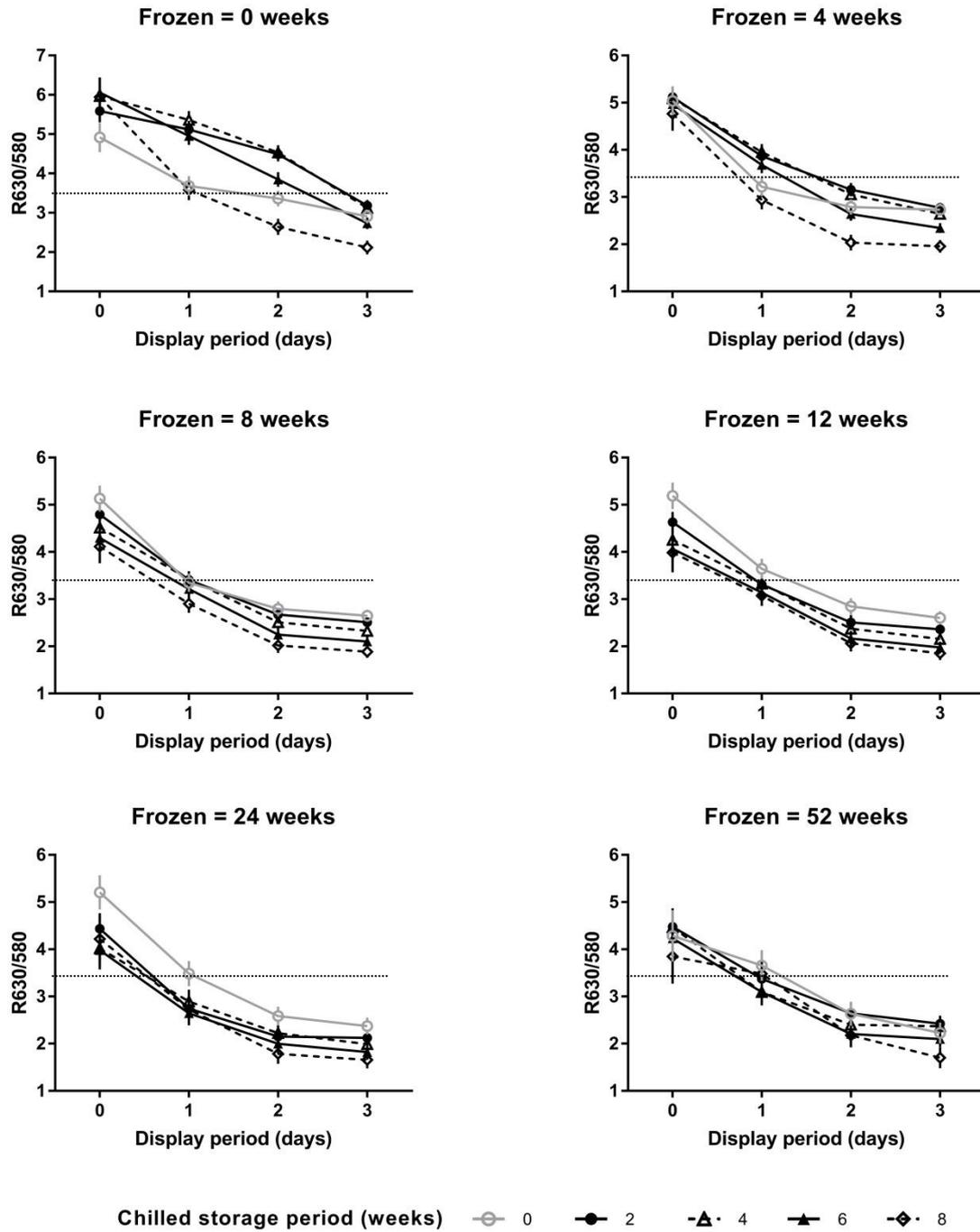
Fig. 6.2.1.4. Predicted mean (± standard error) reflectance ratio (R630/580) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line. The dotted horizontal lines indicate the R630/580 threshold for 50% consumer acceptability (Khliji et al., 2010), with any data below the line indicative of unacceptability.
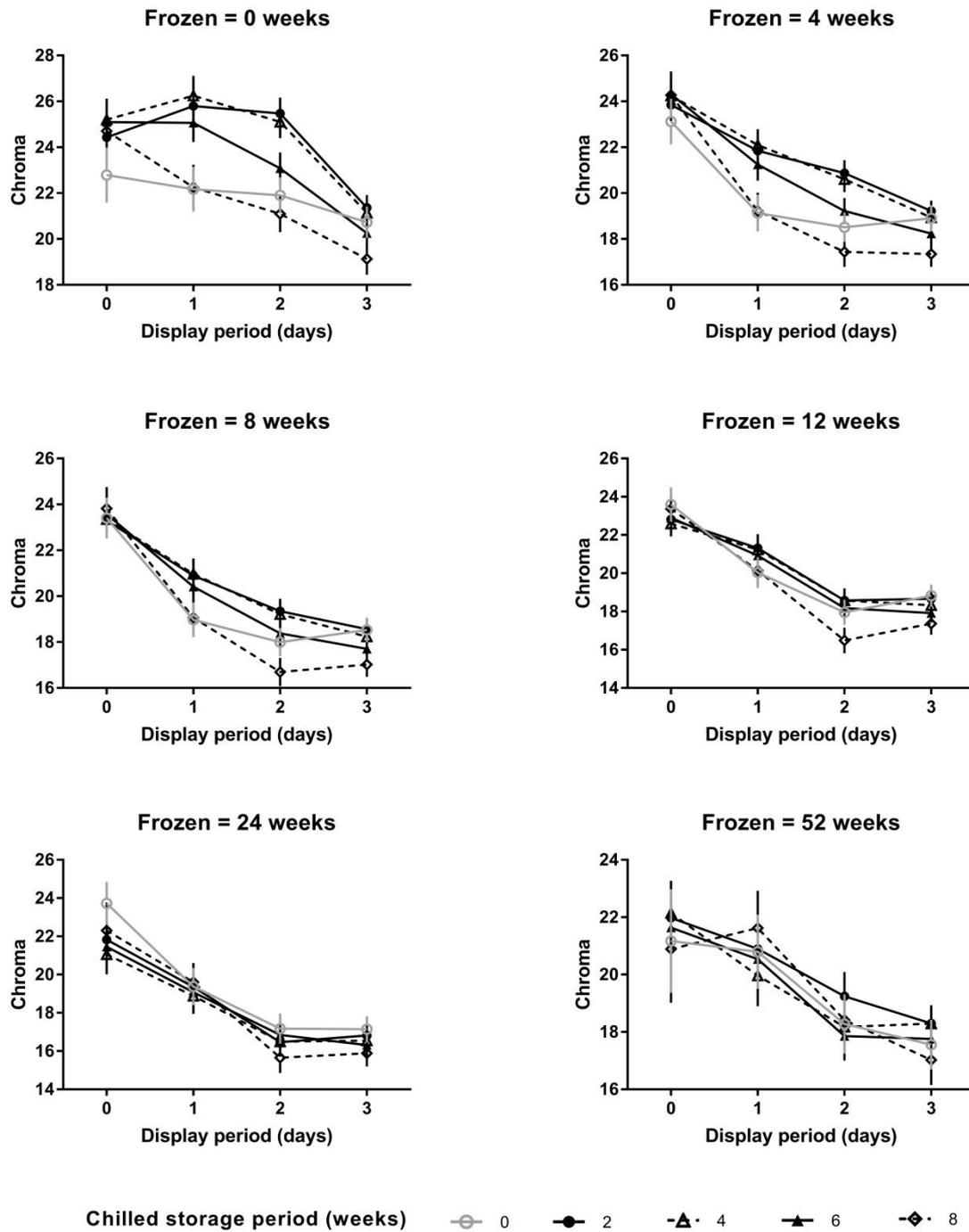
Fig. 6.2.1.5. Predicted mean (± standard error) colour intensity (chroma) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
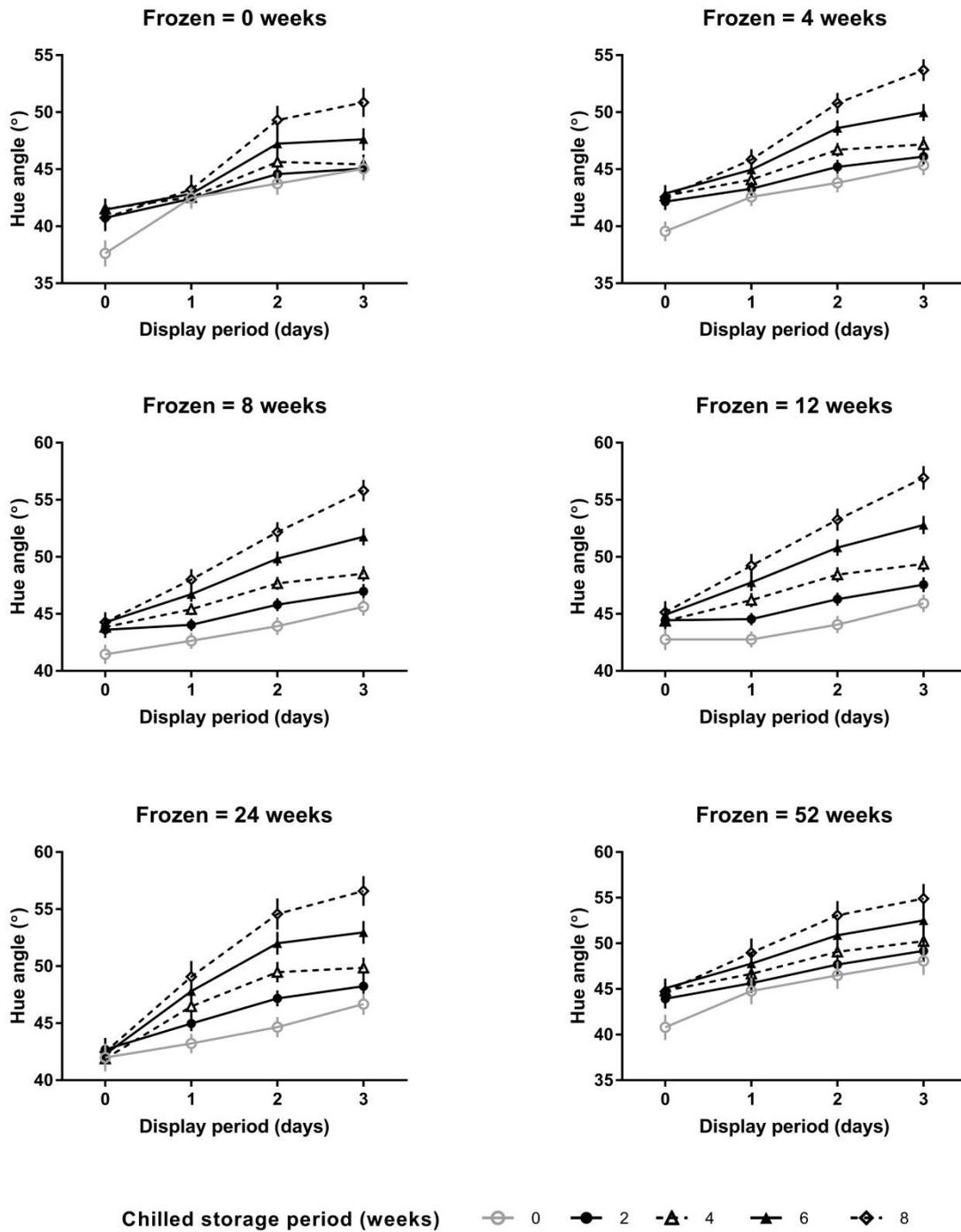
Fig. 6.2.1.6. Predicted mean (± standard error) hue angle of chilled-then-frozen lamb LL stored at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Fluid losses (including, purge, cook and thaw loss)

Purge losses of frozen-only and chilled-then-frozen samples were lower than those of chilled-only samples ($P < 0.001$; Fig. 6.2.1.7). Within chilled-only and chilled-then-frozen samples, purge losses increased with increasing chilled storage period ($P < 0.001$; Fig. 6.2.1.7), though this tended to decrease within chilled-then-frozen samples as the frozen storage period increased ($P = 0.086$; Fig. 6.2.1.7). No treatment effects were observed for thaw losses ($P > 0.05$), although it is worth noting that only LL exposed to frozen storage periods exceeding 0 weeks were tested for thaw loss assessment. Cooking loss increased with corresponding increases in chilled-only storage period ($P = 0.013$), with this effect also evident across chilled-then-frozen storage periods wherein frozen-only LL demonstrated no variation in cooking loss regardless of period ($P > 0.05$). Freezer temperature and interaction effects were not significant ($P > 0.05$).
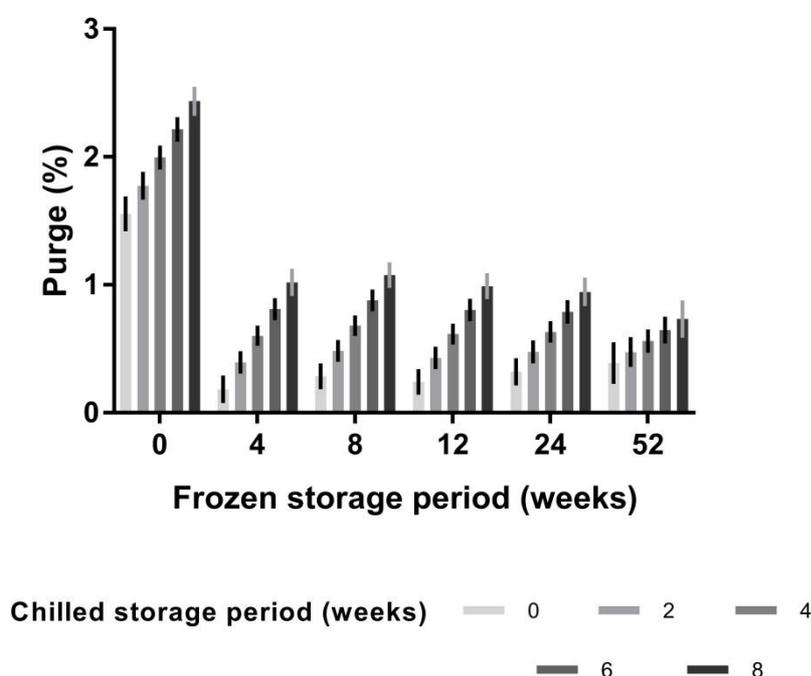
Fig. 6.2.1.7. Predicted mean (± standard error) purge loss (square root transformed) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Glycogen content

Glycogen content was unaffected by chilled-only storage periods ($P > 0.05$). Frozen-only storage period did show an increase in glycogen content ($P < 0.001$), particularly at 52 weeks (Fig. 6.2.1.8). Chilled-then-frozen storage was influential when frozen storage temperature was − 12 °C, resulting in greater glycogen content increases over frozen storage periods compared to − 18 °C, particularly as chilled storage periods increased ($P = 0.05$).
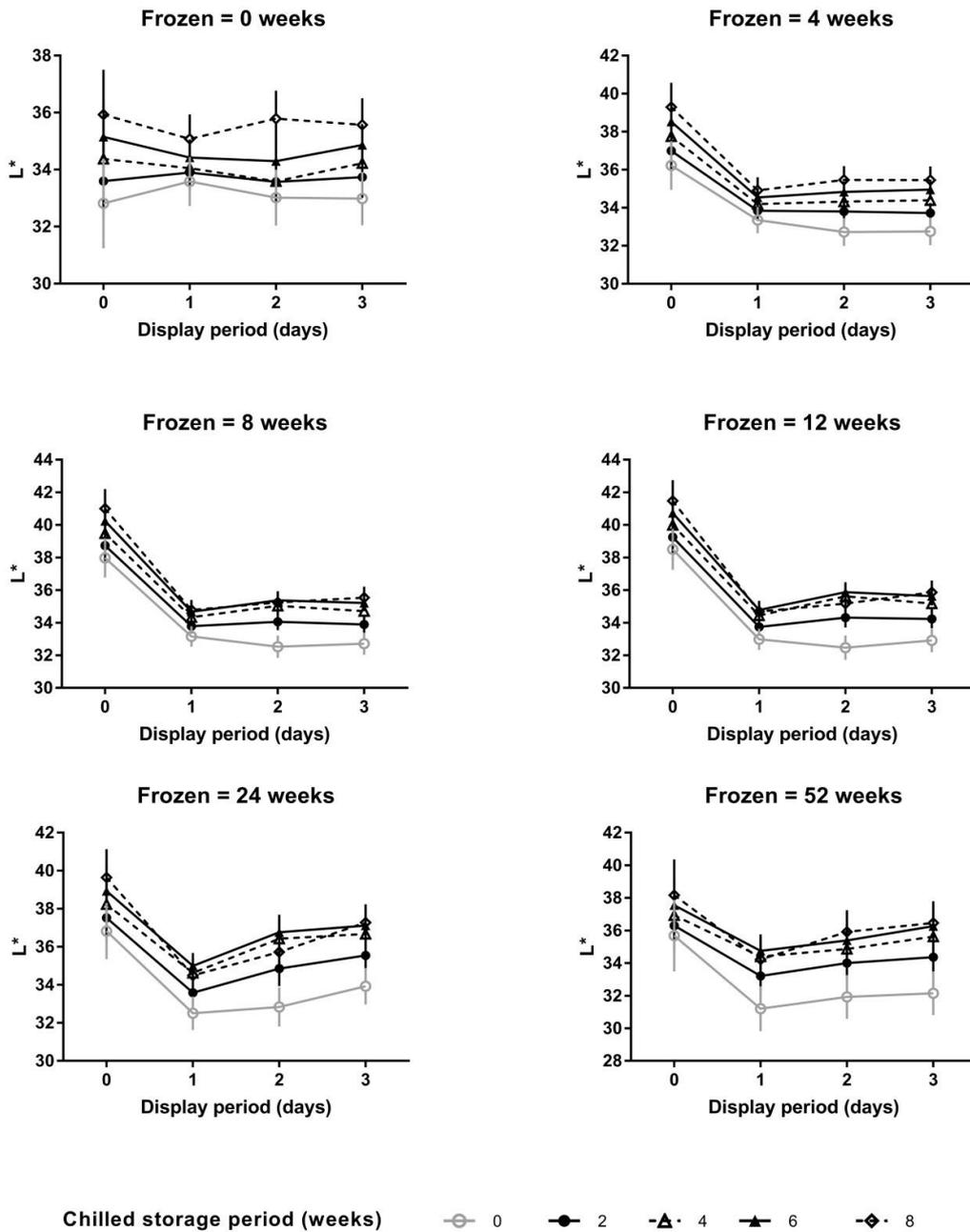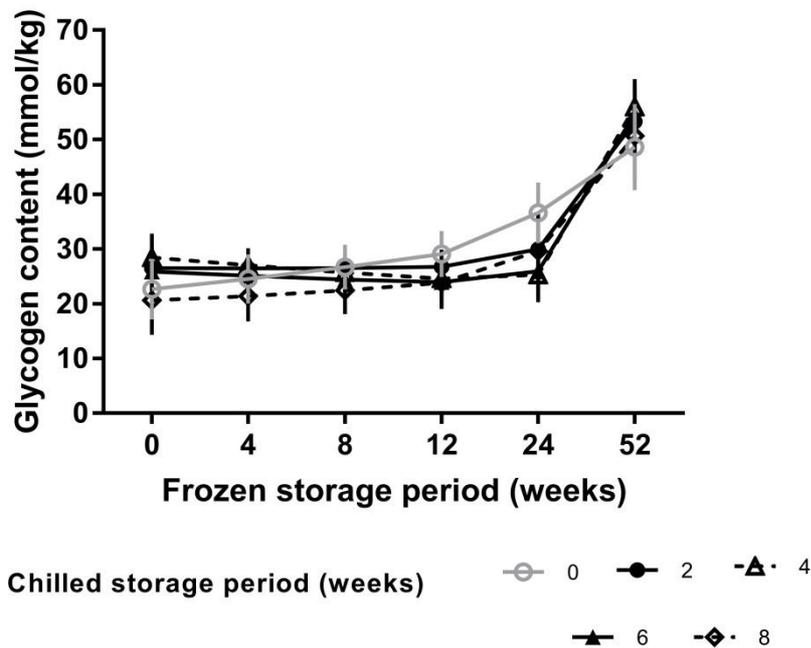
Fig. 6.2.1.8 Predicted mean (± standard error) glycogen content of chilled-then-frozen lamb LL stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Moisture and intramuscular fat contents

Moisture content (MC) and IMF tended to decrease ($P = 0.090$) and increase ($P = 0.052$), respectively, with increased chilled-only storage period (Table 6.2.1.1). This chilled storage period effect was more noticeable in both MC and IMF when LL were frozen for 52 weeks (Table 6.2.1.1). Reflecting this trend, moisture content decreased as frozen-only storage period increased ($P < 0.001$), unlike IMF which did not vary ($P > 0.05$) (Table 6.2.1.1). IMF did increase due to chilled-then-frozen storage interactions with frozen storage temperature ($P = 0.013$), with IMF higher at – 18 °C at shorter chilled-then-frozen periods than – 12 °C counterparts, which, in turn, exhibited higher IMF at longer storage periods.

Table 6.2.1.1. Predicted means (± standard error) for the effect of chilled-then-frozen storage on the proportions of moisture content (MC) and intramuscular fat (IMF). Both are expressed as percentages.

Chilled storage (weeks)		Frozen storage (weeks)					
		0	4	8	12	24	52
0	MC	53.3 ± 0.8[a]	52.8 ± 0.7[ab]	52.4 ± 0.6[ab]	52.0 ± 0.7[b]	51.0 ± 0.7[bc]	50.0 ± 1.3[c]
	IMF	4.80 ± 0.3	4.78 ± 0.3	4.77 ± 0.3	4.75 ± 0.2	4.69 ± 0.2	4.56 ± 0.5
2	MC	52.7 ± 0.6[a]	52.3 ± 0.5[a]	51.9 ± 0.5[ab]	51.6 ± 0.4[ab]	50.4 ± 0.6[b]	47.9 ± 1.0[c]
	IMF	4.86 ± 0.2	4.86 ± 0.2	4.86 ± 0.2	4.86 ± 0.2	4.85 ± 0.2	4.83 ± 0.4
4	MC	52.4 ± 0.6[a]	52.1 ± 0.5[ab]	51.7 ± 0.4[ab]	51.3 ± 0.5[ab]	50.3 ± 0.7[b]	48.1 ± 1.0[c]
	IMF	4.92 ± 0.2	4.94 ± 0.2	4.95 ± 0.1	4.96 ± 0.1	5.00 ± 0.1	5.10 ± 0.3
6	MC	52.4 ± 0.6[a]	51.8 ± 0.5[a]	51.3 ± 0.5[ab]	50.9 ± 0.5[ab]	49.8 ± 0.7[bc]	49.0 ± 1.0[c]
	IMF	4.98 ± 0.2	5.01 ± 0.2	5.04 ± 0.2	5.07 ± 0.2	5.16 ± 0.2	5.37 ± 0.4
8	MC	52.5 ± 0.9[a]	51.7 ± 0.7[ab]	51.0 ± 0.6[abc]	50.3 ± 0.7[bc]	49.2 ± 0.9[c]	50.5 ± 1.3[bc]
	IMF	5.04 ± 0.3	5.09 ± 0.3	5.14 ± 0.3	5.18 ± 0.2	5.32 ± 0.2	5.64 ± 0.5

abc: Different superscript letters in the same row reflects a significant effect of frozen storage upon MC ($P < 0.05$).

Particle size

Particle size (PS) decreased with increasing chilled-only storage ($P < 0.001$). This decrease was non-linear with the majority of PS change occurring in the first 2 weeks of chilled storage, and little to no change at longer chilled-only storage periods (Fig. 6.2.1.9). No difference was observed for PS due to chilled-then-frozen storage, frozen-only storage or temperature, or due to their interactions ($P > 0.05$).
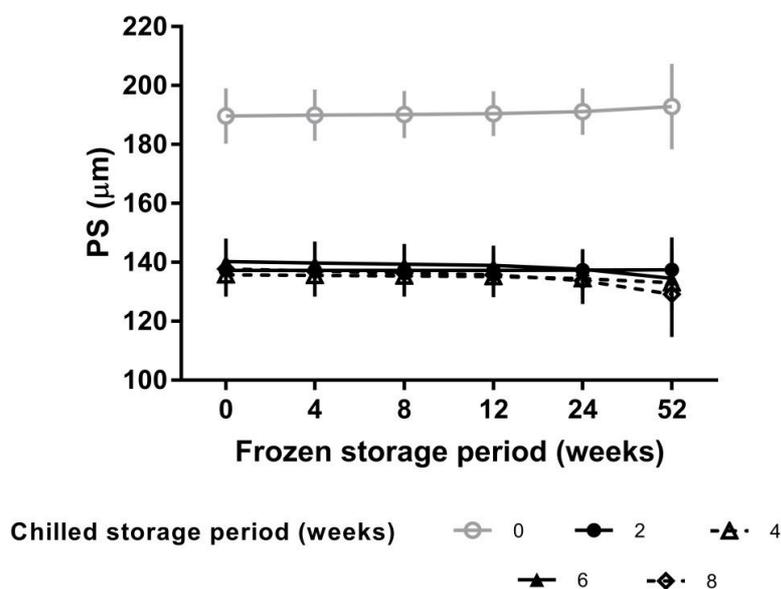
Fig. 6.2.1.9. Predicted mean (± standard error) particle size (PS) of chilled-then-frozen lamb *M. longissimus lumborum* stored at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Protein solubility

Sarcoplasmic protein solubility was found to linearly decrease across increased chilled-only storage periods ($P = 0.003$; Fig. 6.2.1.10), although no change was seen for myofibrillar (predicted mean ± SE: 49.3 ± 0.49 mg/g) or total (predicted mean ± SE: 69.2 ± 2.39 mg/g) protein solubilities ($P > 0.05$). Frozen storage period both alone and when considered following chilled storage, and frozen storage temperature yielded no effects on total, myofibrillar or sarcoplasmic protein solubilities ($P > 0.05$).
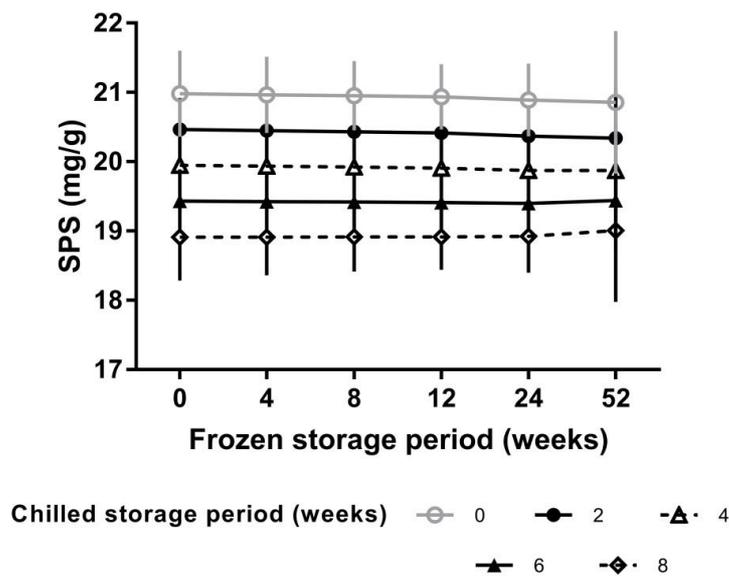
Fig. 6.2.1.10. Predicted mean (± standard error) sarcoplasmic protein solubility (SPS) of chilled-then-frozen lamb *M. longissimus lumborum* stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Shear force determination

Shear force was reduced with increased chilled-only ($P < 0.001$) and frozen-only ($P = 0.028$) storage. There was a noticeable decrease in SF after only 2 weeks of chilled storage, and the relationship between storage period and SF is non-linear (Fig. 6.2.1.11) for both chilled and frozen storage, where SF declined between 0 and 8 weeks frozen storage before levelling out at longer storage periods ($P > 0.05$). Chilled-then-frozen storage period combination, pHu and frozen storage temperature did not have any observed effect on SF ($P > 0.05$). There was also no effect ($P > 0.05$) of SL on SF.
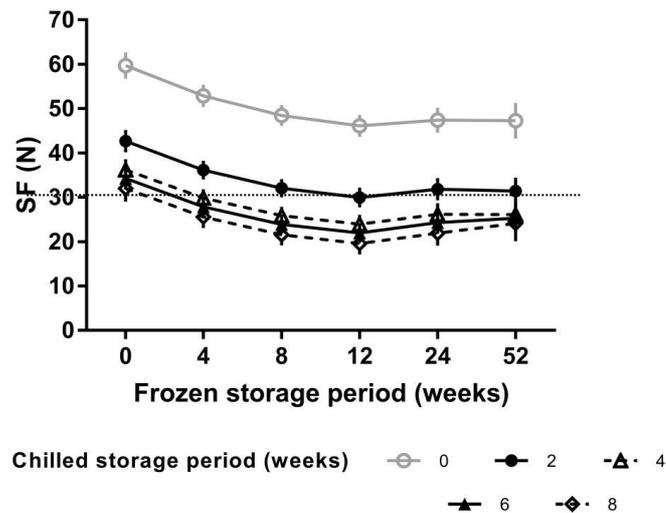
Fig. 6.2.1.11. Predicted mean (± standard error) shear force (SF) of chilled-then-frozen lamb *M. longissimus lumborum* stored at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line. The horizontal dotted line indicates the SF threshold for 50% consumer acceptability (Hopkins, Hegarty, Walker, & Pethick, 2006), with any data below the line indicative of unacceptability.

Sarcomere length

No storage type, period or temperature effects were observed for SL (mean ± standard error (SE): 1.71 ± 0.03 µm; $P > 0.05$).

Ultimate (final) pH

No storage type, period or temperature effects were observed for pHu (mean ± SE: 5.70 ± 0.04; $P < 0.05$). However, pHu was found to exhibit a significant negative relationship with glycogen content, SL, fluid losses and colour on day 0 ($P < 0.001$) and a positive relationship with a* and R630/580 on days 1-3 and chroma on day 1-2 ($P < 0.05$), but this did not change the significance of the fixed terms in the model (e.g. storage type, storage period).

Water activity

No effects of chilled or frozen storage period or temperature were observed for a_w (mean ± SE: 0.94 ± 0.01; $P > 0.05$).

6.2.2 Microbial Status

Brochothrix thermosphacta, LAB, and *Enterobacteriaceae sp.* were all detected (limit of detection = 1 $\log_{10}$ CFU/g) across each storage treatment combination. *Clostridium perfringens* and *E. coli* were not detected. Increased chilled-only storage periods resulted in parallel increases to the loading of these detected microbial types ($P < 0.001$; Fig. 6.2.1.12). LAB generally increased with increasing frozen-only storage periods ($P < 0.001$), albeit a decrease was evident at 24 weeks frozen storage, but this trend was recovered by 52 weeks (Fig. 6.2.1.12a). *B. thermosphacta* increased between 12 and 52 weeks frozen storage when LL were frozen-only ($P < 0.001$; Fig. 6.2.1.12b). Furthermore, *B.*

thermosphacta was observed to decrease within the chilled-then-frozen storage context ($P = 0.004$; Fig. 6.2.1.12b), such that as the period of frozen storage increased, the growth of *B. thermosphacta* was reduced, particularly in LL chilled for between 4-8 weeks. Frozen-only storage had no effect on *Enterobacteriaceae sp.* loadings ($P > 0.05$, Fig. 6.2.1.12c). Likewise, frozen storage temperature had no significant effect on microbial load for any tested species ($P > 0.05$). Higher glycogen content was significantly related to decreased microbial loading of all three species ($P < 0.05$), particularly *Enterobacteriaceae sp.* ($P < 0.001$).
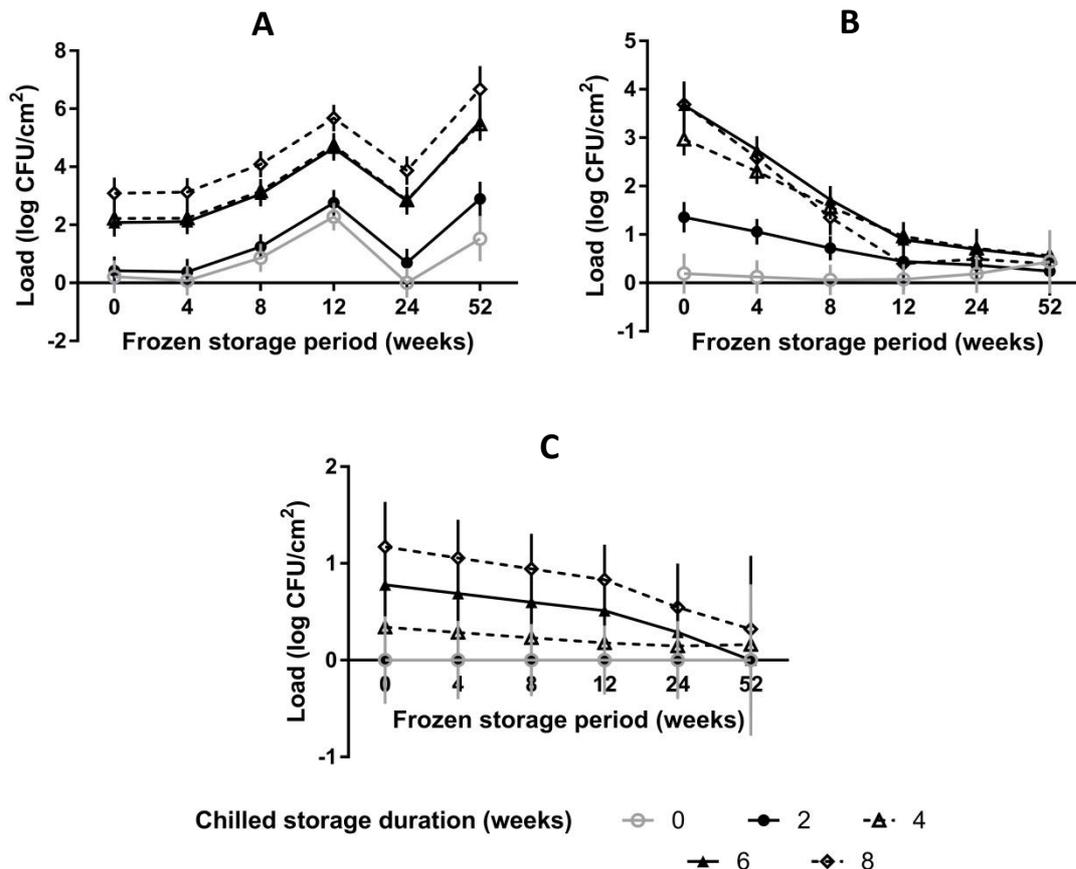
Fig. 6.2.1.12. Predicted mean (± standard error) microbial loadings of A) Lactic Acid Bacteria; B) *Brochothrix thermosphacta*; and C) *Enterobacteriaceae sp.* of chilled-then-frozen lamb *M. longissimus lumborum* stored at two frozen storage temperatures (– 12 and – 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

6.2.3 Fatty Acid Profile

For the most part, saturated fatty acids (SFA) were found to be predominantly palmitic and stearic acids (Table 6.2.3.1). The total levels of SFA did not change throughout the entire storage period ($P > 0.05$; Table 2). Palmitic acid concentration decreased as chilled storage period increased from 0 to 2 weeks ($P = 0.03$). Total monounsaturated fatty acids (MUFA) were primarily comprised of oleic acid (Table 6.2.3.1), with no changes in concentration due to increased storage periods ($P > 0.05$; Table 6.2.3.2). In this study, PUFA was the summation of both omega-3 (n-3) and omega-6 (n-6) FA. Major

n-3 PUFA contributors were α-linolenic acid (ALA) and docosapentaenoic acid (DPA). Major n-6 PUFA contributors were linoleic acid and arachidonic acid (Table 6.2.3.1). Total PUFA tended to increase in concentration across chilled storage periods (P = 0.05; Table 6.2.3.2), particularly n-6 PUFA between 0 and 8 weeks chilled-only storage (P = 0.02).

Table 6.2.3.1. Mean fatty acids found in lamb *M. longissimus lumborum* based on fresh meat (time zero).

Formula	Name	Content (g/100 g total fatty acid)
C10:0	Capric acid	0.15
C12:0	Lauric acid	0.11
C14:0	Myristic acid	2.79
C15:0	Pentadecylic acid	0.33
C16:0	Palmitic acid	25.17
C17:0	Margaric acid	1.08
C18:0	Stearic acid	16.12
C20:0	Arachidic acid	0.64
C21:0	Heneicosylic acid	0.21
Total SFA		**46.60**
C14:1	Myristoleic acid	0.01
C16:1ω7	Palmitoleic acid	2.06
C18:1iso	Iso-oleic acid	1.11
C18:1ω9	Oleic acid	41.67
C18:1ω9t	Elaidic acid	1.31
C20:1	Paullinic acid (ω7) or Gondoic acid (ω9)	0.04
Total MUFA		**46.21**
C18:2ω6	Linoleic acid	3.72
C18:2ω6t	*trans*-Linoleic acid	0.11
C18:3ω6	γ-Linolenic acid	0.05
C20:3ω6	Dihomo-γ-Linolenic acid	0.08
C20:4ω6	Arachidonic acid	1.07
Total n-6 PUFA		*5.04*
C18:3ω3	α-Linolenic acid	1.03
C18:3ω3t	*trans*-Linolenic acid	0.04
C20:5ω3	Eicosapentaenoic acid (EPA)	0.41
C22:5ω3	Docosapentaenoic acid (DPA)	0.52
C22:6ω3	Docosahexaenoic acid (DHA)	0.14
Total n-3 PUFA		*2.15*
n-3/n-6 ratio		0.43
Total PUFA		**7.19**
Total UFA (MUFA + PUFA)		**53.40**
Total (UFA + SFA)		**100**
EPA + DHA		0.55
IMF (% fresh meat)		4.56

Table 6.2.3.2. Predicted mean proportions of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) (g per 100 g total FA) in lamb *M. longissimus lumborum* subjected to different chilled-then-frozen storage periods.

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)					SEM
		0	2	4	6	8	
0	PUFA	7.19	7.97	7.47	7.89	8.44	0.60
	MUFA	46.2	45.6	45.0	45.8	45.2	1.05
	SFA	46.6	46.4	47.5	46.3	46.4	0.86
4	PUFA	8.06	7.23	8.11	9.21	7.30	0.59
	MUFA	46.1	46.5	46.8	45.0	47.9	1.05
	SFA	46.4	46.0	45.6	45.8	47.0	0.86
8	PUFA	8.72	7.59	8.02	7.98	8.03	0.59
	MUFA	44.9	46.5	46.4	43.5	45.0	1.05
	SFA	46.4	46.0	45.6	48.5	47.0	0.86
12	PUFA	8.07	7.94	7.41	8.46	7.25	0.59
	MUFA	45.6	44.8	45.7	44.5	45.2	1.05
	SFA	46.3	47.2	46.9	47.1	47.6	0.86
24	PUFA	10.0[a]	7.46[b]	8.14[ab]	7.76[ab]	6.70[b]	0.59
	MUFA	45.0	46.6	45.8	44.9	45.2	1.05
	SFA	45.0	46.0	46.1	47.4	48.1	0.86
52	PUFA	8.43	7.79	7.54	7.65	8.02	0.59
	MUFA	44.3	45.3	47.0	45.0	45.6	1.05
	SFA	47.3	46.9	45.5	47.3	46.3	0.86

ab: Means with different superscripts are significantly different from others in the same row ($P = 0.05$). LSD: PUFA 1.67 (unfrozen), 2.36 (frozen); MUFA 2.91 (unfrozen), 4.12 (frozen); SFA 2.39 (unfrozen), 3.38 (frozen).

Health-claimable n-3 PUFAs (EPA + DHA) were summed and their concentrations at different chilled-then-frozen storage periods are shown in Table 6.2.3.3. It is worth noting that, while counted towards total PUFA, EPA and DHA were not the two greatest contributors to total PUFA concentration (Table 6.2.3.3). Chilled-only storage maintained the EPA + DHA concentration of fresh meat for 8 weeks, and this continued throughout the frozen storage period ($P > 0.05$; Table 6.2.3.3). Frozen storage period and temperature had no effect on EPA + DHA concentration ($P > 0.05$).

Table 6.2.3.3. Predicted mean sum (g per 100 g total fatty acid) of health claimable fatty acids, eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA), of lamb *M. longissimus lumborum* subjected to different chilled-then-frozen storage periods.

Chilled Storage (weeks)	Frozen Storage (weeks)					
	0	4	8	12	24	52
0	0.550	0.525	0.595	0.520	0.700[a]	0.620
2	0.550	0.410	0.445	0.485	0.380[b]	0.455
4	0.420	0.540	0.520	0.520	0.525[ab]	0.440
6	0.410	0.540	0.465	0.590	0.495[ab]	0.520
8	0.420	0.385	0.500	0.565	0.300[b]	0.570
SEM			0.080			

ab: Means with different superscripts are significantly different from others in the same column ($P = 0.05$). LSD = 0.23 (unfrozen), 0.32 (frozen).

6.2.4 Oxidative Markers

Myoglobin redox fractions

The proportion of MMb increased due to chilled-only storage on days 1 (P = 0.014), 2 (P < 0.001) and 3 (P < 0.001) of display, and due to frozen-only storage on days 0 (P = 0.002), 2 (P = 0.008) and 3 (P = 0.020) of display (Fig. 6.2.4.1). Additionally, temperature affected MMb on day 1 (P = 0.027), wherein MMb proportion was greater in LL stored at − 12 °C than − 18 °C. Chilled-then-frozen storage combination had no effect (P > 0.05).
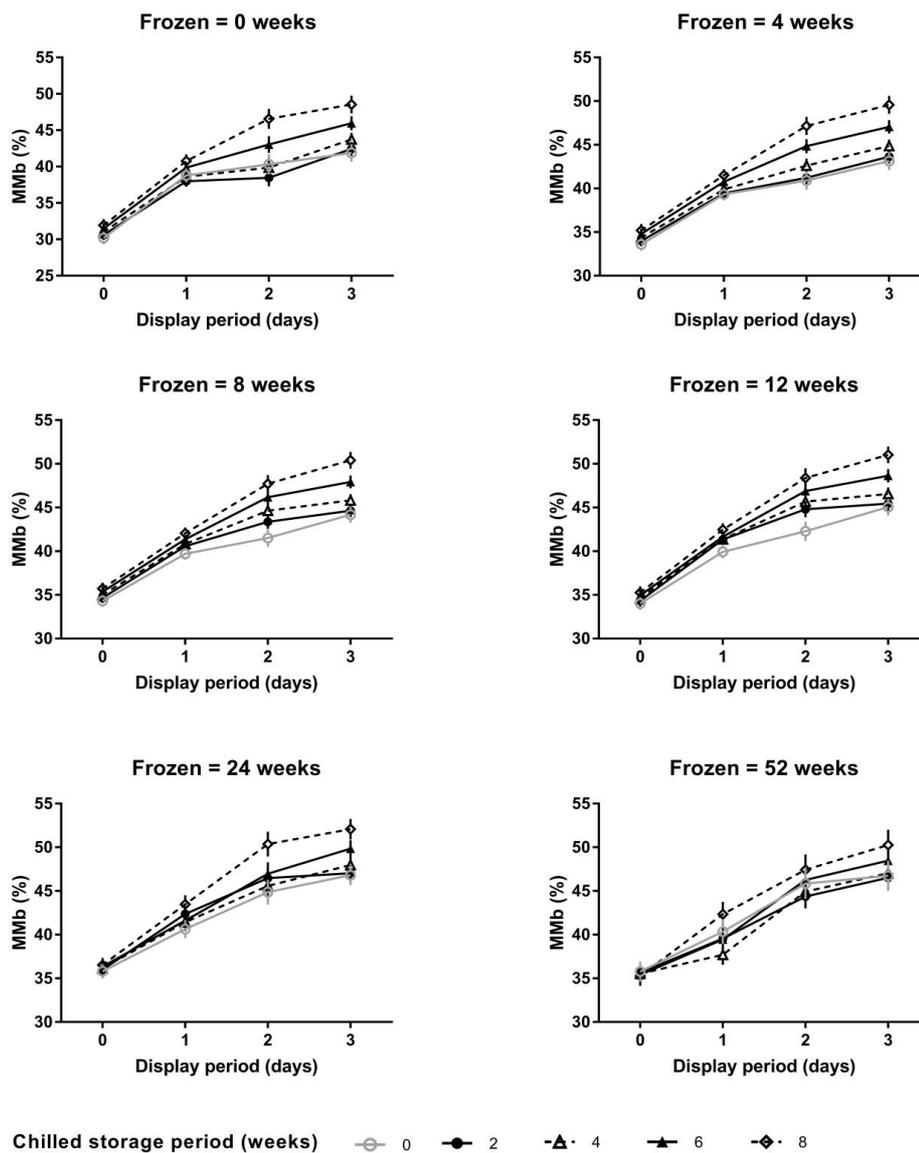
Fig. 6.2.4.1. Predicted mean (± standard error) metmyoglobin (MMb) (%) for lamb *M. longissimus lumborum* stored for different chilled-then-frozen storage periods at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

The proportion of OMb was decreased by chilled-then-frozen storage (*P* = 0.032), although this only occurred on day 0 (Fig. 6.2.4.2). No other effects of chilled- or frozen-only storage period or temperature were observed for OMb (*P* > 0.05).
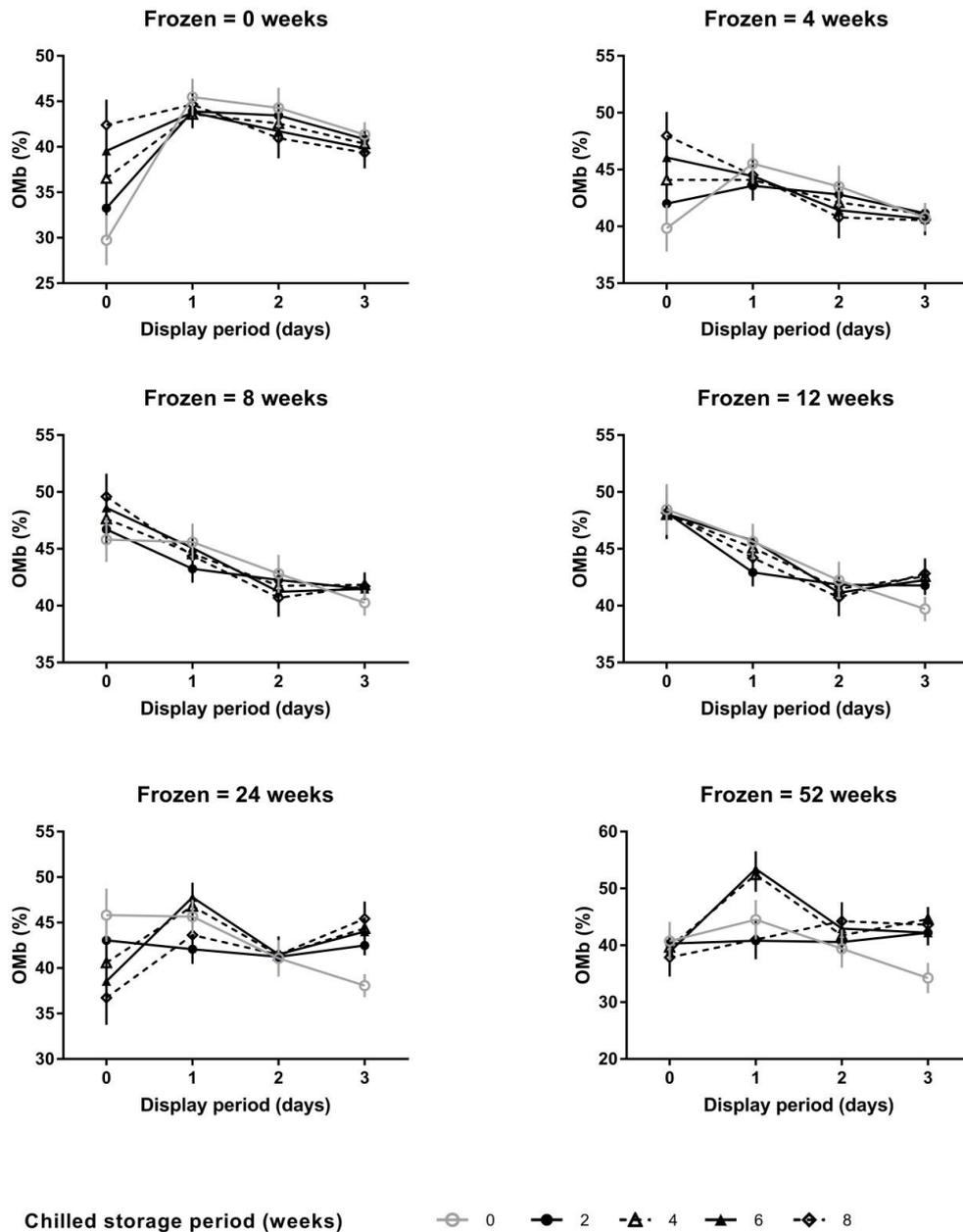
Fig. 6.4.4.2. Predicted mean (± standard error) oxymyoglobin (OMb) (%) for lamb *M. longissimus lumborum* stored for different chilled-then-frozen storage periods at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

The proportion of DMb decreased with increased chilled-only storage on days 2 ($P = 0.008$) and 3 ($P < 0.001$), and increased with frozen-only storage on day 3 ($P = 0.011$) (Fig. 6.4.4.3). Chilled-only and frozen-only storage had no effects on day 0, though chilled-then-frozen storage combination did ($P = 0.027$). No other effects were observed ($P > 0.05$).
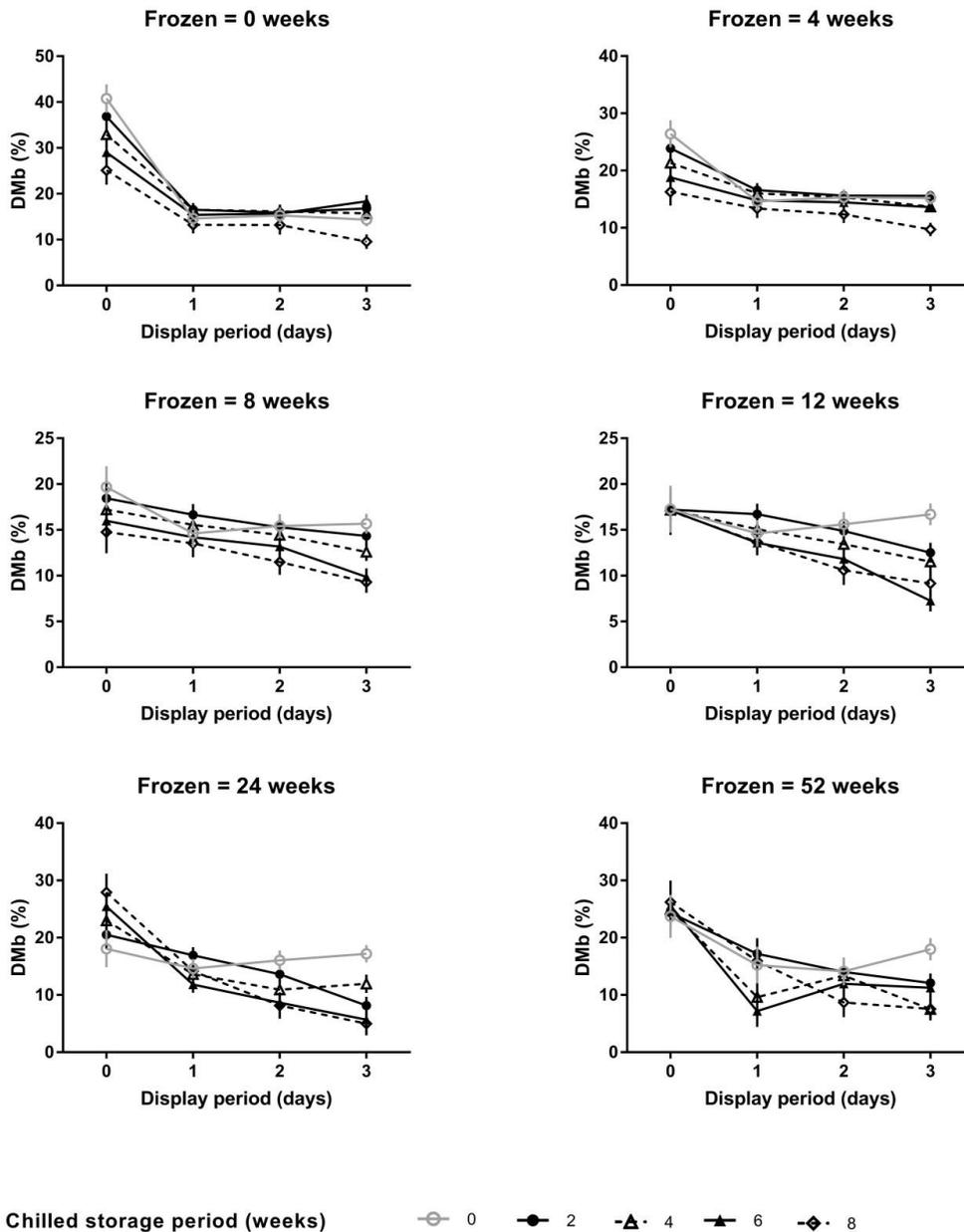
Fig. 6.4.4.3. Predicted mean (± standard error) deoxymyoglobin (DMb) (%) for lamb *M. longissimus lumborum* stored for different chilled-then-frozen storage periods at two frozen storage temperatures (− 12 and − 18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

Nitrate and nitrite content

Nitrate and nitrite contents were not affected by any storage treatment combination investigated ($P > 0.05$). Predicted means ± SE values across all treatments were 38.7 ± 7.76 ppm and 11.6 ± 1.63 ppm for nitrate and nitrite contents, respectively.

Peroxidase activity

Peroxidase activity was affected by chilled-only storage ($P < 0.001$), though minimal variation between means was found between 0 and 6 weeks storage (Table 6). However, peroxidase activity increased in a quadratic manner with increasing frozen-only storage ($P < 0.001$), peaking at 8 weeks (Table 6). Similarly, peroxidase activity increased with increased chilled-then-frozen storage periods ($P < 0.001$), with particular increases at 52 weeks frozen storage (Table 6.4.4.1). No other storage effects were significant ($P > 0.05$).

Table 6.4.4.1. Predicted mean peroxidase activity (U/g) of lamb *M. longissimus lumborum* following different chilled-then-frozen storage periods.

Chilled Storage (weeks)	Frozen Storage (weeks)					
	0	4	8	12	24	52
0	0.31^x	1.01^x	2.75^{az}	1.85^{ay}	0.40^x	0.96^{abx}
2	0.25^x	0.41^x	0.46^{bx}	0.44^{bcx}	0.35^x	1.75^{ay}
4	0.58	0.46	0.35^b	0.55^{bc}	0.37	0.96^{ab}
6	0.63	0.57	0.38^b	1.03^b	0.38	0.79^b
8	0.38^{xy}	1.15^y	0.36^{bxy}	0.14^{cx}	0.20^x	1.09^{aby}
SEM	0.21			0.22		

abc: Means with different superscripts are significantly different from others in the same column ($P < 0.05$). xyz: Means with different superscripts are significantly different from others in the same row ($P < 0.05$). LSD: 0.58 (unfrozen), 0.82 (frozen).

Protein carbonyl content

Protein carbonyl content did not vary nor exceed that observed for fresh meat regardless of sample chilled-then-frozen storage period treatment ($P > 0.05$). The predicted mean ± standard error (SE) carbonyl content averaged across all samples was 3.39 ± 0.46 nmol/mg.

Oxidation-reduction potential

Chilled-only storage had significant effects on ORP ($P < 0.001$); where there was a decrease noted following 6 weeks storage (Table 6.4.4.2). ORP trends across increasing frozen-only and chilled-then-frozen storage periods were significant ($P < 0.001$); although it was difficult to ascertain a consistent trend as ORP levels were observed to decrease over frozen-only storage and increase with chilled-then-frozen storage, but only when the preceding chilled storage period was 8 weeks and this was followed by a frozen storage period of 8 to 52 weeks (Table 6.4.4.2). No other storage effects were significant ($P > 0.05$).

Table 6.4.4.2. Predicted mean oxidation-reduction potential (mV per g) of lamb *M. longissimus lumborum* following different chilled-then-frozen storage periods.

Chilled Storage (weeks)	Frozen Storage (weeks)					
	0	4	8	12	24	52
0	210.1^{bcz}	203.2^{bz}	213.1^{cz}	171.8^{by}	136.8^{ax}	159.2^{ay}
2	217.9^{cz}	200.4^{byz}	163.4^{ax}	213.7^{cz}	197.2^{byz}	190.6^{by}
4	198.9^{bz}	187.5^{byz}	187.2^{byz}	145.2^{ax}	197.9^{bz}	159.6^{axy}
6	177.3^{zy}	154.2^{ax}	215.6^{cz}	208.4^{cz}	209.7^{bz}	201.4^{bcz}
8	202.4^{bcyz}	204.6^{byz}	164.6^{ax}	196.7^{cy}	203.0^{byz}	220.2^{cz}
SEM	5.63			5.56		

abc: Means with different superscripts are significantly different from others in the same column ($P < 0.05$). xyz: Means with different superscripts are significantly different from others in the same row ($P < 0.05$). LSD: 15.66 (unfrozen), 22.14 (frozen).

Thiobarbituric acid reactive substances (TBARS)

The TBARS levels increased with increasing chilled-only storage periods ($P < 0.001$), with a peak at 4 weeks chilled-only storage apparent (Table 6.4.4.3). Frozen-only storage was observed to increase TBARS levels as storage period increased to 8 weeks ($P < 0.001$; Table 6.4.4.3). Chilled-then-frozen storage interactions also increased TBARS, showing a general increase parallel with total storage period ($P < 0.001$). No other storage treatment combination, including frozen storage temperature, significantly influenced TBARS levels ($P > 0.05$).

Table 6.4.4.3. Predicted mean TBARS values (mg malondialdehyde per kg) of lamb *M. longissimus lumborum* following different chilled-then-frozen storage periods.

Chilled Storage (weeks)	Frozen Storage (weeks)					
	0	4	8	12	24	52
0	0.35^{ax}	0.91^{yz}	0.84^{y}	0.74^{y}	0.65^{abxy}	1.26^{z}
2	0.60^{bx}	0.66^{x}	0.73^{x}	0.56^{x}	1.42^{cy}	0.88^{x}
4	1.27^{cz}	0.85^{xy}	0.58^{x}	0.62^{x}	0.55^{ax}	1.06^{yz}
6	0.70^{bx}	0.54^{x}	0.62^{x}	0.51^{x}	0.42^{ax}	1.18^{y}
8	0.74^{bx}	0.65^{x}	0.95^{xy}	0.91^{xy}	1.19^{bcy}	1.04^{xy}
SEM	0.10			0.11		

abc: Means with different superscripts are significantly different from others in the same column ($P < 0.05$). xyz: Means with different superscripts are significantly different from others in the same row ($P < 0.05$). LSD = 0.29 (unfrozen), 0.41 (frozen).

6.2.5 Relationship between Protein Degradation and Protein Oxidation Markers with Shear Force

Shear force was highly positively and linearly ($r^2 = 0.99$) predicted by PS – smaller protein fragments resulted in lower SF across chilled-only storage periods ($P < 0.001$; Table 6.2.5.1), as well as DMb and OMb on day 0 ($P = 0.002$). No other protein degradation or oxidation measures showed a relationship with SF across chilled-only storage periods ($P > 0.05$), with relationships of all predictors in this study to SF shown in Table 6.2.5.1.

Table 6.2.5.1. Relationship of protein degradation and oxidation traits towards shear force across 8 weeks chilled-only storage. Myoglobin fractions are given over 3 days of measurement across chilled-then-frozen storage periods.

Measurement	Coefficient of determination (r^2)	*P*-value
Carbonyl Content	0.04	0.742
Total Protein Solubility	0.26	0.383
Sarcoplasmic Protein Solubility	0.47	0.203
Myofibrillar Protein Solubility	0.63	0.110
Nitrite Content	0.45	0.212
Nitrate Content	0.59	0.129
Particle Size	0.99	<0.001
	Coefficient (± SE)	***P*-value**
MMb (%): 0; 1; 2; 3 d	-0.021 (0.21); 0.340 (0.21); 0.010 (0.16); 0.001 (0.14)	0.945; 0.114; 0.583; 0.964
DMb (%): 0; 1; 2; 3 d	0.244 (0.08); 0.075 (0.14); 0.080 (0.14); 0.279 (0.14)	**0.002**; 0.583; 0.573; **0.046**
OMb (%): 0; 1; 2; 3 d	-0.266 (0.08); -0.170 (0.12); -0.073 (0.13); -0.176 (0.14)	**0.002**; 0.149; 0.562; 0.200

SE: standard error; MMb: metmyoglobin; DMb: deoxymyoglobin; OMb: oxymyoglobin; **bold** indicates a significant effect of each trait on predicting shear force (SF) ($P < 0.05$).

7.0 DISCUSSION

7.1 Beef

7.1.1 Sensory Quality and Microbial Load

Freezing temperatures and rates are already known to influence beef eating quality traits because of the differences in resultant ice crystal size and distribution, and their mechanical impact on meat structure (Grujic, Petrovic, Pikula, & Amidzic, 1993; Kiani & Sun, 2011). In this study these were kept constant for all frozen storage durations and as such, any significant difference would be based on differences in frozen storage holding temperature and their relative capacity to prevent spoilage. The concept of holding temperature effects on frozen beef eating qualities has remained relatively untested, with a few exceptions being; Khan & Lentz (1977) who compared -10 °C and -20 °C on beef portions to find only cooking loss (CL) and drip loss to be influenced, but not SF; Vieira et al. (2009) tested -20 °C and -80 °C and reported no significant difference in their impact on beef sensory quality traits; and Farouk, Wieliczko, & Merts (2003) observed no difference between -18 °C, -35 °C and -75 °C frozen holding temperatures on beef water holding capacity, CL, colour traits or SF, even when evaluated over storage durations up to 12 months. These outcomes reflect many of those observed in the present study, with negligible differences between -12 °C and -18 °C apparent in beef quality parameters. That said, holding temperature should not be confused with frozen storage duration which both here and in the aforementioned studies influenced beef quality parameters, an observation based on temperatures down to -22 °C being insufficient to freeze all available water and therefore halt all biochemical reactions in meat (Rahelic, Gawwad, & Puac, 1985).

The observed variation in SF and absence of significant changes to SL from chilled-then-frozen storage supports the continuance of biochemical reactions within frozen beef. That is, SL only measures muscle shortening and muscle fibre parameter contributions to tenderness (Hopkins, Toohey, Lamb, Kerr, van de Ven, & Refshauge, 2011; Hwang, Park, Cho, & Lee, 2004) and its observed static variance with storage treatments therefore suggests a proteolytic pathway for improved tenderness (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010), evident from SF differences. For example, SF change is driven by calpain activity (Dransfield, 1994), regulated by calpastatin which is, in turn, sensitive to frozen storage so that its ability to limit calpains is reduced as storage duration continues (Kristensen, Christensen, & Ertbjerg, 2006). This and the scope for continued proteolytic reactions when still frozen (Duckett, Klein, Leckie, Thorngate, Busboom, & Snowder, 1998) could outline the pathway to SF variance seen across the experimental storage treatments. However, impacts on SF should be considered in terms of their practical detection – for example, if considered using the Aalhus, Jeremiah, Dugan, Larsen, & Gibson (2004) SF threshold (4.30 kg or 42.17 N for 95% acceptance) for consumer acceptance then all samples regardless of storage treatment would be satisfactory; however only samples that had a chilled storage period > 0 prior to any frozen storage duration would be considered acceptable if the Miller, Carr, Ramsey, Crockett, & Hoover (2001) threshold was used instead (4.0 kg or 39.23 N for 94% acceptance, respectively). This highlights difficulties inherent in broad interpretation, but nevertheless supports the observations made in past research that have used sensory panels to evaluate chilled-then-frozen red meat tenderness which have reported no perceptible difference even when also finding significant SF differences (Lagerstedt, Enfält, Johansson, & Lundström, 2008; Vieira et al., 2009).

It was interesting to note that pHu levels were influenced somewhat inversely to SF across the same chilled-then-frozen storage combinations and doing so reflects similar protein degradation. This is based on pH proximity to isoelectric pH of structural protein resulting in increased protein

denaturation rather than enzymatic processes, both of which can influence moisture and water holding parameters.

Variation was observed in the degree to which chilled-then-frozen storage influenced beef liquid fraction parameters and total moisture. This was thought to have resulted from differences in protein structure capacities to retain water rather than damage from ice-crystal formation, which although previously shown to influence water characteristics (Grayson, King, Shackelford, Koohmaraie, & Wheeler, 2014; Lagerstedt et al., 2008) could have been undermined by the shared freezing rate and method applied to result in the observed thaw loss consistencies. Protein structure degradation, evident from the aforementioned SF and pHu results, compromises its ability to retain water through the capillary forces between actin and myosin filaments (J. Liu, Arner, Puolanne, & Ertbjerg, 2016). Water from this source then contributes to purge and therefore total losses – albeit determination of chilled-then-frozen storage treatment effects on protein oxidation would complement this position. Cooking loss also encapsulates this source, but also intramuscular fat and chemically-bound water that is released as part of the cooking/heating process – i.e. melted and 'squeezed out' from muscle fibre contractions (Hughes, Oiseth, Purslow, & Warner, 2014). Here, therefore, CL may provide a more comprehensive picture of actual water holding parameters as no difference in IMF was found and CL had an inverse reflection of total moisture content trends. In practise, the loss of beef liquid fraction is not solely a yield concern but when, as for the present study, it is held in an impermeable vacuum-packaged environment it can contribute to the risk of microbial spoilage (Mills, Donnison, & Brightwell, 2014).

Microbial spoilage depends on the environmental conditions meat is held within and their interactions on proliferation rate (Gill & Newton, 1978). Consequently, the absence of any significant variation inferred by the absence of sufficient growth to merit analysis suggests chilled-then-frozen storage combinations did inhibit microbial spoilage – especially as LAB, *Enterobacteriaceae sp.*, *C. perfingens* and *B. thermospacta* have each been identified as important contributors to anaerobically stored, refrigerated red meat spoilage (Mills et al., 2014). This was most likely due to frozen storage beneath their collective lower temperature limits which complements other studies that have reported no change in these microbe populations so that 'safe' levels are maintained when meat was stored at comparably sub-limit temperatures (Hinton, Holder, Hudson, Coombs, Allen, & Corry, 1998; Vieira et al., 2009). That said, microbial spoilage also depends on the initial populace and composition, and their capacity to compete within their shared environment. These factors were not assessed in the present study and as such the observed outcomes may have resulted from the hygienic practises of the source abattoir resulting in a low initial loading (a limitation common to these studies and overcome via inoculation trials where a known microbial load is applied to all samples). This latter statement is promoted by the observed variation to a_w and the reported significant differential effect small changes to a_w level have on microbial profiles (Dave & Ghaly, 2011; Gill & Newton, 1978), potentially influenced by pH association with muscle structure water release. Yet, the change in glycogen content over chilled-then-frozen storage treatments does suggest the relative absence of anaerobic microbes that would otherwise metabolise this as a 'fuel reserve' (Mills et al., 2014). The robust cold chain employed by the abattoir is also likely to have contributed to the very low levels of bacteria detected. The practical implications should, however, be considered as to the effects of these other 'dominant' microbial species effect on safety and spoilage thresholds to beef's ultimate market acceptability when the normal candidate species evaluated were unchanged.

There were practical changes in beef colour stability observed, with a noticeable decline in quality that resulted in unacceptable discoloration at earlier display periods when both the preceding chill

and subsequent frozen storage periods increased – a statement based on the consumer acceptance threshold ($a* \geq 14.5$) previously described (Holman et al., 2017b), although other colorimetric thresholds have been defined (Ponnampalam, Hopkins, Bruce, Li, Baldi, & Bekhit, 2017a). The basis for this would involve the myoglobin-redox forms where the release of pro-oxidants as a result of freezing and thawing (Xiong, 2000), and the reduction in metmyoglobin (MMb) reducing enzymes because of freezing (Farouk & Swan, 1998), similar to previously described with calpastatin and other enzymes activities, would skew towards increased and irreversible concentration of MMb. Within this context, MMb is associated with discolouration and browning whereas oxymyoglobin instead infers a bright red colour to red meats (Suman & Joseph, 2013). Other studies have also reported colour stability changes to depend on storage environment and duration (Coombs et al., 2017; Kim et al., 2011), but to better understand those observed in this study would involve the evaluation of oxidative changes for both lipids and proteins across these same chilled-then-frozen storage combinations. This would not only fulfil an underlying mechanism paucity, but could also be useful in explaining structural changes shown to influence SF, water parameters, and other results identified.

7.1.2 Fatty Acid and Lipid Oxidation Markers

SFA and UFA were found to change across chilled and frozen storage durations in comparatively opposite directions – that is, SFA increased as UFA decreased. This suggests that UFA continued to be oxidised even when the beef was 'preserved' and reflects past research which also identified FA profile shifts in beef held frozen for 13 months (Igene, Pearson, & Gray, 1980b) and pork frozen for 2 years (Alonso et al., 2016). PUFA was found, however, not to be significantly influenced by storage treatments. This is thought likely due to the sample portion allocation being balanced within LL and across frozen rather than chilled storage periods and therefore introducing a degree of variance to chilled-only sample comparisons. Based on this knowledge, the observed trend for PUFA to decline with increasing chilled storage becomes noteworthy, if not concerning due to the implications on health claimable FAs.

Depending on the dietary body, the recommended daily adult consumption of EPA plus DHA levels equates to 250-2000 mg (World Health Organisation), 90-160 mg (Australia and New Zealand National Health and Medical Research Council) or 500-1000 mg (American Diabetic Association, Spanish Society of Community Nutrition) every day (Aranceta & Pérez-Rodrigo, 2012), and much effort has been invested in promoting beef as a nutritious source of these FAs (Howes et al., 2015). Yet applying the findings from this study, we can observe that beef's value as a source or good source of these and other healthy FAs depends on the nature and duration of its preservation. This interpretation is supported by considering peroxidation parameter responses to these same storage combinations. These can also explain the difference in the rate of FA profile change evident between chilled and frozen storage.

Peroxides are a primary product from lipid oxidation and are in turn, broken down by the action of peroxidase enzymes to form low molecular weight products that include ketones, esters, alkanes and malondialdehydes – the latter measured as TBARS (Faustman, Sun, Mancini, & Suman, 2010). Within this context, the increase in PA over chilled storage suggests an increase in substrate availability (peroxides) and therefore lipid peroxidation that had not progressed to secondary oxidation (TBARS). Vieira et al. (2009) also found chilled storage to have no influence on TBARS, but ensuing frozen storage to result in increased levels, and suggested the oxygen-limited packaging environment to have inhibited oxidation rate (Rogers, 2007). This too could be true here, but then not for primary lipid oxidation. The reduction in PA with frozen storage suggests a slowing rate, but continuation of lipid oxidation that would be illustrated in TBARS concentrations which was observed in the present

study. An alternative view is based on the Gray & Monahan (1992) suggestion that PA may underestimate lipid oxidation in meat held for long durations because they themselves break down and therefore TBARS as a secondary product is a more reliable indicator. The same could be true for ORP results, which followed a similar trend to PA across the frozen treatment combinations, but again did not reflect TBARS findings – albeit the 'breakdown' of ORP would be a function of beef's oxygen-scavenging/anti-oxidative capacities (Zhang et al., 2013). This would explain the results from the present study and better suits comparisons to consumer thresholds, particularly as peroxides, unlike TBARS, are tasteless and odourless compounds (Gray & Monahan, 1992).

Consumer thresholds that describe meat eating quality, acceptability and consumer perception of rancidity have been developed using TBARS. These include Campo, Nute, Hughes, Enser, Wood, & Richard (2006) proposing for TBARS exceeding 2.28 as unacceptable for beef; and Igene, Pearson, Merkel, & Coleman (1979) and McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell (2005) both using the arbitrary TBARS threshold of 1.0. From these we can observe in the present study that TBARS levels were 'acceptable' over all chilled storage durations, but became 'unacceptable' with frozen storage and seemingly earlier when frozen storage followed a longer chilled storage period. It must be noted that consumer thresholds are not infallible as Hughes et al. (2015) found when beef TBARS levels reached 2.6, upon 20 weeks chilled storage, the meat had acceptable eating quality when tested using a consumer, but not trained sensory panel. Consequently, this could also be the case for the samples investigated in the present study.

TBARS and other markers of lipid oxidation have been associated with myoglobin oxidative status in many previous studies (Baron & Andersen, 2002; Chaijan, 2008; Faustman et al., 2010). These have defined myoglobin and lipid oxidation as reciprocal reactions because of the capacity for secondary oxidative products released upon the oxidation of one to accelerate the reactivity of the other. Consequently, we could assume colour variation to reflect lipid oxidation in these same beef samples (Colle, Richard, Killinger, Bohlscheid, Gray, Loucks, Day, Cochran, Nasadox, & Doumit, 2015; Suman & Joseph, 2013), and when doing so find the rate of discoloration over display periods reported in Holman, Coombs, Morris, Kerr, & Hopkins (2017a) to be comparable to the evidence of lipid oxidation found here. Furthermore and akin to this past study of frozen beef biochemical reactions, lipid oxidation occurs within the unfrozen water fraction of otherwise frozen beef (Leygonie et al., 2012).

Frozen holding temperatures down to − 22 °C have been reported as insufficient to freeze all available water and therefore prevent all biochemical reactions; which occur within this medium, generate reactive oxidative species, and contribute to lipid oxidation (Rahelic et al., 1985; Rogers, 2007). Building on this, lipid oxidation is thought to be accelerated by frozen storage because of the inherent disruptions from freezing and thawing on muscle cell ultrastructures (Igene et al., 1980b; Leygonie et al., 2012) – processes which were standard for both frozen holding temperatures tested and potentially resulting in similar unfrozen water availability between − 12 °C and − 18 °C and thus their negligible differences. This supports beef sensory quality observations (Holman et al., 2017a) and the adoption of − 12 °C as the minimum frozen storage temperature for beef provided by the EU Commission Implementing Regulation (Anonymous, 2013). The focus of freezing and thawing damage on muscle cell ultrastructures would result in lipid oxidation occurring at the cellular membrane or phospholipid level rather than the triglyceride fraction (Awad et al., 1968; Leygonie et al., 2012). These cellular membranes are rich in UFA and therefore these would be more susceptible to peroxidation and could result in the FA profile shift from UFA to SFA (Igene et al., 1979). This mechanical disruption focus is also the likely source of FA profile differences between chilled and frozen samples (Rogers, 2007).

7.1.3   Indicators of Protein Oxidation and Degradation

The extent of beef protein structure degradation was observed to be influenced by chilled-then-frozen storage combination, and when considered within the context of past research, this was not unexpected. Already the enzymatic pathways of protein degradation, including calpains systems, heat shock protein contributions, etc. (Hopkins & Thompson, 2002; Koohmaraie, Wheeler, & Shackelford, 1995; Lomiwes, Farouk, Wiklund, & Young, 2014; Lund et al., 2011), have been reported to continue throughout chilled storage (ageing) and their action/rate slowed as holding temperatures decrease, before these are eventually halted upon freezing (Dransfield, 1994; Kim, Liesse, Kemp, & Balan, 2015). The act of freezing further contributes to protein degradation as it facilitates the formation of intracellular ice crystals between degraded myofibrils and in turn, these mechanically weaken protein structures and induce additional fragmentation (Zaritzky, 2012). Influence from these pathways was also evident from the shear force (tenderness) values reported for these same samples (Holman et al., 2017a) that reflect the PS and protein solubility results reported here. This too supports past research which identified a strong association between PS and protein solubility to tenderness (Karumendu et al., 2009; Marino, Albenzio, della Malva, Santillo, Loizzo, & Sevi, 2013; Wu, Farouk, Clerens, & Rosenvold, 2014) – it is interesting that protein solubility had been suggested to impact ice formation dynamics (Farouk et al., 2003), and could consequently drive the observed chilled-then-frozen effect.

Ice crystal size and location within a beef structural matrix depends on freezing rate and method and would therefore influence the extent of protein deterioration (Grayson et al., 2014; Leygonie et al., 2012). These were standard within the present study and consequently provide a probable basis for the relative absence of sub-zero holding temperature effects on the protein degradation measures PS and protein solubility. In some respects, however, this finding proves contrary to other studies that reported sarcoplasmic and myofibrillar protein solubility to decrease and increase respectively as frozen storage holding temperature declined (Farouk et al., 2003); but – 12 °C and – 18 °C may not be sufficiently different to repeat these observations for – 18 °C, – 35 °C, and – 75 °C. In addition, frozen holding temperatures above – 22 °C (Rahelic et al., 1985) or – 30 °C (Rogers, 2007) are reportedly insufficient to freeze all available water and restrict biochemical reactions in meat. This was evident in the variation to protein oxidation results across frozen storage periods.

As aforementioned, protein oxidation has been the topic of recent review, but to summarise, it is thought to result from ROS chain reactions which cause peptide side chains and/or amino acid backbone modifications to the detriment of normal protein function (Estévez, 2011; Lund et al., 2011). Lipid oxidation can provide a source of ROS, including superoxides, hydroxyl and peroxyl radicals, which 'feed into' these protein oxidation reactions (Estévez, 2011; Park, Xiong, Alderton, & Oozumi, 2006). As a consequence, we observe similarities between the peroxidase activity and oxidation-reduction potential for these same samples as observed in their carbonyl content (Holman, Coombs, Morris, Bailes, & Hopkins, 2018) – an important indicator of protein oxidation (Estévez, 2011). Admittedly, these oxidative marker trends are only broadly consistent across the experimental chilled-then-frozen storage treatments and again this is thought to be due to the allocation of experimental portions across treatments failing to account for all individual LL effects in addition to potential for inconsistent vacuum-packaging, because of the commercial settings of sample preparation, impacting the degree of anaerobic storage and its inhibition of oxidative processes. It is also noteworthy that lipid oxidation is not the sole source of ROS as it is derived from normal metabolic processes and exogenous factors (Zhang et al., 2013). Nevertheless, the general trends observed do support past research. For example, Utrera et al. (2014c) used HPLC-FLD, a more precise method than applied herein, to find α-aminoadipic and γ-glutamic semialdehydes concentrations

(two specific carbonyl forms) increased with continued storage of frozen beef patties. Popova, Marinova, Vasileva, Gorinov, & Lidji (2009) reported an increased total carbonyl substance level as beef was aged (chilled storage) and although this was an insignificant trend this was suggested to result from individual animal contributions to variation as per the present study. The implications from carbonyl concentration increases on meat eating quality (specifically tenderness) is, however, negative (Huff-Lonergan & Lonergan, 2005; Lund et al., 2011). This is because of their indication of protein denaturation which includes the deactivation of proteolytic and antioxidant functioning enzymes – the latter contributing to the accumulation of ROS and nitrogenous species (Zhang et al., 2013).

Nitrate and nitrite are the oxidised forms of nitric oxide (NO) (Shiva, 2013) – a reactive nitrogenous species with the potential to 'drive' oxidative pathways akin to ROS (Villaverde, Ventanas, & Estévez, 2014). Applying this to the experimental samples, we assumed nitrate and nitrite concentrations to reflect the potential NO contributions to oxidation and to some degree they do – i.e. nitrate levels tended to increase across frozen storage periods and doing so reflect the trend of other oxidative markers assessed here and previously on the same samples (Holman et al., 2018). That said, these trends are largely inconsistent and using these as 'oxidation biomarkers' in uncooked and unprocessed red meat is comparatively novel. Therefore, we acknowledge the need for further investigation and refinement, in terms of quantification precision, before their usefulness in this application can be confirmed.

Mb redox proportions reflect the shifts in colour stability measured on these same samples previously (Holman et al., 2017a). Furthermore, when interpreting the MMb portions against the Hood & Riordan (1973) threshold, whereby beef with MMb concentrations ≥ 20% are considered unacceptable by prospective consumers, we reached the same observation that beef colour discolouration breached acceptability at earlier display periods when both the preceding chilled and subsequent frozen storage durations increased (Holman et al., 2017a). Several other studies have reported similar colour acceptability and changes to OMb and MMb concentrations over chilled and frozen storage (Aroeira, Filho, Fontes, Ramos, Gomide, Ladeira, & Ramos, 2017; Coombs et al., 2017; Vieira et al., 2009). The basis for these outcomes is assumed to be the increased intracellular concentration of pro-oxidants caused by the ageing (chilled storage) and freezing processes (Estévez, 2011; Lynch & Faustman, 2000), as evident from the protein oxidation marker variation and lipid oxidation in these samples (Baron & Andersen, 2002; Holman et al., 2018), denaturing the Mb molecule and reducing colour stability (Holman et al., 2017a). This inference may not be unidirectional as the haem-iron is an efficient promotor of protein carbonylation (Baron & Andersen, 2002; Estévez, 2011; Utrera, Morcuende, & Estevez, 2014a), and its release upon Mb denaturation could have underpinned the carbonyl content observations that respond paradoxically to Mb content across the studied chilled-then-frozen storage durations.

7.2   Lamb

7.2.1   Sensory Quality and Microbial Load

Both chilled and frozen storage affected the meat quality of lamb LL in this study. The relative absence of any interaction effect from chilled-then-frozen storage on meat quality was interesting. Additionally, frozen storage temperature only had negligible effects on meat quality parameters and no effect on microbial loading.

The use of the warmer frozen storage temperature (– 12 °C) compared to the internationally recognised temperature (– 18 °C) (MLA, 2016) tested the potential to maintain lamb LL quality and

safety at different frozen storage temperatures. Differences in quality due to temperature were minimal, and combined with no difference in the load of spoilage microbes suggests – 12 °C could be used to provide comparable safe frozen storage to the – 18 °C temperature for the maintenance of the quality of conventionally chilled meat. A basis for this observation could be the use of a commercial freezer/freezing rate, which prevented excess damage to muscle ultrastructure (Fernandez, Sanz, Molina-Garcia, Otero, Guignan, & Vaudagna, 2007; Grujic et al., 1993; Ngapo, Barbare, Reynolds, & Mawson, 1999; Petrovic, Gruijic, & Petrovic, 1993) and maintained a uniform ice crystal size (Grujic et al., 1993; Ngapo et al., 1999). This was also evident in the lack of frozen storage period effects upon thaw losses in the present study. Similarly, most prior frozen storage studies have found that freezing method and rate, not storage period or temperature; have resulted in alterations to meat quality (Bueno, Resconi, Campo, Cacho, Ferreira, & Escudero, 2013; Muela et al., 2010, 2012). This result means that there is potential to reduce the costs associated with maintaining freezers at lower temperatures, and even higher frozen storage temperatures such as – 10 °C could be tested for use, provided good temperature control is used, which can fulfil paucities arising from prior studies examining eating quality of lamb stored at – 10 °C (Hagyard, Keiller, Cummings, & Chrystall, 1993; Winger, 1984). It would, however, be advantageous to validate this result further through consumer studies focusing on odour and flavour or through measurement of ice crystal size.

In terms of eating quality type traits, the decreased SF and increased IMF proportion showed the positive effects of both chilled- and frozen-only storage periods. The increased proportion of IMF may be related to the proportional moisture loss from experimental LL, as purge and thaw losses, and the corresponding effect of 'concentrating' IMF levels. This could, in turn, result in improved juiciness and flavour traits (Hopkins et al., 2006; Pannier, Gardner, Pearce, McDonagh, Ball, Jacob, & Pethick, 2014). In practice, however, consumers have been found to identify IMF > 4.5% as being preferable in terms of juiciness (Pannier et al., 2014); in this study all mean IMF values were higher than this regardless of treatment. This observation is supported with juiciness found to rarely increase across frozen storage periods (Fernandes et al., 2013; Muela, Monge, Sanudo, Campo, & Beltrán, 2016; Muela et al., 2012) and even decrease in some instances (Bueno et al., 2013).

Tenderness increased due to increased chilled storage periods, based on the decreased SF in this study showing consistency with prior studies on chilled lamb (Belles, Alonso, Roncalés, & Beltrán, 2017). The decrease was most significant following 2 weeks chilled storage, which is consistent with prior studies suggesting tenderisation occurs within 7-10 d from slaughter in lamb (Dransfield, 1994; Starky, Geesink, Oddy, & Hopkins, 2015; Thompson, Hopkins, D'Souza, Walker, Baud, & Pethick, 2005). Frozen lamb has rarely increased in sensory tenderness (Bueno et al., 2013; Fernandes et al., 2013; Muela et al., 2016; Muela et al., 2012) despite decreases in SF in the short term (Muela et al., 2015). Nevertheless, SF results in the current study remained under the consumer acceptability threshold (40 N) based on the modelling of (Hopkins et al., 2006) if chilled for more than 4 weeks, irrespective of the period of frozen storage. By contrast the unchilled lamb was not acceptable based on the threshold, and two weeks chilling provided a significant improvement. It is believed that under the extended storage periods, sufficient proteolysis had occurred to tenderise the meat via calpains in the case of chilled storage (Dransfield, 1994; Hopkins & Thompson, 2002; Huff-Lonergan, Mitsuhashi, Beekman, Parrish, & Olson, 1996) and tissue damage in the case of frozen storage (Leygonie et al., 2012). It was likely; however, that tenderisation via proteolysis did also occur in frozen-only meat, albeit at a slower rate due to diminished calpain activity at frozen temperatures (Crouse & Koohmaraie, 1990; Dransfield, 1994; Duckett et al., 1998). Furthermore, PS analysis can confirm changes in protein structure due to chilled and frozen storage (Coombs et al., 2017; Starky et al., 2015). Protein oxidation could potentially explain increases in SF (Rowe et al., 2004b) and

decreased colour stability (Estévez, 2011) which may have occurred following prolonged frozen storage periods (Coombs et al., 2017).

Upon display, both chilled and frozen storage periods increased the rate of discolouration compared to fresh meat, reflected by a decrease in a*, R630/580 and chroma values, which is consistent with prior studies examining chilled (Ponnampalam, Trout, Sinclair, Egan, & Leury, 2001) and frozen (Muela et al., 2015) storage of lamb. Denaturation of myoglobin from cellular disruption during chilled and frozen storage results in its more rapid oxidation to metmyoglobin and accumulation on the meat surface upon exposure to oxygen, resulting in a brown colour (Leygonie et al., 2012; Muela et al., 2015) – frozen storage additionally reduces stability due to the metmyoglobin reducing system being compromised which thereby affects the ability of these anaerobically stored samples to bloom upon display (Ledward, 1985). Chilled-only storage did, however, result in higher a* values on day 1 of display after periods of 2-6 weeks compared to zero weeks, which likely presented as a stabilised bloomed red colour due to peak oxygenation from myoglobin to oxymyoglobin, consistent with Ponnampalam et al. (2001). This oxygenation effect from the chilled storage was preserved when the lamb was then frozen for 4 weeks, having increased redness compared to frozen-only storage as per Kim et al. (2011), however this effect did not continue with increased frozen storage period.

The presence of spoilage microbial species increased as the chilled storage period lengthened, although all microbial species were below safety thresholds even after 8 weeks; 8 log CFU/g for LAB (Bell, 2001; Kiermeier, Tamplin, May, Holds, Williams, & Dann, 2013) and 6 log CFU/g for *B. thermosphacta* and *Enterobacteriaceae sp.* (Gill, 2014; Gribble, Mills, & Brightwell, 2014). *B. thermosphacta* decreased with subsequent frozen storage, particularly following 12 weeks, which indicates one advantage of freezing. Similarly, proliferation of *Enterobacteriaceae sp.* decreased and proliferation of LAB increased at a slower rate as applied frozen storage periods increased beyond 12 weeks. Microbial profiles in this study indicate good management practices (initial load < 2 log CFU/g) (Gill, 2014), safety (no *E. coli*) (Mills et al., 2014) and good temperature control (maintained safety), even though mean chilled storage temperatures were above the ideal temperature for export safety of around − 1.5 °C (Bell, 2001; Mills et al., 2014; Sumner, 2016; Sumner & Jenson, 2011). This good management may have contributed to the observation that frozen-stored LL (regardless of prior chilled storage period) exhibited low microbial loading that remained below spoilage thresholds for up to 52 weeks, which is consistent with prior frozen storage studies (Fernandes et al., 2013; Hinton et al., 1998). This reflects the findings previously discussed for beef. The implications from this suggest a maintenance of meat quality, as microbial spoilage can result in greening (Egan, Eustace, & Shay, 1988), discolouration (Li, Zamaratskaia, Roos, Bath, Meijer, Borch, & Johansson, 2015) and off-odours and flavours (Gill, 2014; Gribble et al., 2014; Mills et al., 2014). Even so, the observed discolouration (decreased a* and R630/580, increased hue angle) for 8 weeks chilled storage may be attributable to this microbial proliferation despite safety being assured. Additionally, glycogen was observed to be a favourable substrate for spoilage microbes through its negative relationship with microbial loading; its consumption by microbes prevents spoilage where amino acids become consumed by bacteria resulting in off-odours (Coombs et al., 2017). This is to be interpreted with caution, however, as the relationship was more evident between glycogen content and growth of *Enterobacteriaceae sp.* and *B. thermosphacta* at 24 to 52 weeks frozen storage, rather than with LAB across the experimental period. It is also worth noting that different LL were used at different storage period combinations, which may have yielded differences within the data due to different pHu and muscle glycogen levels (Gill, 2014).

In comparison to existing thresholds for meat quality measures and microbial levels we can suggest that the temperatures and periods for chilled storage and frozen storage (− 12 °C) used in this study

could be used in commercial chilled and frozen storage for export and lamb would remain below spoilage thresholds. Meat quality effects were mostly negligible, although decreased colour stability and increased water losses (purge and thaw) can affect the marketability of chilled and frozen lamb LL at a retail level, particularly when displayed aerobically. Colour acceptability was likely to be the most limiting factor towards adoption of these long-term chilled-then-frozen storage combinations with all experimental LL considered as too discoloured following 3 d display based on R630/580 limits (Khliji et al., 2010), while at 1 d display LL were acceptable up to and including c2:f52, c4:f12, c6:f4 and c8:f0. In terms of a* limits from this same study, chilled-only storage for 0-4 weeks would result in acceptable colour for 3 d and c2:f52, c4:f8, c6:f4 and c8:f0 would result in acceptable colour for only 1 d. However given the display of previously frozen lamb at retail is not a practice to be encouraged (although applied by some importing countries) the relevance of this effect in practical terms is minimal.

7.2.2   Fatty Acid and Lipid Oxidation Markers

Chilled and frozen storage conditions, both alone and in combination, largely did not affect the fatty acid profiles (FAP), although they increased lipid oxidation parameters of lamb LL in this study. Storage temperature had minimal effects on either FAP or lipid peroxidation parameters of lamb LL, which further suggests evidence for the use of a warmer frozen storage temperature of -12 °C for the preservation of meat quality over long periods; this is corroborated by previous work in meat quality (*7.2.1 Sensory Quality and Microbial Load*).

The summation of FA categories revealed comparative levels to that previously reported for supermarket lamb LL (Enser, Hallett, Hewitt, Fursey, & Wood, 1996). The only significant point of PUFA increase and diversion from fresh meat FAP occurred at 24 weeks frozen-only storage, where PUFA increased compared to all other storage periods. One possible explanation for this outlier was the use of different LL for each chilled-then-frozen storage period, rather than continued sampling of the same LL (which was not feasible in the current study given the number of assays applied). This was different to the study of Alonso et al. (2016), who found that frozen-only storage of pork for 6 months to 2 years resulted in PUFA oxidation to MUFA and SFA. Prior studies on frozen-only storage of lamb LL for 3-6 months have similarly not reported changes in PUFA, MUFA or SFA concentrations (Popova, 2013; Samouris, Kasapidou, Ioannidou, & Eleftheriadou, 2011). Oxidation of PUFA has been suggested to precede formation of TBARS (Popova & Marinova, 2013), suggesting that lipid oxidation commencing in the chilled storage period continued into frozen storage, or that microbial agents may have contributed to rapid oxidation of frozen-only lamb LL (*7.2.1 Sensory Quality and Microbial Load*). PUFA decreases more commonly occur due to oxidation over chilled-only storage periods, and are concurrent with increased TBARS (Wood, Enser, Fisher, Nute, Richardson, & Sheard, 1999).

The lack of change in FAP due to chilled and frozen storage similarly did not impact on the nutritional quality of lamb in terms of health-claimable n-3 FA (EPA + DHA) levels, however the levels of PUFA, including EPA + DHA, were not sufficient to be considered as a "source" of n-3 PUFA (Alvarenga, Chen, Furusho-Garcia, Perez, & Hopkins, 2016). The nutritional quality was, however, reduced due to the increase in TBARS due to chilled-then-frozen storage, MDA known to have cytotoxic and mutagenic properties (Esterbauer, Schaur, & Zollner, 1991). It is also noteworthy that lamb higher in PUFA through dietary measures (Ponnampalam et al., 2014a) will exhibit more rapid oxidation and will therefore have different nutritional and eating qualities (Cifuni, Napolitano, Pacelli, Riviezzi, & Girolami, 2000; Hopkins et al., 2014) when antioxidant status of muscles are below the threshold (Ponnampalam et al., 2017). Although this lamb LL would not be marketable as "healthy" or a "source of long-chain omega-3 FA", its nutritional value is unlikely to change from a marketing

perspective (FSANZ, 2012). Previous research has found that EPA decreased in concentration in lamb LL frozen for 3-6 months at − 20 °C, though DHA and shorter frozen storage periods were not measured (Popova, 2013).

Chilled-then-frozen storage effects upon lipid oxidation biomarkers (TBARS, ORP and peroxidase activity) are consistent with prior studies which agree that anaerobic storage of red meat increases lipid peroxidation, though not to the extent of aerobic storage (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013; Coombs et al., 2017; Kim et al., 2011; Popova & Marinova, 2013). The observed inconsistencies in all these parameters likely arose from the use of different loins for each storage treatment combination. Meanwhile, the use of anaerobic storage in this study, and the sampling procedure occurring from the frozen state rather than thawed (Muela et al., 2015; Ponnampalam et al., 2017b), likely prevented excess lipid oxidation from occurring in long-term chilled and frozen lamb. This lack of excess lipid oxidation is reflected in the TBARS levels not breaching the rancidity threshold of 2.0 mg MDA per kg of meat suggested by (Campo et al., 2006) at any chilled-then-frozen storage period. Despite this, TBARS exhibited a general increase due to both chilled and frozen storage in the present study, which is consistent with prior lamb studies that observed TBARS increases with longer chilled-only storage periods (Berruga, Vergara, & Gallego, 2005; Fernandes, Freire, de Paula, Kanashiro, Catunda, Rosa, Balieiro, & Trindade, 2014; Kim et al., 2011; Ponnampalam et al., 2017b; Samouris et al., 2011). This increase in oxidation occurred more rapidly in chilled-stored meat compared to frozen meat most likely due to the increases of free PUFA and greater instability of lipid free radicals during chilled storage as previously determined (Igene, Pearson, Dugan, & Price, 1980a). Frozen storage, meanwhile, has been found to result in slower, albeit continuing, lipid oxidation (Kanner, 1994), evidenced by increased TBARS following frozen-only storage periods for 3-9 months (Coombs et al., 2017; Kim et al., 2011; Muela et al., 2015; Muela et al., 2010; Popova & Marinova, 2013). Beyond 9 months frozen storage, however, TBARS has been found to decrease or stabilise (Leygonie et al., 2012; Muela et al., 2015), which did not occur in this study. Results of these previous studies, however, should be interpreted with caution when compared to the present study due to the TBARS measurement taking place on thawed and/or aerobically displayed meat.

One potential reason for TBARS stabilisation or decrease with increased frozen storage periods in prior studies is that MDA acted as a precursor to protein oxidation (Xiong, 2000), and this can be reflected in myoglobin oxidation, causing colour changes over a display period (*7.2.1 Sensory Quality and Microbial Load*). This area of integrative oxidative processes, particularly the effect of lipid oxidation facilitating myoglobin oxidation, and *vice versa*, has been extensively reviewed (Faustman et al., 2010; Kanner, 1994; Min & Ahn, 2005), although the interaction has not been examined in chilled-then-frozen meat. Greater muscle myoglobin content can increase ORP and thus myoglobin oxidation (Min & Ahn, 2005), while increased peroxidase activity may infer a greater ORP of the muscle involved and therefore increase its susceptibility to increased TBARS (Daun & Akesson, 2004).

Rancidity (Martínez-Cerezo, Sañudo, Medel, & Olleta, 2005) and confinement odour (Bell & Garout, 1994; Sumner & Jenson, 2011) have been noted in meat stored chilled-only for periods beyond 8 weeks. Although short-term chilled-only storage periods (< 8 weeks) and associated minor increases in TBARS have been found to improve species-specific flavour (Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard, & Enser, 2003), this may also be linked to increases in medium-chain SFA (6-12 carbon atoms) for sheep meat flavour (Rousset-Akrim et al., 1997; Young, Berdague, Viallon, Rousset-Akrim, & Theriez, 1997), however in the present study no measures on these FA nor sensory panels were conducted. In the present study, all TBARS levels remained below the rancidity threshold regardless of treatment period, which is supported by an accompanying sensory study finding flavour

and overall liking acceptability following 8 weeks chilled-only storage (Coombs, Holman, van de Ven, Friend, & Hopkins, 2016). Regarding frozen-only meat, prior sensory studies have noted acceptability and a lack of difference between lamb meat frozen for one year compared to fresh meat (Fernandes et al., 2013; Hagyard et al., 1993; Muela et al., 2016; Ponnampalam, Sinclair, Egan, Ferrier, & Leury, 2002). It is noteworthy that the majority of the aforementioned studies reported lower TBARS values than the present study at corresponding storage periods, which may have occurred due to the lack of a standardised TBARS measurement procedure (Ponnampalam, Norng, Burnett, Dunshea, Jacobs, & Hopkins, 2014b). Based on prior lamb sensory results, the rancidity threshold (2.0 mg/kg) could potentially be greater, as evidenced by a majority of consumer odour acceptability at TBARS levels ranging from 2.0 to 4.2 mg/kg (Berruga et al., 2005). The resolution of this discrepancy in scientific literature is a potential future investigation.

Based on the general lack of consumer undesirability shown in these results, the frozen storage period could potentially be extended to 2 years or greater (Winger, 1984), even with prior chilled storage, but this remains to be confirmed. At such long frozen storage periods it is unlikely the FAP will change further (Alonso et al., 2016), although the health-claimable FA did not significantly differ at shorter frozen storage periods in the present study. Aerobic display under retail conditions post-thawing should also be tested, as previous studies have reported increases to TBARS (Muela et al., 2015), ORP (Kim, Min, & Ahn, 2002) and rancidity (Muela et al., 2016) as a result of aerobic storage. Such increases were particularly significant following long-term (> 9 months) frozen storage, adding further weight to the argument of testing the effects of aerobic display following chilled-then-frozen storage.

7.2.3   Indicators of Protein Oxidation and Degradation Parameters

Chilled-then-frozen storage periods in this study did not influence carbonyl, nitrate or nitrite contents. Increases in nitrate due to oxidation of nitrite ions (Honikel, 2008) and increased carbonyl contents (Lund et al., 2011) and SF (Coombs et al., 2017) would be expected from protein oxidation, as proteins such as myosin and troponin T resist degradation by calpains under oxidative conditions (Martinaud, Mercier, Marinova, Tassy, Gatellier, & Renerre, 1997; L. J. Rowe, K. Maddock, S. M. Lonergan, & E. J. Huff-Lonergan, 2004a). This outcome could be due to the levels of nitrate and nitrite in lamb LL being low (maxima below 70 ppm and 20 ppm, respectively) and mean values can be considered as "compliant" for fresh meat, being below 40 ppm for nitrate (Iammarino & Di Taranto, 2012). Similarly, carbonyl content remained below 4 nmol/mg for all storage period combinations, which is consistent for pasture-fed lamb stored chilled-only (De Brito et al., 2017; Sante-Lhoutellier, Engel, Aubry, & Gatellier, 2008), highlighting the lack of increase due to frozen storage in the present study.

The lack of effect of frozen storage upon carbonyl content results may also have occurred due to the use of frozen rather than thawed meat for the carbonyl assay, whereas most other studies employed thawing (Utrera et al., 2014c; Xia, Kong, Liu, & Liu, 2009) and/or cooking of the sample (Bueno et al., 2013; Promeyrat, Sayd, Laville, Chambon, Lebret, & Gatellier, 2011) prior to carbonyl quantification, both of which facilitate increased exposure to ROS due to oxidative conditions (Bekhit et al., 2013; Xiong, 2000) and consequently could result in increased carbonyl formation. Meanwhile, the use of a colorimetric assay rather than other methods of carbonyl content quantification such as volatile extraction followed by derivation via gas chromatography-olfactometry-mass spectrometry (Bueno et al., 2013; Utrera et al., 2014c); gel electrophoresis followed by identification via mass spectrometry (Estévez, 2011; Promeyrat et al., 2011); or reactivity with 2,4-dinitrophenylhydrazine (Martinaud et al., 1997; Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987; Sante-Lhoutellier et al.,

2008) means carbonyl content from the present study should be interpreted with caution due to differences in sensitivity from these assays.

Some previous frozen storage results have differed from this study, for example Popova & Marinova (2013) reported carbonyl content to increase following a 12 week frozen storage period and proposed that this occurred due to the freezing process damaging muscle ultrastructure, releasing mitochondrial and lysosomal enzymes and haem iron, allowing for carbonyl formation following exposure to ROS (Xiong, 2000). Similar results were found for frozen beef patties, with specific carbonyl increases at 4 and 12 weeks of frozen storage (Utrera et al., 2014c). The commercial freezing rate used in this study likely minimised, or at least standardised this ultrastructure damage and resulted in uniform ice crystal size (Farouk et al., 2003; Petrovic et al., 1993) (*7.2.1. Sensory Quality and Microbial Load*), which prevented excess protein changes resulting from frozen storage periods or different holding temperatures. The lack of frozen storage temperature effects in this study and *7.2.1. Sensory Quality and Microbial Load* provides an incentive to decrease commercial freezer running costs by increasing the frozen storage temperature to -12 °C given the lack of effect upon quality or safety parameters.

This study suggests that long-term chilled-then-frozen storage combinations do not affect meat quality of lamb LL due to the lack of detrimental effect on protein degradation parameters which have been found to be strongly related with SF, namely PS (Karumendu et al., 2009; Starky et al., 2015) and protein solubility (Farouk et al., 2003; Hopkins & Thompson, 2001). Similarly, protein cross-linking has been previously linked to decreased water holding capacity (Huff-Lonergan & Lonergan, 2005; Z. Liu, Xiong, & Chen, 2010) and juiciness (Bueno et al., 2013). The relative lack of negative effects upon protein oxidation or meat quality (*7.2.1 Sensory Quality and Microbial Load*) provides further opportunity for the frozen storage period to be increased beyond one year until durations successfully tested previously such as 15 months (Hagyard et al., 1993), 21 months (Muela et al., 2016; Muela et al., 2015) and even 24 months (Winger, 1984), although in this case preceded by up to eight weeks of chilled storage. It is also worth mentioning that instrumental measures such as SF and PS are proxies for meat tenderness, and for this reason, consumer sensory panels for long-term stored meat should also be investigated.

Protein solubility research has been scarce for frozen lamb, but for frozen beef myofibrillar soluble protein content increased with storage duration whereas sarcoplasmic protein content decreased (Farouk & Swan, 1998). This was not found to occur in lamb in the present study; the lack of change in myofibrillar protein content can be related to the lack of increase in carbonyl content and PS, as myofibrillar proteins such as myosin and titin have been implicated in cross-linking and oxidation (Huff-Lonergan, Zhang, & Lonergan, 2010; Xiong, 2000). Similarly, these proteins did not degrade due to frozen storage, as they had most likely already degraded from the chilled storage period, as indicated by decreased PS in the present study. Decreased sarcoplasmic protein content with chilled storage period can be related to the decreased PS from proteolysis and associated protein degradation (Farouk & Swan, 1998), and was most likely lost in the increased purge losses with increased chilled storage period (*7.2.1. Sensory Quality and Microbial Load*). Furthermore, the lack of effect of frozen storage on soluble protein content could be explained by the proteolysis taking place as a result of prior chilled storage or from an increased *post mortem* rigor temperature, from which decreases in sarcoplasmic and increases in total and myofibrillar solubilities have been previously reported in beef (Farouk & Swan, 1998). Meanwhile, increased total and myofibrillar protein solubility are related to degradation and the decreased SF associated with ageing (Hopkins & Thompson, 2001) and decreases in these are related to protein denaturation and decreased tenderness/increased SF (Farouk et al., 2003; Petrovic et al., 1993). In the present study this ageing

effect of proteolysis was seen via the strong positive relationship between PS and SF, wherein the decreased PS due to degradation with chilled-only storage resulted in decreased SF, although no effects on protein solubility were observed, possibly due to the low sensitivity of the technique employed.

The relationship of protein degradation markers to SF was predicted in the present study, reaffirming previous studies examining PS (Karumendu et al., 2009; Lametsch, Knudsen, Ertbjerg, Oksbjerg, & Therkildsen, 2007; Starky et al., 2015) and total soluble protein content (Hopkins & Thompson, 2001; Petrovic et al., 1993). Starky et al. (2015) suggested PS and desmin degradation to be the two best predictors of lamb SF when compared to ultimate pH and sarcomere length following 2 weeks chilled storage. The lack of storage period effects on PS beyond 2 weeks chilled-only storage in the present study reflected the lack of difference beyond 7 d chilled storage by Starky et al. (2015), suggesting no further proteolysis or tenderisation occurred beyond this point (Dransfield, 1994). The lack of increase in PS and associated carbonyl content (Xiong, 2000) from prolonged storage periods in this study indicates a lack of protein oxidation and can be related to the preservation of meat quality from the same storage periods (*7.2.1. Sensory Quality and Microbial Load*), which is certainly a positive finding for exporters and consumers.

Oxidation of Mb to the MMb form was seen to occur across display duration (1-3 d) due to chilled storage and on days 0, 2 and 3 following frozen storage. It is worth noting, however, that the Mb fractions in the present study are estimations based on wavelength ratios rather than direct examinations. The MMb fraction can negatively affect consumer appraisal if above 40% of the total Mb concentration (Khliji et al., 2010; Kropf, Hunt, & Piske, 1996), and increased Mb content can lead to more rapid protein oxidation (Promeyrat et al., 2011). The increased MMb to proportions greater than 40% on day 1 in the present study is the largest limiting factor in marketing chilled-then-frozen lamb at a retail level, as meat often requires a retail shelf life of at least 3 days (Coombs et al., 2017) (*7.2.1 Sensory Quality and Microbial Load*). It is also clear that even fresh lamb (not chilled or frozen) in the present study is close to the 40% MMb threshold following 1 d retail display and has breached this threshold by 2 d. These changes in Mb fractions with storage periods reflect the browning (decreased a* and R630/580) from *7.2.1 Sensory Quality and Microbial Load*.

It remains to be seen whether other oxidative conditions resulting from aerobic display will affect protein degradation and oxidation parameters; as has been demonstrated in the present study with further Mb oxidation (decreased DMb and OMb and increased MMb) over 3 d display. It is believed that the anaerobic storage used in this study may explain the lack of protein oxidation, as protein oxidation is more prevalent in aerobic conditions (Lund, Hviid, & Skibsted, 2007; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Aerobic conditions can include retail display, where protein oxidation has been previously observed as a carbonyl increase (Rowe et al., 2004b; Sante-Lhoutellier et al., 2008) and has decreased sarcoplasmic protein solubility over 3 d display period compared to 0 d (Xiong, 2000). Furthermore, decreased SF and increased lipid oxidation have been observed across 6 d aerobic display in addition to discolouration (Muela et al., 2015), inferring associations between lipid and protein oxidation and protein degradation. Measurement of PS and protein solubility post-display can explain this muscle degradation or lack thereof with aerobic storage, particularly in meat that did not sufficiently tenderise under anaerobic storage, such as those not stored chilled prior (Karumendu et al., 2009; Muela et al., 2015). Similarly, carbonyl content can be measured following aerobic storage, with freezing and thawing previously implicated in increased carbonyl content of pork (Xia et al., 2009) and aerobic storage of 4 to 7 d in the increased carbonyl content of fresh lamb (Sante-Lhoutellier et al., 2008).

8.0 CONCLUSIONS/RECOMMENDATIONS

8.1 Key Research Findings

Beef

We found holding temperature effects to be negligible, which suggest – 12 °C could deliver comparable quality LL to -18 °C across these same storage periods. Meat quality parameters varied significantly, but when compared to existing consumer thresholds these may not be perceptible, colour being the exception which proved unacceptable, earlier into retail display when either chilled and subsequent frozen storage periods were increased. There was insufficient detection of key spoilage microbes to allow for statistical analysis, potentially due to the hygienic and commercially representative LL source, although variation in water activity, glycogen content, pH and other moisture parameters conducive to microbial proliferation were influenced by chilled-then-frozen storage.

FA profile variation was observed, with increasing frozen storage periods resulting in unsaturated FA levels declining as saturated FA levels increased. Polyunsaturated and health claimable FA levels also tended to decline with increasing chilled storage period, albeit insignificant within the constraints of the experimental design. Peroxidase activity, TBARS and oxidation-reduction potential analyses reflected these FA changes. These, when compared against existing consumer thresholds, suggest a perceptible detraction from LL held under long-term frozen storage durations that are less evident earlier as dependent on the preceding chilled storage period. Negligible impact of frozen storage holding temperatures was observed on measured traits. These results suggest long-term chilled-then-frozen storage can influence beef lipid stability, healthy FA profile and therefore the healthiness of beef.

Chilled-then-frozen storage was found to impact the extent of protein oxidation and protein structure degradation in beef held over 'long term' periods. Protein degradation, determined as PS and protein solubility, increased over chilled and ensuing frozen storage periods, although at a lesser extent during the latter. The influence of frozen storage holding temperatures (– 12 °C and – 18 °C) was negligible. Protein oxidation continued with storage durations, but generally following an inconsistent trend. Chilled-then-frozen storage also contributed to Mb content and redox proportion variation across display to the detriment of retail appeal. These reflect the sensory quality, fatty acid profile and lipid oxidation results previously reported and doing so strengthen and support chilled-then-frozen storage regimes as a viable industry option.

Lamb

Chilled storage prior to conventional frozen storage can improve and preserve the meat qualities of lamb, evident as tenderness (SF) and colour stability parameters. Furthermore, the optimal pre-freeze chilled storage duration was identified as 2 to 4 weeks – this facilitating the least negative effects in terms of promoting microbial spoilage. Chilled storage beyond 4 weeks is not recommended based on the results presented here as there was negligible improvement in shear force and increased development of discolouration (upon aerobic display) and spoilage microbial proliferation. Frozen storage, meanwhile, proved acceptable for up to one year at both – 12 °C and – 18 °C regardless of prior chilled storage period with minimal quality deterioration based upon the results of this study, although it would be beneficial if future studies investigated odour/flavour properties, retail storage and display conditions. Consequently, food service markets, such as

restaurants or additional processing, would be suitable destinations for long-term chilled-then-frozen meat given excessive colour deterioration and thaw losses.

It can be concluded that chilled-then-frozen storage of lamb LL did not affect the nutritional quality (health-claimable fatty acids EPA + DHA, nor total n-3 PUFA), meaning that LL can be stored for up to 8 weeks chilled-then-52 weeks frozen, and potentially longer, and retain these fatty acids, although it is worth noting the meat in the present study did not constitute a "source" of n-3 PUFA and consequently, variation to initial levels could result in alternative response to chilled-then frozen storage treatments. Increases in lipid oxidation were observed with longer storage periods; however TBARS levels remained below the rancidity threshold regardless of storage period. Furthermore, frozen storage temperatures of $-18\,°C$ and $-12\,°C$ did not result in significant differences in either lipid oxidation or FAP of chilled-then-frozen lamb. It is recommended that further research be undertaken to investigate the linkages between lipid oxidation and oxidation of myoglobin and other proteins, as well as the relationship between TBARS and rancidity and flavour determination of chilled-then-frozen lamb LL using consumer sensory panels.

Conventional chilled and frozen storage, both alone and in combination, posed no significant effect upon the carbonyl, nitrate or nitrite contents of lamb. Similarly, differences in frozen storage temperature (-12 vs. $-18\,°C$) did not yield tangible effects upon the protein properties of the LL. PS did not increase with frozen storage, instead it decreased, along with sarcoplasmic protein solubility, across chilled-only storage periods. This was expected given the proteolysis of meat during this period, along with the associated decreases in SF. The absence of change from frozen storage effects supports the observation that frozen storage periods for up to, including, and potentially beyond one year can be used to preserve the quality of conventionally chilled lamb. The only limiting protein factor from those tested in this study that may restrict the export of long-term chilled-then-frozen lamb is the increased myoglobin oxidation from both forms of storage and its contribution to unsightly discolouration.

8.2   Masters Student Training Outcomes

The project-linked Masters (by research) student Cassius Coombs actively contributed to this project and doing so was successful in satisfying all requirements for his degree – noting his principal supervisor was Dr David Hopkins. Cassius's reflections on this study have been included below:

"In 2015 I commenced my work on the AMPC-funded project "Identifying Storage Thresholds for Frozen and Chilled Red Meat" at Cowra. So far this project, and its funding towards my stipend, have contributed to the submission of my thesis for the degree of Master of Philosophy, as well as the publication of three scientific research papers (Meat Science), four submitted scientific research papers (Meat Science), and six papers in scientific conference proceedings (62nd and 63rd International Congresses of Meat Science and Technology, the former of which I attended; and the Animal Production 2016 conference). I was also named a Young Guns finalist during LambEX 2016.

In addition to attending these conferences and publishing these papers, the project and its associated financial support have furthered my personal and professional development by allowing me to learn many new laboratory and scientific writing techniques, work with top-level scientists, visit several NSW abattoirs and to network with worldwide experts in their respective fields. This project has also inspired me to further my career as a young scientist and professional, by applying for further AMPC-funded projects in similar industries, although my overall career goal is to continue to contribute towards the sustainability of the Australian red meat industry.

8.3   Future Complementary Research

Research is dynamic, with the results from one study often highlighting the need for another. This study was no different. Consequently, at the completion of this project, several paucities or knowledge gaps became apparent that if investigated would bolster and complement its findings. These included:

//   **Can we go longer?** From the present study we see that lamb and beef remains respectively *viable* for up 8 and 5 weeks chilled storage followed by 12 months frozen storage. It would be valuable explore the potential to extend both these chilled and frozen storage durations and provide industry with greater scientifically-based flexibility when managing product distribution and marketing.

//   **Better microbial modelling?** The good cold-chain and initial loads for the assessed microbes are thought to have contributed to the positive outcomes observed, but these conditions are not uniform across the industry. Therefore, additional insight into alternative microbial species and potential use of inoculation trials to standardise the experimental samples across storage combinations would broaden the application of these findings.

//   **Warmer storage?** Frozen storage holding temperatures − 12 °C and − 18 °C were found to have negligible differences in their effect on the analysed parameters. While this finding is exciting, in terms of its potential adoption within industry to improve the cost efficiency of long term storage, it prompts the question: Can we store at warmer temperatures (say − 10 °C as some research has suggested in literature) and further these economic benefits while maintaining acceptable quality?

//   **Are health claimable FA declining in chilled-only beef?** The trend towards health claimable long chain omega-3 fatty acid declines over chilled storage is alarming. Although found to be insignificant here, a more robust and focused experimental design must be applied to confirm or disprove this observation so that Australia's beef industry is informed when marketing its product to an increasing health conscious consumer.

//   **Review of instrumental measure thresholds for red meat's consumer acceptability?** Evident when scrutinising literature was a disparity or relative absence of well-designed and experimentally founded instrumental thresholds defining the acceptability of beef or lamb to the end user. This ambiguity contributes to confusion when interpreting the results of this study (i.e. which threshold should we use to discriminate quality level?) and could be solved with a critical review paper that amalgamates these to identify valid benchmarks and highlight the need to invest in the development of others.

//   **What are driving these observations?** The findings presented herein demonstrate

underlying biochemical processes that drive variation in organoleptic and oxidative traits. By increasing our understanding of these processes the Australian industry could be provided with novel and useful insight into optimising chilled and frozen storage as technological developments 'catch-up'.

// **Different cuts or processed product types?** Only loins were investigated in this study because of their relative value compared to other cuts, ease of use and widespread application as a marker muscle, and uniformity. However many other cuts and product types are held under chilled and frozen storage and their *behaviour* as a result of processing method, biochemical status and interactions with preservative environment may prove different. This should be explored.

9.0 BIBLIOGRAPHY

Aalhus, J. L., Jeremiah, L. E., Dugan, M. E. R., Larsen, I. L., & Gibson, L. L. (2004). Establishment of consumer thresholds for beef quality attributes. *Canadian Journal of Animal Science, 84*, 631-638.

Alonso, V., Muela, E., Tenas, J., Calanche, J. B., Roncalés, P., & Beltrán, J. A. (2016). Changes in physiochemical properties and fatty acid composition of pork following long-term frozen storage. *European Food Research and Technology, 242*, 2119-2127.

Alvarenga, T. I. R. C., Chen, Y., Furusho-Garcia, I. F., Perez, J. R. O., & Hopkins, D. L. (2016). Manipulation of omega-3 PUFAs in lamb: Phenotypic and genotypic views. *Comprehensive Reviews in Food Science and Food Safety, 14*, 189-204.

AMSA. (2012). *Meat color measurement guidelines* (2nd ed.). Champaign, Illinois USA: American Meat Science Association (AMSA).

Anonymous. (2007). Australian standards for the hygienic production and transportation of meat and meat products for human consumption (Vol. AS 4696, pp. 74). AUS: Commonwealth of Australia and each of its States and Territories.

Anonymous. (2013). Opening and providing for the administration of tariff quotas for high-quality fresh, chilled and frozen beef and for frozen buffalo meat *Commission implementing regulation* (pp. 1-12). BEL: European Union.

Aranceta, J., & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *British Journal of Nutrition, 107*, 8-22.

Aroeira, C. N., Filho, R. A. T., Fontes, P. R., Ramos, A. L. S., Gomide, L. A. M., Ladeira, M. M., & Ramos, E. M. (2017). Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle. *Meat Science, 125*, 16-21.

Awad, A., Powrie, W. D., & Fennema, O. (1968). Chemical deterioration of frozen bovine muscle at -4°C. *Journal of Food Science, 33*, 227-235.

Baron, C. P., & Andersen, H. J. (2002). Myoglobin-induced lipid oxidation. A review. *Journal of Agricultural and Food Chemistry, 50*(14), 3887-3897.

Bekhit, A. E. D., Hopkins, D. L., Fahri, F. T., & Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers and remedies. *Comprehensive Reviews in Food Science and Food Safety, 12*, 565-597.

Bell, R. G. (2001). Protection, preservation and presentation. In Y. H. Hui, W. K. Nip, R. W. Rogers & O. A. Young (Eds.), *Meat science and applications*. New York, USA: Marcel-Decker.

Bell, R. G., & Garout, A. M. (1994). The effective product life of vacuum-packaged beef imported into Saudi Arabia by sea, as assessed by chemical, microbiological and organoleptic criteria. *Meat Science, 36*, 381-396.

Belles, M., Alonso, V., Roncalés, P., & Beltrán, J. A. (2017). A review of fresh lamb chilling and preservation. *Small Ruminant Research, 146*, 41-47.

Berruga, M. I., Vergara, H., & Gallego, L. (2005). Influence of packaging conditions on microbial and lipid oxidation in lamb meat. *Small Ruminant Research, 57*, 257-264.

Bouton, P. E., Carrol, F. D., Harris, P. V., & Shorthose, W. R. (1973). Influence of pH and fiber contraction state upon factors affecting the tenderness of bovine muscle. *Journal of Food Science, 38*, 404-407.

Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V., & Escudero, A. (2013). Effect of freezing method and frozen storage duration on odor-active compounds and sensory perception of lamb. *Food Research International, 54*(1), 772-780.

Butler, D. (2009). asreml: asreml() fits the linear mixed model (Version 3.0-1). Retrieved from www.vsni.co.uk

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richard, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*, 303-311.

Chaijan, M. (2008). Review: Lipid and myoglobin oxidations in muscle foods. *Songklanakarin Journal of Science and Technology, 30*, 47-53.

CIE. (1978). Recommendations on uniform color spaces - color equations, psychometric color terms (pp. 15 (E-11.13.L)). Paris: France: Commission Internationale de l'eclairage (CIE).

Cifuni, G. F., Napolitano, F., Pacelli, C., Riviezzi, A. M., & Girolami, A. (2000). Effect of age at slaughter on carcass traits, fatty acid composition and lipid oxidation of Apulian lambs. *Small Ruminant Research, 35*, 65-70.

Colle, M. J., Richard, R. P., Killinger, K. M., Bohlscheid, J. C., Gray, A. R., Loucks, W. I., Day, R. N., Cochran, A. S., Nasadox, J. A., & Doumit, M. E. (2015). Influence of extended aging on beef quality characteristics and sensory perception of steaks from the *gluteus medius* and *longissimus lumborum*. *Meat Science, 110*, 32-39.

Coombs, C. E. O., Holman, B. W. B., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science, 125*, 84-94.

Coombs, C. E. O., Holman, B. W. B., van de Ven, R. J., Friend, M. A., & Hopkins, D. L. (2016). *Comparing chilled and frozen storage on lamb sensory quality parameters.* Paper presented at the 62nd International Congress of Meat Science and Technology (ICoMST), Bangkok, THA.

Crouse, J. D., & Koohmaraie, M. (1990). Effect of freezing of beef on subsequent post-mortem aging and shear force. *Journal of Food Science, 55*, 573-574.

Daun, C., & Akesson, B. (2004). Comparison of glutathione peroxidase activity, and of total and soluble selenium content in two muscles from chicken, turkey, duck, ostrich and lamb. *Food Chemistry, 85*, 295-303.

Dave, D., & Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences, 6*(4), 486.

De Brito, G. F., Holman, B. W. B., McGrath, S. R., Friend, M. A., van de Ven, R. J., & Hopkins, D. L. (2017). The effect of forage-types on the fatty acid profile, lipid and protein oxidation, and retail colour stability of muscles from White Dorper lambs. *Meat Science, 130*, 81-90.

De Brito, G. F., McGrath, S. R., Holman, B. W. B., Friend, M. A., Fowler, S. M., van de Ven, R. J., & Hopkins, D. L. (2016). The effect of forage type on lamb carcass traits, meat quality and sensory traits. *Meat Science, 119*, 95-101.

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science, 36*, 105-121.

Duckett, S. K., Klein, T. A., Leckie, R. K., Thorngate, J. H., Busboom, J. R., & Snowder, G. D. (1998). Effect of freezing on calpastatin activity and tenderness of callipyge lamb. *Journal of Animal Science, 76*, 1869-1874.

Egan, A. F., Eustace, I. J., & Shay, B. J. (1988). Meat Packaging - Maintaining the quality and proloning the storage life of chilled beef, port and lamb. *Meat 88: Proceedings of the industry day, pp. 68-75*.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J., & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science, 42*, 443-456.

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, amlondialdehyde and related aldehydes. *Free Radical Biology and Medicine, 11*, 81-128.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*, 259-279.

Farouk, M. M., & Swan, J. E. (1998). Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Science, 49*, 233-247.

Farouk, M. M., Wieliczko, K. J., & Merts, I. (2003). Ultra-fast freezing and low storage temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Science, 66*, 171-179.

Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science, 86*, 86-94.

Fernandes, R. P. P., Freire, M. T. A., Carrer, C. C., & Trindade, M. A. (2013). Evaluation of physicochemical, microbiological and sensory stability of frozen stored vacuum-packed lamb meat. *Journal of Integrative Agriculture, 12*, 1946-1952.

Fernandes, R. P. P., Freire, M. T. A., de Paula, E. S. M., Kanashiro, A. L. S., Catunda, F. A. P., Rosa, A. F., Balieiro, J. C. C., & Trindade, M. A. (2014). Stability of lamb loin stored under refrigeration and packed in different modified atmosphere packaging systems. *Meat Science, 96*, 554-561.

Fernandez, P. P., Sanz, P. D., Molina-Garcia, A. D., Otero, L., Guignan, B., & Vaudagna, S. R. (2007). Conventional freezing plus high pressure-low temperature treatment: Physical properties, microbial quality and storage stability of beef meat. *Meat Science, 77*, 616-625.

FSANZ. (2012). Nutrition Claims *Nutrition Information Users Guide to Standard 1.2.8 - Nutritional Information Requirements*. Retrieved from www.foodstandards.gov.au: Food Standards Australia and New Zealand.

Gill, C. O. (2014). Spoilage, factors affecting - microbiological. In C. E. Devine & M. Dikeman (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., Vol. 3). Oxford, UK: Elsevier Ltd.

Gill, C. O., & Newton, K. G. (1978). The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Science, 2*, 207-217.

Gray, J. I., & Monahan, F. J. (1992). Measurement of lipid oxidation in meat and meat products. *Trends in Food Science & Technology, 3*, 315-319.

Grayson, A. L., King, D. A., Shackelford, S. D., Koohmaraie, M., & Wheeler, T. L. (2014). Freezing and thawing or freezing, thawing, and aging effects on beef tenderness. *Journal of Animal Science, 92*, 2735-2740.

Gribble, A., Mills, J., & Brightwell, G. (2014). The spoilage characteristics of *Brochothrix thermosphacta* and two psychrotolerant *Enterobacteriacae* in vacuum packed lamb and the comparison between high and low pH cuts. *Meat Science, 97*, 83-92.

Grujic, R., Petrovic, L., Pikula, B., & Amidzic, L. (1993). Definition of the optimum freezing rate - 1. Investigation of structure and ultrastructure of beef m. longissimus dorsi frozen at different freezing rates. *Meat Science, 33*, 301-318.

Hagyard, C. J., Keiller, A. H., Cummings, T. L., & Chrystall, B. B. (1993). Frozen storage conditions and rancid flavour development in lamb. *Meat Science, 35*, 305-312.

Hinton, M., Holder, J. R., Hudson, W. R., Coombs, E., Allen, V., & Corry, J. E. L. (1998). The bacteriological quality of British beef 3. Frozen primal joints. *Meat Science, 50*, 403-409.

Holman, B. W. B., Alvarenga, T. I. R. C., van de Ven, R. J., & Hopkins, D. L. (2015). A comparison of technical replicate (cuts) effect on lamb Warner–Bratzler shear force measurement precision. *Meat Science, 105*, 93-95.

Holman, B. W. B., Coombs, C. E. O., Morris, S., Bailes, K., & Hopkins, D. L. (2018). Effect of long term chilled (up to 5 weeks) then frozen (up to 12 months) storage at two different sub-zero holding temperatures on beef: 2. Lipid peroxidation and fatty acid profiles. *Meat Science*, (In Press).

Holman, B. W. B., Coombs, C. E. O., Morris, S., Kerr, M. J., & Hopkins, D. L. (2017a). Effect of long term chilled (up to 5 weeks) then frozen (up to 12 months) storage at two different sub-zero holding temperatures on beef: 1. Meat quality and microbial loads. *Meat Science, 133*, 133-142.

Holman, B. W. B., Ponnampalam, E. N., van de Ven, R. J., Kerr, M. G., & Hopkins, D. L. (2015). Lamb meat colour values (HunterLab CIE and reflectance) are influenced by aperture size (5mm v. 25mm). *Meat Science, 100*, 202-208.

Holman, B. W. B., van de Ven, R., Mao, Y., Coombs, C. E. O., & Hopkins, D. L. (2017b). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127*, 57-62.

Holman, B. W. B., Van de Ven, R. J., Coombs, C. E. O., & Hopkins, D. L. (2017c). Effect of beef pre-cooking status (frozen v. thawed) and sample weight on shear force evaluation. *Food Analytical Methods, 10*, 3235-3238.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science, 49*, 447-457.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science, 78*, 68-76.

Hood, D. E., & Riordan, E. B. (1973). Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. *Journal of Food Technology, 8*, 333-343.

Hopkins, D. L., Clayton, E. H., Lamb, T. A., Van de Ven, R. J., Refshauge, G., Kerr, M. J., Bailes, K., Lewandowski, P., & Ponnampalam, E. N. (2014). The impact of supplementing lambs with algae on growth, meat traits and oxidative status. *Meat Science, 98*, 135-141.

Hopkins, D. L., Hegarty, R. S., Walker, P. J., & Pethick, D. W. (2006). Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep. *Animal Production Science, 46*, 879-884.

Hopkins, D. L., Lamb, T. A., Kerr, M. J., van de Ven, R. J., & Ponnampalam, E. N. (2013). Examination of the effect of ageing and temperature at rigor on colour stability of lamb meat. *Meat Science, 95*, 311-316.

Hopkins, D. L., & Thompson, J. M. (2001). The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. *Meat Science, 57*(1), 1-12.

Hopkins, D. L., & Thompson, J. M. (2002). Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact of tenderisation in beef and sheep meat. *Australian Journal of Agricultural Research, 53*, 149-166.

Hopkins, D. L., Toohey, E. S., Lamb, T. A., Kerr, M. J., van de Ven, R. J., & Refshauge, G. (2011). Explaining the variation in the shear force of lamb meat using sarcomere length, the rate of rigor onset and pH. *Meat Science, 88*, 794-796.

Howes, N. L., Bekhit, A. E. D., Burritt, D. J., & Campbell, A. W. (2015). Opportunities and implications of pasture-based lamb fattening to enhance the long-chain fatty acid composition in meat. *Comprehensive Reviews in Food Science and Food Safety, 14*, 22-36.

Huff-Lonergan, E. J., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of post-mortem biochemical and structural changes. *Meat Science, 119*, 32-40.

Huff-Lonergan, E. J., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., & Olson, O. E. (1996). Proteolysis of specific muscle structural prtoeins by mu-calpain at low pH and temperature is similar to degradation in post-mortem bovine muscle. *Journal of Animal Science, 74*, 993-1008.

Huff-Lonergan, E. J., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - Lessons on mechanisms of meat tenderization. *Meat Science, 86*, 184-195.

Hughes, J. M., McPhail, N. G., Kearney, G., Clarke, F., & Warner, R. D. (2015). Beef *longissimus* eating quality increased up to 20 weeks of storage and is unrelated meat colour at carcass grading. *Animal Production Science, 55,* 174-179.

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science, 98*, 520-532.

Hwang, I. H., Park, B. Y., Cho, S. H., & Lee, J. M. (2004). Effects of muscle shortening and proteolysis on Warner-Bratzler shear force in beef *longissimus* and *semitendinosus*. *Meat Science, 68*, 497-505.

Iammarino, M., & Di Taranto, A. (2012). Nitrate and nitrite in fresh meats: A contribution to the estimation of admissible maximum limits to induce in directive 95/2/EC. *International Journal of Food Science and Technology, 47*, 1852-1858.

Igene, J. O., Pearson, A. M., Dugan, L. R., & Price, J. F. (1980a). Role of triglycerides and phospholipids on development of rancidity in meat model systems during frozen storage. *Food Chemistry, 5*, 263-276.

Igene, J. O., Pearson, A. M., & Gray, J. I. (1980b). Effect of length of frozen storage, cooking and holding temperatures upon component phospholipids and the fatty acid composition of meat triglycerides and phospholipids. *Food Chemistry, 7*, 289-303.

Igene, J. O., Pearson, A. M., Merkel, R. A., & Coleman, T. H. (1979). Effect of frozen storage time, cooking and holding temperature upon extractable lipids and TBA values of beef and chicken. *Journal of Animal Science, 49*, 701-707.

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stabiliy, retail properties and case-life of retail-ready beef. *Food Research International, 34*, 815-826.

Kanner, R. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Science, 36*, 169-189.

Karumendu, L. U., van de Ven, R., Kerr, M. J., Lanza, M., & Hopkins, D. L. (2009). Particle size analysis of lamb meat: Effect of homogenisation speed, comparison with myofibrillar fragmentation index and its relationship with shear force. *Meat Science, 82*, 425-431.

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J., & Parr, T. (2010). Tenderness – An enzymatic view. *Meat Science, 84*, 248-256.

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effect estimators from restricted maximum likelihood. *Biometrics, 53*, 983-997.

Khan, A. W., & Lentz, C. P. (1977). Effects of freezing, thawing and storage on some quality factors for portion-size beef cuts. *Meat Science, 1*, 263-270.

Khliji, S., van de Ven, R. J., Lamb, T. A., Lanza, M., & Hopkins, D. L. (2010). Relationship between consumer ranking of lamb colour and objective measures of colour. *Meat Science, 85*, 224-229.

Kiani, H., & Sun, D. W. (2011). Water crystallization and its importance to freezing of foods: A review. *Trends in Food Science & Technology, 22*, 407-426.

Kiermeier, A., Tamplin, M., May, D., Holds, G., Williams, M., & Dann, A. (2013). Microbial growth, communities and sensory characteristics of vacuum and modified atmosphere packaged lamb shoulders. *Food Microbiology, 36*, 305-315.

Kim, Y. H. B., Frandsen, M., & Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. *Meat Science, 88*, 332-337.

Kim, Y. H. B., Liesse, C., Kemp, R., & Balan, P. (2015). Evaluation of combined effects of ageing period and freezing rate on quality attributes of beef loins. *Meat Science, 110*, 40-45.

Kim, Y. H. B., Min, K. C., & Ahn, D. U. (2002). Color, oxidation-reduction potential, and gas production of irradiated meats from different animal species. *Journal of Food Science, 67*, 1692-1695.

Koohmaraie, M., Wheeler, T. L., & Shackelford, S. D. (1995). *Beef tenderness: regulation and prediction*. Paper presented at the Proc. Meat.

Kristensen, L., Christensen, M., & Ertbjerg, P. (2006). Activities of calpastatin, µ-calpain and m-calpain are stable during frozen storage of meat. *Meat Science, 72*, 116-120.

Kropf, D. H., Hunt, M. C., & Piske, D. (1996). *Color formation and retention in fresh meat.* Paper presented at the Meat Industry Research Conference, Washington DC, USA.

Lagerstedt, Å., Enfält, L., Johansson, L., & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi*. *Meat Science, 80*, 457-461.

Lametsch, R., Knudsen, J. C., Ertbjerg, P., Oksbjerg, N., & Therkildsen, M. (2007). Novel method for determiantion of myofibril fragmentation post-mortem. *Meat Science, 75*, 719-724.

Ledward, D. A. (1985). Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science, 15*, 149-171.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*, 93-98.

Li, S., Zamaratskaia, G., Roos, S., Bath, K., Meijer, J., Borch, E., & Johansson, M. (2015). Inter-relationships between the metrics of instrumental color and microbial graoth during aerobic storage of beef at 4°C. *Acta Agriculturae Scandinavica, Section A - Animal Science, 65*, 97-106.

Liu, J., Arner, A., Puolanne, E., & Ertbjerg, P. (2016). On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation. *Meat Science, 119*, 32-40.

Liu, Z., Xiong, Y. L., & Chen, J. (2010). Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. *Journal of Agricultural and Food Chemistry, 58*, 10697-10704.

Lomiwes, D., Farouk, M. M., Wiklund, E., & Young, O. A. (2014). Small heat shock proteins and their role in meat tenderness: A review. *Meat Science, 96*, 26-40.

Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*, 83-95.

Lund, M. N., Hviid, M. S., & Skibsted, L. H. (2007). The combined effect of antioxidants and modified atmospheric packaging on protein and lipid oxdiation in beef patties during chilled storage. *Meat Science, 76*, 226-233.

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science, 77*, 295-393.

Lynch, M. P., & Faustman, C. (2000). Effect of aldehyde lipid oxidation products on myoglobin. *Journal of Agricultural and Food Chemistry, 48*, 600-604.

Marino, R., Albenzio, M., della Malva, A., Santillo, A., Loizzo, P., & Sevi, A. (2013). Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time. *Meat Science, 95*, 281-287.

Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry, 45*, 2481-2487.

Martínez-Cerezo, S., Sañudo, C., Medel, I., & Olleta, J. L. (2005). Breed, slaughter weight and ageing time effects on sensory characteristics. *Meat Science, 69*, 571-578.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70*, 665-682.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science, 79*, 3062-3068.

Mills, J., Donnison, A., & Brightwell, G. (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science, 98*, 71-80.

Min, B., & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products - A review. *Food Science and Biotechnology, 14*, 152-163.

MLA. (2016). *Shelf life of Australian red meat* (2 ed.). Retrieved from www.mla.com.au: Meat & Livestock Australia (MLA).

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science, 49*, 73-86.

Muela, E., Monge, P., Sanudo, C., Campo, M. M., & Beltrán, J. A. (2016). Sensory quality of lamb following long-term frozen storage. *Meat Science, 114*, 32-37.

Muela, E., Monge, P., Sañudo, C., Campo, M. M., & Beltrán, J. A. (2015). Meat quality of lamb frozen stored up to 21 months: Instrumental analyses on thawed meat during display. *Meat Science, 102*, 35-40.

Muela, E., Sañudo, C., Campo, M. M., Medel, I., & Beltrán, J. A. (2010). Effect of freezing method and frozen storage duration on instrumental quality of lamb throughout display. *Meat Science, 84*, 662-669.

Muela, E., Sañudo, C., Campo, M. M., Medel, I., & Beltrán, J. A. (2012). Effect of freezing method and frozen storage duration on lamb sensory quality. *Meat Science, 90*, 209-215.

Mulley, R., Lean, I., & Wright, V. E. (2014). Market preparation. In D. Cottle & L. Kahn (Eds.), *Beef Cattle Production and Trade* (pp. 47-63). Collingwood, AUS: CSIRO Publishing.

Ngapo, T. M., Barbare, I. H., Reynolds, J., & Mawson, R. F. (1999). Freezing and thawing rate effects on drip loss from samples of pork. *Meat Science, 53*, 149-158.

Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S., & Stadtman, E. R. (1987). Age-related changes in oxidized proteins. *The Journal of Biological Chemistry, 262*, 5488-5491.

Pannier, L., Gardner, G. E., Pearce, K. L., McDonagh, M., Ball, A. J., Jacob, R. H., & Pethick, D. W. (2014). Association of sire estimated breeding values and objective meat quality measurements with sensory scores in Australian lamb. *Meat Science, 96*, 1076-1087.

Park, D., Xiong, Y. L., Alderton, A. L., & Oozumi, T. (2006). Concentration effects of hydroxyl radical oxidizing systems on biochemical properties of porcine muscle myofibrillar protein. *Food Chemistry, 101*, 1239-1246.

Petrovic, L., Gruijic, R., & Petrovic, M. (1993). Definition of the optimal freezing rate - 2. Investigation of the physico-chemical properties of beef M. longissimus dorsi frozen at different freezing rates. *Meat Science, 33*, 319-331.

Ponnampalam, E. N., Butler, K. L., Pearce, K. M., Mortimer, S. I., Pethick, D. W., Ball, A. J., & Hopkins, D. L. (2014a). Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights. *Meat Science, 96*, 1095-1103.

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. D. (2017a). Causes and contributing factors to "dark cutting" meat: Current Trends and Future Directions: A review. *Comprehensive Reviews in Food Science and Food Safety, 00*, 1-31.

Ponnampalam, E. N., Norng, S., Burnett, V. F., Dunshea, F. R., Jacobs, J. L., & Hopkins, D. L. (2014b). The synergism of biochemical components controlling lipid oxidation in lamb muscle. *Lipids, 49*, 757-766.

Ponnampalam, E. N., Plozza, T., Kerr, M. G., Linden, N., Mitchell, M., Bekhit, A. E. D., Jacobs, J. L., & Hopkins, D. L. (2017b). Interaction of diet and long ageing period on lipid oxidation and colour stability of lamb meat. *Meat Science, 129*, 43-49.

Ponnampalam, E. N., Sinclair, A. J., Egan, A. R., Ferrier, G. R., & Leury, B. J. (2002). Dietary manipulation of muscle long-chain omega-3 and omega-6 fatty acids and sensory properties of lamb meat. *Meat Science, 60*, 125-132.

Ponnampalam, E. N., Trout, G. R., Sinclair, A. J., Egan, A. R., & Leury, B. J. (2001). Comparison of the color stability and lipid oxidative stability of fresh and vacuum packaged lamb muscle containing elevated omega-3 and omega06 fatty acid levels from dietary manipulation. *Meat Science, 58*, 151-161.

Popova, T. (2013). Fatty acid composition during storage in m. *Longissimus dorsi* and m. *Semimembranosus* in lambs, reared indoors and on pasture. *Emirates Journal of Food and Agriculture, 26*(3), 302-308.

Popova, T., & Marinova, P. (2013). Protein oxidation in *M. longissimus dorsi* and *M. semimembranosus* lambs reared indoors and on pasture. *Iranian Journal of Applied Animal Science, 3*, 673-677.

Popova, T., Marinova, P., Vasileva, V., Gorinov, Y., & Lidji, K. (2009). Oxidative changes in lipids and proteins in beef during storage. *Archiva Zootechnica, 12*, 30-38.

Promeyrat, A., Sayd, T., Laville, E., Chambon, C., Lebret, B., & Gatellier, P. (2011). Early post-mortem sarcoplasmic proteome of porcine muscle related to protein oxidation. *Food Chemistry, 127*, 1097-1104.

R Core Team. (2016). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from www.R-project.org

Rahelic, S., Gawwad, A. H., & Puac, S. (1985). Structure of beef longissimus dorsi muscle frozen at various temperatures: Part 2 - Ultrastructure of muscles frozen at -10, -22, -33, -78 and -115°C. *Meat Science, 14*, 73-81.

Rogers, R. W. (2007). Packaging and freezing of beef as related to sensory properties. In D. W. Sun (Ed.), *Handbook of Frozen Food Processing and Packaging* (pp. 3-38). Boca Raton, USA: Taylor & Francis Publishing.

Rousset-Akrim, S., Young, O. A., & Berdague, J. L. (1997). Diet and growth effects in panel assessment of sheepmet odour and flavour. *Meat Science, 45*, 169-181.

Rowe, L. J., Maddock, K., Lonergan, S. M., & Huff-Lonergan, E. J. (2004a). Oxidative environments decrease tenderization of beef steaks through inactivation of u-calpain. *Journal of Animal Science, 82*, 3254-3266.

Rowe, L. J., Maddock, R. J., Lonergan, S. M., & Huff-Lonergan, E. J. (2004b). Influence of early postmortem protein oxidation on beef quality. *Journal of Animal Science, 82*, 785-793.

Samouris, G., Kasapidou, E., Ioannidou, M., & Eleftheriadou, A. (2011). Changes in lipid oxidation levels and fatty acid composition in the meat of milk-fed lambs during refrigerated and frozen storage - Nutritional indices. *Journal of Food Safety and Food Quality, 62*, 217-222.

Sante-Lhoutellier, V., Engel, E., Aubry, L., & Gatellier, P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Science, 79*, 777-783.

Shiva, S. (2013). Nitrite: A physiological store of nitric oxide and modulator of mitochondrial function. *Redox Biology, 1*, 40-44.

Sigma-Aldrich. (2015a). Bicinchoninic acid kit for protein determination assay kit *Technical Bulletin BCA1*. Sigma-Adrich Co. LLC. Retrieved from www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/1/bca1.html.

Sigma-Aldrich. (2015b). Glycogen assay kit *Technical Bulletin*. Sigma-Adrich Co. LLC. Retrieved from www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/1/mak016bul.pdf.

Sigma-Aldrich. (2015c). Nitrite/nitrate assay kit *Technical Bulletin no. 23479*. Sigma-Adrich Co. LLC. Retrieved from www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/1/23479.html.

Sigma-Aldrich. (2015d). Peroxidase activity assay kit *Technical Bulletin*. Sigma-Adrich Co. LLC. Retrieved from www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/1/mak092bul.pdf.

Sigma-Aldrich. (2015e). Protein carbonyl assay kit *Technical Bulletin MAK094*. Sigma-Adrich Co. LLC. Retrieved from www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/1/mak094.html.

Small, A. H., Jenson, I., Kiermeier, A., & Sumner, J. (2012). Vacuum-packed beef primals with extremely long shelf life have unusual microbiological counts. *Journal of Food Protection, 75*, 1524-1527.

Smith, M. A., Bush, R. D., van de Ven, R. J., Hall, E. J. S., Greenwood, P. L., & Hopkins, D. L. (2017). The impact of gender and age on the nutritional parameters of alpaca (Vicugna pacos) meat, colour stability and fat traits. *Meat Science, 123*, 21-28.

Starky, C. P., Geesink, G. H., Oddy, V. H., & Hopkins, D. L. (2015). Explaining the variation in lamb *longissimus* shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Science, 105*, 32-37.

Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology, 4*, 79-99.

Sumner, J. (2016). The impact of transport to Australia's distant markets: Australian Meat Processor Corporation.

Sumner, J., & Jenson, I. (2011). The effect of storage temperature on shelf-life of vacuum-packaged lamb shoulders. *Food Australia, 63*, 249-251.

Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Advances in Nutrition, 3*, 1-7.

Thompson, J. M., Hopkins, D. L., D'Souza, D. N., Walker, P. J., Baud, S. R., & Pethick, D. W. (2005). The impact of processing on sensory and objective measurements of sheep meat eating quality. *Animal Production Science, 45*, 561-573.

Utrera, M., Morcuende, D., & Estevez, M. (2014a). Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. *Meat Science, 96*, 1250-1257.

Utrera, M., Morcuende, D., & Estévez, M. (2014b). Fat content has a significant impact on protein oxidation occured during frozen storage of beef patties. *LWT - Food Science and Technology, 56*, 62-68.

Utrera, M., Parra, V., & Estévez, M. (2014c). Protein oxidation during frozen storage and subsequent processing of different beef muscles. *Meat Science, 96*, 812-820.

Verbyla, A. P., Cullis, B. R., Kenward, M. G., & Welham, S. J. (1999). The analysis of designed experiments and longitudinal data by using smoothing splines. *Applied Statistics, 48*, 269-311.

Vieira, C., Diaz, M. T., Martínez, B., & García-Cachán, M. D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science, 83*, 398-404.

Villaverde, A., Ventanas, J., & Estévez, M. (2014). Nitrite promotes protein carbonylation and Strecker aldehyde formation in experimental foermented sausages: Are both events connects? *Meat Science, 98*, 665-672.

Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics, 64*, 113-119.

Winger, R. J. (1984). Selection of judges and assessment of storage-related flavours in frozen sheepmeats. *International Journal of Food Science & Technology, 19*, 17-20.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I., & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society, 58*, 363-370.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., & Enser, M. (2003). Effects of fatty acids on meat quality: A review. *Meat Science, 66*, 21-32.

Wu, G., Farouk, M. M., Clerens, S., & Rosenvold, K. (2014). Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Science, 98*, 637-645.

Wyness, L., Weichselbaum, E., O'Connor, A., Williams, E. B., Benelam, B., Riley, H., & Stanner, S. (2011). Red meat in the diet: an update. *Nutrition Bulletin, 36*, 34-77.

Xia, X., Kong, B., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influence by different freeze-thaw cycles. *Meat Science, 83*, 239-245.

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. In E. Decker & C. Faustman (Eds.), *Antioxidants in muscle foods* (pp. 85-111). UK: John Wiley & Sons.

Young, O. A., Berdague, J. L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-bourne volatiles and sheepmeat odours. *Meat Science, 45*, 183-200.

Zaritzky, N. (2012). Physical-chemical principles in freezing. In D. W. Sun (Ed.), *Handbook of Frozen Food Processing and Packaging* (2 ed., pp. 4-37). New York, USA: CRC Press.

Zeptometrix. (2006). Thiobarbituric acid reactive substances. *OXltek TBARS Assay Kit, 2*, 1-11.

Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition, 53*, 1191-1201.

10.0 APPENDICES

10.1 List of Publications

Holman BWB, Coombs CEO, Hopkins DL (2015) The effect of long-term chilled ageing duration on lamb meat water activity. Proceedings of the '61th International Congress of Meat Science and Technology', 23-28th August, Clermont-Ferrand: FRA.

Coombs CEO, Holman BWB, Collins D, Friend MA, Hopkins DL (2016) Examining the relationship between colorimetric measurements and microbial loading of beef meat. Proceedings of the '31st Biennial Conference of the Australian Society of Animal Production', 5-7th July, Adelaide: AUS. *www.asap.asn.au/wp-content/uploads/abstract-2015/101/attach_brief.pdf*

Coombs CEO, Holman BWB, van de Ven RJ, Friend MA, Hopkins DL (2016) Effect of eight weeks chilled or frozen storage on consumer-defined sensory quality traits of lamb. Proceedings of the '31st Biennial Conference of the Australian Society of Animal Production', 5-7th July, Adelaide: AUS. *www.asap.asn.au/wp-content/uploads/abstract-2015/169/attach_brief.pdf*

Coombs CEO, Holman BWB, van de Ven RJ, Friend MA, Hopkins DL (2016) Comparing chilled and frozen storage on lamb sensory quality parameters. Proceedings of the '62th International Congress of Meat Science and Technology', 15-19th August, Bangkok: THA (P03-06; pp. 1-4)

Coombs CEO, Holman BWB, Collins D, Friend MA, Hopkins DL (2016) Using beef meat colorimetrics to predict microbial loading following chilled-then-frozen storage. Proceedings of the '62th International Congress of Meat Science and Technology', 15-19th August, Bangkok: THA (P06-06: pp. 1-4)*.*

Coombs CEO, Holman BWB, van de Ven RJ, Friend MA, Hopkins DL (2016) Effect of chilled storage (up to 8 weeks) on lamb meat quality traits. Proceedings of the '62th International Congress of Meat Science and Technology', 15-19th August, Bangkok: THA (P01-01; pp. 1-4).

Coombs CEO, Holman BWB, Friend MA, Hopkins DL (2017) Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science* 125: 84-94.

Holman BWB, Coombs CEO, Morris S, Kerr MJ, Hopkins DL (2017) Effect of long term chilled (up to 5 weeks) then frozen (up to 12 months) storage at two different sub-zero holding temperatures on beef: 1. Meat quality and microbial loads. *Meat Science* 133: 133-142.

Coombs CEO, Holman BWB, Collins D, Friend MA, Hopkins DL (2017) Effects of chilled-then-frozen storage (up to 52 weeks) on lamb *M. longissimus lumborum* quality and safety parameters. *Meat Science* 134: 86-97.

Holman BWB, Coombs CEO, van de Ven RJ, Hopkins DL (2017). Effect of frozen storage (up to 24 weeks) at different temperatures on beef loin eating quality. Proceedings of the '63rd International Congress of Meat Science and Technology' *Proc. 63rd International Congress of Meat Science and Technology'*, (Eds. Troy D, McDonnell C, Hinds L & Kerry J) Cork: IRE (pp. 81-82)*.*

Coombs CEO, Holman BWB, Collins D, Kerr MJ, Friend MA, Hopkins DL (2018) Effects of chilled-then-frozen storage (up to 52 weeks) on an indicator of protein oxidation and indices of protein degradation in lamb *M. longissimus lumborum*. *Meat Science* 135: 134-141.

Holman BWB, Coombs CEO, Morris S, Bailes K, Hopkins DL (2018). Effect of long term chilled (up to 5 weeks) then frozen (up to 12 months) storage at two different sub-zero holding temperatures on beef: 2. Lipid peroxidation and fatty acid profiles. *Meat Science (In press).*

10.2 Snapshot

This has been provided as a separate document and included herein.

SNAPSHOT

IDENTIFYING STORAGE THRESHOLDS IN FROZEN AND CHILLED RED MEATS

Project Report Reference: 2014-1048   **Date: 13 December 2017**

Project Description

Red meat (lamb and beef) are important global commodities and as such, industry must deliver safe and quality product to often distant end-users – to do otherwise would incur substantial market access and economic penalties. Chilled and frozen storage have each been applied to fulfil this need and have proven independently successful; evident from their universal use to preserve red meat and their broad scientific validation. Their combination, however, and usefulness across long-term durations has not received comparable exploration and as a consequence the associated advantages and implications on red meat quality may have been overlooked.

Project Content

This project aimed to study the effect of different chilled-then-frozen storage durations on red meat quality. This entailed two staggered and complementary experiments (including two simultaneous replicates) testing lamb and beef respectively. It should be noted that sample collection, preparation, processing and chilled (ageing) storage occurred at and with the cooperation of commercial Australian abattoirs.

// *Experiment 1*: Lamb loins (360) were randomly selected and assigned to 5 chilled storage periods (0, 2, 4, 6 and 8 weeks) x six ensuing frozen storage periods (0, 4, 8, 12, 24 and 52 weeks) set at two different frozen storage holding temperatures (-12 and -18 °C).

// *Experiment 2*: Beef loins (48) which met Japanese market specifications were selected; divided into 4 equal portions (192) which were then assigned to each of 4 chilled storage periods (0, 2, 3 and 5 weeks) followed by the same frozen storage periods and holding temperatures as Experiment 1.

These were analysed for sensory traits; microbial loads; lipid and fatty acid composition; and protein degradation and oxidation characteristics – which were in turn compared to existing thresholds defining consumer acceptability.

Project Outcome

This project demonstrated that there are no apparent quality limitations with holding lamb or beef frozen across extended periods of time at -12 °C. There were expected improvements in tenderness of both meats from ageing, but significant improvements in lamb beyond 2 weeks chilled storage were not observed. Microbial thresholds were not breached during the chilled and frozen storage of either meat types, with the beef cold chain especially noteworthy because of its very low levels of bacteria detected and this demonstrating the controls available from good management.

There was a notable decline in unsaturated fatty acid levels in the beef over chilled and frozen storage durations – a result that requires future investigation to protect the 'healthy' image of grass-fed beef. Displaying either lamb or beef after longer chilled and frozen storage durations will lead to a rapid deterioration in colour stability and although this in not a recommended practice, anecdotal intelligence suggests this practice does occur in some importing countries and it would be opportune to inform these markets of the likely negative effect on consumer acceptance and preferential purchase.

Benefit for Industry

It is apparent, provided lamb and beef is managed to have low microbial loading, that chilling and extended frozen storage can be used to stabilise or even the supply of these meats, without a reduction in the tenderness, the development of rancidity or other adverse effects. This provides industry with flexibility (esp. when managing production gluts, and unforeseen market shifts or closures). It also provides a scientific basis from which claims of reduced quality as a result of chilled product moving to a frozen state for an extended period, to be countered. It is imperative, however, that an appropriate chilled storage period is applied prior to freezing to maximise improvements in tenderness.

The finding that product can be held long term at -12 °C instead of -18 °C offers considerable energy saving potential to industry. This would reduce 'waste' and improve the efficiencies of long term storage and transportation of lamb and beef – and consequently alone represents a significant return on investment from this project. Furthermore, a Masters student in Meat Science was trained as a result of this study; an important achievement as industry moves to employ more skilled people to move meat quality to new heights.

USEFUL RESOURCES

// www.sciencedirect.com/science/article/pii/S0309174016305952
// www.sciencedirect.com/science/article/pii/S0309174017307453
// www.sciencedirect.com/science/article/pii/S030917401730333

93

10.3 Supplementary Tables

Table 10.3.1. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* L* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks) 0	2	3	5	SEM
0	0	(unfrozen)	35.2	37.2	39.5	40.5	1.7
	4	-12	43.9	42.6	41.9	52.2	
		-18	38.9	46.4	42.6	52.2	
	8	-12	39.6	46.2	49.9	44.4	
		-18	40.5	52.1	50.2	49.4	
	12	-12	41.4	43.0	42.5	50.3	2.7
		-18	47.4	44.7	45.1	51.0	
	24	-12	43.8	45.2	45.2	44.0	
		-18	44.7	49.2	48.3	44.5	
	52	-12	37.5	45.0	55.8	49.7	
		-18	37.0	45.7	48.0	53.6	
1	0	(unfrozen)	37.0	40.1	40.5	41.8	1.1
	4	-12	41.1	42.8	42.6	42.8	
		-18	39.1	41.9	39.8	42.6	
	8	-12	37.3	41.8	46.6	41.3	
		-18	39.6	41.4	44.1	42.4	
	12	-12	38.7	41.8	41.3	43.7	1.8
		-18	38.3	41.6	43.9	43.2	
	24	-12	38.0	43.7	43.5	44.0	
		-18	38.9	41.7	41.7	42.8	
	52	-12	39.6	43.1	48.7	45.0	
		-18	37.0	43.5	45.7	46.7	
2	0	(unfrozen)	39.2	40.1	40.5	41.8	1.1
	4	-12	41.1	42.8	42.6	42.8	
		-18	39.1	41.9	39.8	42.6	
	8	-12	39.5	41.9	44.9	41.5	
		-18	40.6	41.6	44.9	41.5	
	12	-12	41.4	42.7	44.2	44.9	1.7
		-18	40.0	41.0	43.7	45.7	
	24	-12	40.4	44.9	44.2	46.5	
		-18	40.2	40.7	42.3	44.5	
	52	-12	41.1	43.6	49.2	45.1	
		-18	38.6	43.3	45.6	46.9	
3	0	(unfrozen)	39.0	39.5	39.8	41.0	1.0
	4	-12	41.3	42.1	42.2	42.6	
		-18	39.2	43.1	48.9	42.9	
	8	-12	40.1	40.9	43.9	42.1	
		-18	40.3	41.1	42.4	43.2	
	12	-12	41.2	42.9	44.8	44.2	1.6
		-18	41.6	41.6	44.8	44.5	
	24	-12	40.4	41.9	44.8	43.6	
		-18	40.7	40.9	42.0	44.2	
	52	-12	41.3	43.7	48.8	44.5	
		-18	37.6	42.8	44.3	45.8	

Table 10.3.2. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* a* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	18.9	21.1	20.1	19.5	1.1
	4	-12	12.7	17.3	19.9	10.6	1.9
		-18	13.3	13.9	17.1	11.0	
	8	-12	15.6	16.4	11.3	15.9	
		-18	17.8	12.7	9.1	12.8	
	12	-12	16.3	15.7	19.0	15.4	
		-18	10.6	13.5	16.3	14.6	
	24	-12	14.4	15.6	16.7	17.8	
		-18	12.5	12.6	14.8	16.1	
	52	-12	21.7	15.5	11.2	14.1	
		-18	18.7	14.0	14.4	11.0	
1	0	(unfrozen)	24.7	25.8	23.6	24.2	0.6
	4	-12	19.3	17.1	18.9	17.5	0.8
		-18	18.2	17.8	21.3	19.0	
	8	-12	18.9	18.7	17.8	19.2	
		-18	18.7	20.5	17.1	20.8	
	12	-12	19.6	18.9	19.0	15.8	
		-18	18.2	19.9	20.4	16.7	
	24	-12	18.7	18.5	18.6	16.4	
		-18	18.3	19.9	18.4	18.0	
	52	-12	21.0	17.9	12.8	15.7	
		-18	19.1	17.3	15.8	15.2	
2	0	(unfrozen)	23.2	23.5	21.7	21.8	0.5
	4	-12	17.9	16.7	15.4	16.5	0.8
		-18	16.0	16.2	18.2	17.7	
	8	-12	15.9	16.3	16.5	17.6	
		-18	15.5	17.2	15.9	18.9	
	12	-12	15.6	15.7	14.1	13.0	
		-18	14.7	18.0	16.8	13.2	
	24	-12	15.2	16.6	15.6	13.2	
		-18	15.5	17.4	15.6	14.5	
	52	-12	16.6	15.7	11.2	13.8	
		-18	15.9	15.4	13.7	13.5	
3	0	(unfrozen)	23.3	24.5	21.6	22.0	0.6
	4	-12	16.8	15.8	14.5	15.1	0.8
		-18	15.2	14.6	17.3	16.3	
	8	-12	14.8	15.5	16.3	16.1	
		-18	15.4	16.1	15.3	16.9	
	12	-12	15.8	14.7	12.3	12.2	
		-18	13.4	16.6	15.1	12.3	
	24	-12	14.9	15.8	13.5	14.0	
		-18	14.9	16.4	14.4	13.7	
	52	-12	16.5	14.5	10.8	13.0	
		-18	15.4	15.3	13.7	12.6	

Table 10.3.3. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* b* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	15.3	17.5	17.8	16.6	0.9
	4	-12	11.3	18.0	19.9	13.4	
		-18	11.7	15.6	16.2	14.8	
	8	-12	14.3	17.9	13.0	15.5	
		-18	18.1	15.9	12.0	14.0	
	12	-12	15.9	15.9	18.0	16.3	1.5
		-18	11.7	13.7	16.0	16.0	
	24	-12	14.1	15.8	15.1	18.0	
		-18	13.0	13.4	14.9	15.3	
	52	-12	19.4	16.6	15.4	15.9	
		-18	16.2	14.5	14.9	14.9	
1	0	(unfrozen)	20.4	21.9	19.4	20.0	0.6
	4	-12	17.5	14.7	18.0	14.9	
		-18	16.1	15.9	19.7	17.3	
	8	-12	18.0	17.3	16.7	19.1	
		-18	18.4	18.7	15.4	20.9	
	12	-12	17.5	16.8	17.2	16.3	0.9
		-18	16.6	17.3	18.3	17.2	
	24	-12	15.5	17.1	17.5	16.9	
		-18	16.8	17.4	17.3	16.8	
	52	-12	18.4	17.5	14.5	16.2	
		-18	16.7	17.0	15.4	16.8	
2	0	(unfrozen)	18.6	18.6	17.0	18.1	0.6
	4	-12	16.4	14.7	14.7	15.7	
		-18	14.1	15.4	17.0	17.1	
	8	-12	14.5	15.4	15.8	18.4	
		-18	15.6	15.7	15.0	19.4	
	12	-12	13.4	14.0	12.3	13.5	0.9
		-18	12.9	15.7	15.3	12.3	
	24	-12	13.3	16.2	14.9	13.9	
		-18	14.7	16.5	15.3	13.4	
	52	-12	14.1	16.5	13.5	14.3	
		-18	14.6	15.4	14.4	15.4	
3	0	(unfrozen)	19.1	20.4	17.9	19.2	0.7
	4	-12	15.8	15.0	14.3	14.8	
		-18	14.6	14.7	16.6	16.5	
	8	-12	14.2	15.5	16.9	16.5	
		-18	16.9	15.8	15.2	17.2	
	12	-12	13.1	13.7	11.4	12.8	0.9
		-18	11.4	15.2	14.2	11.7	
	24	-12	13.7	15.7	14.2	14.4	
		-18	14.3	15.6	14.2	13.2	
	52	-12	14.8	14.9	13.8	13.7	
		-18	14.7	15.8	14.7	15.2	

Table 10.3.4. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* chroma values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

| Display Period (d) | Frozen Storage (weeks) | Frozen Temperature (°C) | Chilled Storage (weeks) | | | | SEM |
			0	2	3	5	
0	0	(unfrozen)	24.2	27.4	26.9	25.6	1.3
	4	-12	17.1	25.0	28.2	17.2	
		-18	17.7	20.9	23.5	18.6	
	8	-12	21.2	24.3	17.3	22.2	
		-18	25.4	20.5	15.2	19.0	
	12	-12	22.8	22.4	26.3	22.4	2.3
		-18	15.9	19.2	22.9	21.8	
	24	-12	20.2	22.2	22.5	25.5	
		-18	18.1	18.4	21.2	22.3	
	52	-12	29.2	22.7	19.1	21.3	
		-18	24.8	20.2	20.7	18.6	
1	0	(unfrozen)	32.0	33.9	30.6	31.3	0.8
	4	-12	26.1	22.5	26.1	22.9	
		-18	24.3	23.9	29.0	25.7	
	8	-12	26.1	25.5	24.4	27.1	
		-18	26.3	27.8	23.0	29.5	
	12	-12	26.3	25.3	25.6	22.7	1.1
		-18	24.6	26.4	27.4	24.0	
	24	-12	24.3	25.2	25.5	23.6	
		-18	24.8	26.5	25.3	24.6	
	52	-12	27.9	25.1	19.3	22.6	
		-18	25.4	24.3	22.1	22.6	
2	0	(unfrozen)	29.8	30.0	27.6	28.3	0.7
	4	-12	24.4	22.3	21.4	22.8	
		-18	21.3	22.4	24.9	24.6	
	8	-12	21.6	22.4	22.8	25.5	
		-18	22.0	23.3	21.8	27.1	
	12	-12	20.6	21.1	18.7	18.7	1.1
		-18	19.5	23.9	22.8	18.1	
	24	-12	20.2	23.2	21.5	19.2	
		-18	21.4	24.0	21.9	19.8	
	52	-12	21.8	22.8	17.6	19.9	
		-18	21.5	21.8	19.8	20.5	
3	0	(unfrozen)	30.1	31.9	28.1	29.2	0.8
	4	-12	23.0	21.9	20.4	21.2	
		-18	21.2	20.7	24.1	23.2	
	8	-12	20.5	22.0	23.5	23.0	
		-18	23.0	22.7	21.6	24.1	
	12	-12	20.4	20.1	16.8	17.7	1.1
		-18	17.7	22.5	20.7	17.0	
	24	-12	20.3	22.2	19.6	19.9	
		-18	20.7	22.7	20.1	19.1	
	52	-12	22.1	20.7	17.6	18.9	
		-18	21.3	22.0	20.2	19.8	

Table 10.3.5. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* hue values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	0.68	0.69	0.72	0.70	0.03
	4	-12	0.73	0.81	0.80	0.90	
		-18	0.72	0.85	0.76	0.94	
	8	-12	0.73	0.85	0.86	0.78	
		-18	0.79	0.93	0.93	0.84	
	12	-12	0.78	0.78	0.76	0.82	0.04
		-18	0.85	0.81	0.78	0.83	
	24	-12	0.80	0.80	0.74	0.80	
		-18	0.80	0.82	0.81	0.77	
	52	-12	0.73	0.81	0.96	0.85	
		-18	0.71	0.80	0.80	0.94	
1	0	(unfrozen)	0.69	0.70	0.69	0.69	0.01
	4	-12	0.74	0.71	0.76	0.70	
		-18	0.72	0.73	0.75	0.74	
	8	-12	0.76	0.75	0.75	0.78	
		-18	0.78	0.74	0.73	0.79	
	12	-12	0.73	0.72	0.73	0.80	0.02
		-18	0.74	0.72	0.73	0.80	
	24	-12	0.69	0.74	0.76	0.81	
		-18	0.74	0.72	0.76	0.75	
	52	-12	0.72	0.77	0.84	0.80	
		-18	0.72	0.78	0.77	0.84	
2	0	(unfrozen)	0.68	0.67	0.66	0.69	0.01
	4	-12	0.74	0.72	0.76	0.76	
		-18	0.72	0.76	0.75	0.77	
	8	-12	0.73	0.76	0.76	0.80	
		-18	0.79	0.74	0.76	0.80	
	12	-12	0.71	0.73	0.72	0.70	0.02
		-18	0.71	0.72	0.74	0.75	
	24	-12	0.72	0.77	0.76	0.82	
		-18	0.76	0.75	0.77	0.74	
	52	-12	0.70	0.81	0.88	0.80	
		-18	0.74	0.78	0.81	0.85	
3	0	(unfrozen)	0.69	0.69	0.69	0.72	0.01
	4	-12	0.76	0.75	0.78	0.77	
		-18	0.76	0.79	0.76	0.79	
	8	-12	0.76	0.78	0.80	0.80	
		-18	0.83	0.76	0.78	0.80	
	12	-12	0.70	0.75	0.75	0.80	0.02
		-18	0.70	0.74	0.76	0.76	
	24	-12	0.74	0.79	0.81	0.81	
		-18	0.76	0.76	0.78	0.77	
	52	-12	0.73	0.80	0.90	0.82	
		-18	0.76	0.80	0.82	0.87	

Table 10.3.6. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* reflectance at 630 nm and 580 nm ratio values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

| Display Period (d) | Frozen Storage (weeks) | Frozen Temperature (°C) | Chilled Storage (weeks) | | | | SEM |
			0	2	3	5	
0	0	(unfrozen)	5.8	6.2	5.5	5.5	0.4
	4	-12	3.0	4.7	5.8	2.4	
		-18	3.4	3.4	4.1	2.6	
	8	-12	4.1	3.8	2.7	4.2	
		-18	4.7	2.8	2.4	3.0	
	12	-12	4.8	4.0	5.3	3.3	0.7
		-18	2.6	3.2	3.9	3.4	
	24	-12	3.5	3.9	3.8	4.4	
		-18	3.1	2.8	3.5	3.8	
	52	-12	6.5	4.2	2.6	3.1	
		-18	5.1	3.5	3.2	2.5	
1	0	(unfrozen)	7.1	7.2	6.0	6.3	0.3
	4	-12	4.3	3.5	4.3	3.7	
		-18	4.2	3.9	5.1	4.1	
	8	-12	4.8	4.2	3.6	4.7	
		-18	4.5	4.7	3.7	4.9	
	12	-12	4.8	4.0	4.5	3.3	0.4
		-18	4.5	4.4	4.5	3.6	
	24	-12	4.4	4.1	4.1	3.5	
		-18	4.3	4.4	4.2	4.0	
	52	-12	5.1	3.9	2.7	3.3	
		-18	4.7	3.7	3.2	3.3	
2	0	(unfrozen)	5.8	5.6	4.8	5.0	0.2
	4	-12	3.7	3.4	3.1	3.4	
		-18	3.4	3.4	3.9	3.7	
	8	-12	3.6	3.5	3.4	3.9	
		-18	3.4	3.6	3.4	4.1	
	12	-12	3.2	3.1	2.8	2.7	0.2
		-18	3.1	3.8	3.4	2.5	
	24	-12	3.3	3.3	3.2	2.6	
		-18	3.4	3.7	3.3	3.0	
	52	-12	3.3	3.2	2.4	2.8	
		-18	3.4	3.1	2.9	2.9	
3	0	(unfrozen)	6.2	6.4	5.0	5.2	0.2
	4	-12	3.4	3.1	2.9	3.0	
		-18	3.3	2.8	3.8	3.2	
	8	-12	3.2	3.5	3.5	3.4	
		-18	3.4	3.6	3.2	3.4	
	12	-12	3.3	2.9	2.4	2.5	0.3
		-18	2.8	3.4	2.9	2.4	
	24	-12	3.2	3.2	2.7	2.7	
		-18	3.1	3.4	2.9	2.8	
	52	-12	3.3	2.9	2.2	2.6	
		-18	3.4	3.1	2.8	2.7	

Table 10.3.7. Predicted means and their standard error (SEM) for beef m. longissimus lumborum particle size (µm) for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp)

Frozen Storage (weeks)	Temp (°C)	Chilled Storage (weeks)				
		0	2	3	5	SEM
0	(unfrozen)	181.4	117.0	104.9	90.1	14.1
4	-12	178.0	101.3	111.9	19.8	
	-18	223.3	118.3	118.3	114.9	
8	-12	216.9	184.9	130.6	128.9	
	-18	188.4	164.7	137.9	157.0	
12	-12	216.9	230.2	130.7	165.3	20.3
	-18	208.2	219.7	113.4	136.0	
24	-12	214.6	153.9	143.2	143.9	
	-18	206.4	198.4	150.6	124.1	
52	-12	267.0	141.2	137.7	180.6	
	-18	250.4	157.6	134.0	165.5	

Table 10.3.8. Predicted means and their standard error (SEM) for beef m. longissimus lumborum carbonyl content (nmole per g protein) for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp)

Frozen Storage (weeks)	Temp (°C)	Chilled Storage (weeks)				
		0	2	3	5	SEM
0	(unfrozen)	2.2	2.4	3.7	1.6	0.6
4	-12	2.1	5.2	2.6	5.6	
	-18	1.9	6.1	0.8	4.4	
8	-12	4.5	8.3	7.8	2.6	
	-18	6.1	7.5	5.4	2.4	
12	-12	1.0	2.2	1.9	2.5	0.8
	-18	1.2	2.1	2.9	2.3	
24	-12	0.8	2.2	8.2	1.7	
	-18	1.1	2.4	6.3	1.6	
52	-12	0.8	2.0	1.0	8.7	
	-18	1.1	1.5	0.9	6.2	

Table 10.3.9. Predicted means and their standard error (SEM) for beef m. longissimus lumborum protein solubility (mg/g) for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperature (Temp)

Soluble Protein	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
Sarcoplasmic	0	(unfrozen)	20.3	17.8	17.6	16.7	0.7
	4	-12	19.6	17.1	18.3	19.0	
		-18	21.1	17.7	16.6	17.2	
	8	-12	19.2	17.6	16.0	16.7	
		-18	18.3	19.4	16.3	15.3	
	12	-12	21.8	16.3	17.5	16.3	1.0
		-18	18.6	16.4	15.5	16.8	
	24	-12	21.7	19.3	17.9	17.4	
		-18	19.3	18.9	17.1	17.9	
	52	-12	21.3	19.8	16.5	17.3	
		-18	20.1	18.1	16.5	18.3	
Total	0	(unfrozen)	67.7	85.5	83.3	70.6	4.2
	4	-12	60.4	79.2	105.6	74.1	
		-18	64.0	77.0	109.2	64.6	
	8	-12	61.5	87.6	80.2	65.2	
		-18	76.9	89.4	78.0	75.2	
	12	-12	85.9	98.8	98.4	91.1	6.0
		-18	68.6	94.4	99.1	77.5	
	24	-12	84.2	88.0	67.6	69.2	
		-18	81.3	78.2	67.7	69.9	
	52	-12	78.6	99.6	98.8	68.9	
		-18	64.9	93.2	86.8	67.4	
Myofibrillar	0	(unfrozen)	47.3	67.2	65.0	53.8	4.2
	4	-12	40.8	62.1	87.2	55.1	
		-18	42.9	58.5	92.6	47.6	
	8	-12	42.3	70.0	64.2	48.5	
		-18	58.5	69.9	61.6	59.8	
	12	-12	64.2	72.7	83.7	74.7	6.0
		-18	50.2	78.0	83.6	60.6	
	24	-12	62.4	68.7	49.7	51.7	
		-18	61.8	60.4	51.4	50.9	
	52	-12	55.6	79.8	43.3	51.5	
		-18	44.8	75.2	70.3	49.1	

Table 10.3.9. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* oxymyoglobin percentage (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	41.7	47.1	48.8	51.8	1.6
	4	-12	38.5	50.6	49.1	54.8	
		-18	40.2	50.2	49.6	56.1	
	8	-12	40.9	46.9	47.5	40.8	
		-18	43.3	47.1	47.3	41.2	
	12	-12	44.0	51.1	50.8	50.2	2.3
		-18	46.5	44.2	51.6	44.9	
	24	-12	52.3	51.7	47.1	47.0	
		-18	49.6	50.7	47.6	46.9	
	52	-12	41.9	47.0	54.9	45.6	
		-18	46.6	46.5	54.7	47.6	
1	0	(unfrozen)	39.3	49.1	45.3	47.9	1.0
	4	-12	51.8	47.0	45.3	50.4	
		-18	52.2	47.1	49.6	52.4	
	8	-12	46.7	46.4	52.0	49.2	
		-18	49.8	45.8	48.6	49.3	
	12	-12	42.7	47.6	50.7	45.8	1.4
		-18	44.8	46.4	48.7	48.7	
	24	-12	49.7	48.2	51.4	48.7	
		-18	49.4	49.9	50.0	45.4	
	52	-12	42.6	46.8	48.4	50.0	
		-18	44.6	45.8	47.9	48.6	
2	0	(unfrozen)	51.0	45.1	43.0	42.9	1.0
	4	-12	44.4	45.8	47.5	42.0	
		-18	42.2	48.9	46.3	42.1	
	8	-12	44.1	51.8	49.7	46.7	
		-18	44.9	54.1	47.7	46.4	
	12	-12	53.0	51.4	46.7	44.5	1.4
		-18	51.8	51.9	43.4	47.2	
	24	-12	45.4	49.0	48.3	44.2	
		-18	46.2	50.1	46.4	45.8	
	52	-12	41.4	48.8	48.1	48.4	
		-18	40.4	47.1	45.5	49.4	
3	0	(unfrozen)	48.8	48.4	45.6	47.0	1.1
	4	-12	49.6	46.5	44.7	44.1	
		-18	50.2	46.2	43.3	46.6	
	8	-12	46.5	46.8	46.7	49.9	
		-18	43.3	49.3	42.4	51.1	
	12	-12	41.6	51.1	41.7	51.5	1.5
		-18	42.4	48.1	43.1	49.4	
	24	-12	39.2	48.8	46.8	51.5	
		-18	43.2	49.6	47.5	52.7	
	52	-12	44.1	42.5	44.9	44.8	
		-18	39.1	42.7	42.6	47.8	

Table 10.3.10. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* metmyoglobin percentage (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	30.1	32.4	31.3	29.1	0.5
	4	-12	33.2	33.6	32.4	35.3	
		-18	32.3	35.1	33.1	34.8	
	8	-12	35.2	34.4	34.2	34.4	
		-18	33.3	36.4	35.4	34.7	
	12	-12	34.0	32.2	34.4	35.1	0.8
		-18	34.3	33.0	34.6	36.6	
	24	-12	32.4	32.6	34.8	33.7	
		-18	31.3	34.1	34.2	33.9	
	52	-12	34.3	35.0	33.9	34.5	
		-18	34.5	34.4	33.4	35.4	
1	0	(unfrozen)	35.3	33.1	34.6	33.6	0.3
	4	-12	32.7	35.0	35.6	35.0	
		-18	32.7	35.2	33.6	35.3	
	8	-12	36.0	35.8	34.8	33.7	
		-18	34.9	36.2	35.3	34.3	
	12	-12	37.2	34.1	36.3	36.1	0.5
		-18	37.1	33.7	36.6	35.4	
	24	-12	34.7	33.7	35.9	36.8	
		-18	35.2	33.2	36.4	36.7	
	52	-12	39.0	37.3	36.3	34.8	
		-18	38.5	37.6	35.3	35.2	
2	0	(unfrozen)	35.0	34.3	36.0	35.1	0.4
	4	-12	37.0	37.5	37.0	37.5	
		-18	38.3	37.7	36.2	37.1	
	8	-12	37.0	35.7	36.2	36.5	
		-18	36.5	35.1	36.5	36.6	
	12	-12	35.1	37.0	38.1	39.4	0.6
		-18	35.6	36.4	38.0	38.9	
	24	-12	38.2	35.7	35.9	37.8	
		-18	38.3	35.9	36.4	35.9	
	52	-12	39.2	37.0	37.7	36.6	
		-18	38.6	36.2	37.6	36.1	
3	0	(unfrozen)	33.6	33.9	33.4	35.1	0.5
	4	-12	36.5	37.9	38.4	38.1	
		-18	36.5	39.4	38.0	38.0	
	8	-12	37.6	37.0	37.4	35.1	
		-18	39.1	36.3	38.6	35.4	
	12	-12	37.4	35.5	41.4	38.8	0.7
		-18	37.8	35.6	40.0	39.5	
	24	-12	39.0	36.7	37.1	37.5	
		-18	38.8	36.5	36.8	35.9	
	52	-12	38.0	40.0	40.8	38.6	
		-18	38.9	39.5	39.3	37.2	

Table 10.3.11. Predicted means and their standard error (SEM) for beef *m. longissimus lumborum* deoxymyoglobin percentage (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) across display periods (0-3 days)

Display Period (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				SEM
			0	2	3	5	
0	0	(unfrozen)	28.3	20.5	20.1	19.2	1.7
	4	-12	28.2	15.8	18.6	10.1	
		-18	27.7	14.8	17.3	9.0	
	8	-12	23.9	18.5	18.5	24.9	
		-18	23.6	16.5	17.5	24.0	
	12	-12	22.0	16.6	14.9	14.9	2.4
		-18	18.9	23.1	13.7	18.2	
	24	-12	15.4	15.6	18.1	19.5	
		-18	19.1	15.3	17.9	19.1	
	52	-12	23.7	18.0	11.0	20.0	
		-18	18.9	19.2	12.2	16.9	
1	0	(unfrozen)	25.5	17.9	20.0	18.7	0.9
	4	-12	15.1	18.1	19.3	14.8	
		-18	15.3	17.9	16.9	12.3	
	8	-12	17.5	18.0	13.2	17.1	
		-18	15.5	18.0	16.0	16.1	
	12	-12	20.0	18.0	12.9	18.2	1.3
		-18	18.1	19.5	14.7	15.6	
	24	-12	15.8	18.0	12.8	14.5	
		-18	15.4	17.1	13.7	17.9	
	52	-12	18.3	15.7	15.5	15.3	
		-18	16.9	16.8	16.7	16.2	
2	0	(unfrozen)	14.1	20.5	21.0	22.1	0.9
	4	-12	18.6	16.7	15.5	20.6	
		-18	19.5	13.3	17.4	20.7	
	8	-12	18.9	12.5	14.2	16.8	
		-18	18.6	11.1	15.9	17.1	
	12	-12	11.8	11.6	15.2	16.3	1.2
		-18	12.7	11.5	18.7	13.9	
	24	-12	16.3	15.1	15.8	18.0	
		-18	15.5	14.1	17.2	18.2	
	52	-12	19.3	14.2	14.2	15.0	
		-18	21.1	16.6	16.9	14.5	
3	0	(unfrozen)	17.8	17.9	20.8	18.1	1.0
	4	-12	13.8	15.6	17.1	17.9	
		-18	13.4	14.6	18.9	15.7	
	8	-12	16.1	16.6	16.0	14.9	
		-18	17.7	14.1	18.6	13.2	
	12	-12	20.9	13.0	17.1	9.7	1.4
		-18	19.6	16.3	16.6	10.7	
	24	-12	22.0	14.4	16.2	10.6	
		-18	17.9	14.1	15.9	11.7	
	52	-12	17.9	17.4	14.6	16.5	
		-18	21.8	18.0	18.2	15.4	

Table 10.3.12. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* purge loss (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	-12	1.57 (0.13)	1.78 (0.11)	1.99 (0.09)	2.21 (0.10)	2.42 (0.11)
	-18	1.54 (0.14)	1.77 (0.11)	2.00 (0.09)	2.23 (0.10)	2.45 (0.11)
4	-12	0.20 (0.11)	0.41 (0.09)	0.62 (0.08)	0.83 (0.09)	1.04 (0.10)
	-18	0.17 (0.11)	0.38 (0.09)	0.59 (0.08)	0.79 (0.09)	1.00 (0.11)
8	-12	0.30 (0.10)	0.50 (0.08)	0.71 (0.08)	0.92 (0.08)	1.13 (0.10)
	-18	0.27 (0.10)	0.46 (0.08)	0.65 (0.08)	0.84 (0.09)	1.02 (0.10)
12	-12	0.25 (0.10)	0.46 (0.09)	0.66 (0.08)	0.86 (0.09)	1.06 (0.10)
	-18	0.23 (0.10)	0.40 (0.09)	0.57 (0.08)	0.74 (0.09)	0.92 (0.10)
24	-12	0.33 (0.11)	0.51 (0.09)	0.69 (0.08)	0.87 (0.09)	1.05 (0.11)
	-18	0.31 (0.11)	0.44 (0.09)	0.58 (0.08)	0.71 (0.09)	0.84 (0.11)
52	-12	0.40 (0.16)	0.49 (0.11)	0.57 (0.09)	0.66 (0.10)	0.75 (0.15)
	-18	0.38 (0.17)	0.46 (0.12)	0.55 (0.09)	0.63 (0.10)	0.72 (0.15)

Table 10.3.13. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* shear force (N) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	-12	58.2 (3.0)	41.7 (2.5)	35.8 (2.4)	34.4 (2.5)	32.7 (3.0)
	-18	61.2 (3.0)	43.6 (2.5)	36.6 (2.4)	34.1 (2.5)	31.3 (3.0)
4	-12	51.7 (2.5)	35.4 (2.1)	29.5 (2.0)	28.0 (2.1)	26.2 (2.5)
	-18	54.1 (2.5)	36.9 (2.1)	30.2 (2.0)	27.7 (2.1)	25.0 (2.5)
8	-12	47.6 (2.4)	31.5 (2.1)	25.7 (2.0)	24.0 (2.1)	22.1 (2.4)
	-18	49.4 (2.4)	32.6 (2.1)	26.1 (2.0)	23.7 (2.1)	21.1 (2.4)
12	-12	45.5 (2.4)	29.6 (2.2)	23.8 (2.2)	22.1 (2.2)	20.0 (2.5)
	-18	46.7 (2.4)	30.3 (2.2)	24.0 (2.2)	21.8 (2.2)	19.1 (2.5)
24	-12	47.7 (2.8)	32.1 (2.5)	26.4 (2.5)	24.5 (2.5)	22.1 (2.8)
	-18	47.1 (2.8)	31.6 (2.5)	25.9 (2.5)	24.1 (2.5)	21.7 (2.8)
52	-12	49.7 (4.1)	33.2 (3.1)	27.1 (2.7)	25.6 (3.1	23.7 (4.1)
	-18	44.8 (4.1)	29.7 (3.1)	25.1 (2.7)	25.0 (3.1)	24.5 (4.1)

Table 10.3.14. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* glycogen content (mg/g) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	-12	19.3 (5.6)	24.1 (4.2)	26.2 (4.4)	22.3 (4.6)	14.9 (6.3)
	-18	26.0 (5.6)	29.0 (4.2)	30.7 (4.4)	29.4 (4.6)	26.4 (6.3)
4	-12	21.7 (4.5)	25.0 (3.2)	26.2 (3.1)	23.4 (3.3)	17.9 (4.6)
	-18	27.3 (4.5)	27.9 (3.2)	27.9 (3.1)	26.8 (3.3)	24.9 (4.6)
8	-12	24.5 (4.1)	26.0 (2.9)	26.2 (2.9)	24.4 (3.1)	21.0 (4.3)
	-18	28.9 (4.1)	26.9 (2.9)	25.2 (2.9)	24.3 (3.1)	23.8 (4.3)
12	-12	27.5 (4.2)	27.1 (3.2)	26.2 (3.4)	25.3 (3.5)	23.9 (4.8)
	-18	30.7 (4.3)	26.4 (3.2)	23.0 (3.4)	22.6 (3.6)	23.7 (4.8)
24	-12	36.7 (5.6)	31.8 (4.3)	28.0 (5.0)	28.1 (5.1)	30.2 (6.8)
	-18	36.4 (5.5)	28.1 (4.3)	22.6 (5.0)	23.8 (5.2)	29.0 (6.8)
52	-12	52.9 (7.9)	54.7 (5.6)	53.7 (5.1)	47.3 (5.8)	37.2 (8.1)
	-18	44.4 (7.9)	51.9 (5.6)	58.2 (5.1)	62.0 (5.8)	64.1 (8.1)

Table 10.3.15. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* moisture content (%) and intramuscular fat (IMF; %) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Parameter	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
Moisture	0	-12	53.4 (0.8)	52.7 (0.6)	52.5 (0.6)	52.6 (0.7)	53.1 (0.9)
		-18	53.2 (0.8)	52.7 (0.6)	52.3 (0.6)	52.1 (0.7)	51.9 (0.9)
	4	-12	53.0 (0.7)	52.4 (0.5)	52.1 (0.5)	52.1 (0.5)	52.2 (0.7)
		-18	52.7 (0.7)	52.3 (0.5)	52.0 (0.5)	51.6 (0.5)	51.2 (0.7)
	8	-12	52.7 (0.6)	52.0 (0.5)	51.8 (0.4)	51.6 (0.5)	51.4 (0.6)
		-18	52.2 (0.6)	51.9 (0.5)	51.6 (0.4)	51.1 (0.5)	50.6 (0.6)
	12	-12	52.3 (0.6)	51.7 (0.5)	51.4 (0.5)	51.1 (0.5)	50.7 (0.7)
		-18	51.7 (0.6)	51.4 (0.5)	51.3 (0.5)	50.7 (0.5)	50.0 (0.7)
	24	-12	51.5 (0.7)	50.7 (0.6)	50.4 (0.7)	49.9 (0.7)	49.3 (0.9)
		-18	50.5 (0.7)	50.2 (0.6)	50.3 (0.7)	49.8 (0.7)	49.0)0.9)
	52	-12	51.0 (1.3)	48.3 (1.0)	48.1 (1.0)	48.7 (1.0)	50.0 (1.3)
		-18	49.0 (1.3)	47.4 (1.0)	48.1 (1.0)	49.2 (1.0)	50.9 (1.3)
IMF	0	-12	4.65 (0.31)	4.72 (0.22)	4.79 (0.18)	4.86 (0.22)	4.92 (0.31)
		-18	4.95 (0.31)	5.00 (0.22)	5.06 (0.18)	5.11 (0.22)	5.16 (0.31)
	4	-12	4.60 (0.28)	4.73 (0.20)	4.85 (0.16)	4.97 (0.20)	5.09 (0.28)
		-18	4.96 (0.28)	5.00 (0.20)	5.03 (0.16)	5.06 (0.20)	5.09 (0.28)
	8	-12	4.56 (0.25)	4.73 (0.18)	4.90 (0.15)	5.08 (0.18)	5.25 (0.25)
		-18	4.98 (0.25)	4.99 (0.18)	5.00 (0.15)	5.01 (0.18)	5.02 (0.25)
	12	-12	4.51 (0.23)	4.73 (0.17)	4.96 (0.14)	5.19 (0.17)	5.42 (0.23)
		-18	4.99 (0.23)	4.98 (0.17)	4.97 (0.14)	4.96 (0.17)	4.95 (0.24)
	24	-12	4.36 (0.25)	4.75 (0.17)	5.13 (0.14)	5.52 (0.17)	5.91 (0.25)
		-18	5.02 (0.25)	4.95 (0.17)	4.88 (0.14)	4.80 (0.17)	4.73 (0.25)
	52	-12	4.01 (0.51)	4.77 (0.36)	5.54 (0.29)	6.30 (0.36)	7.06 (0.51)
		-18	5.11 (0.51)	4.89 (0.36)	4.66 (0.29)	4.44 (0.36)	4.22 (0.51)

Table 10.3.16. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* microbial load (log CFU/cm^2) values for A) Lactic Acid Bacteria; B) *Brochothrix thermosphacta*; and C) *Enterobacteriaceae sp.*, for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp). Not detected is shown as ND.

Parameter	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
A	0	-12	ND	0.26 (0.50)	2.16 (0.47)	2.12 (0.48)	3.22 (0.54)
		-18	0.48 (0.56)	0.59 (0.49)	2.28 (0.47)	2.04 (0.48)	2.95 (0.54)
	4	-12	ND	0.21 (0.45)	2.16 (0.43)	2.13 (0.43)	3.24 (0.48)
		-18	0.33 (0.51)	0.54 (0.45)	2.30 (0.45)	2.09 (0.44)	3.01 (0.48)
	8	-12	0.62 (0.48)	1.09 (0.43)	3.09 (0.42)	3.07 (0.42)	4.18 (0.45)
		-18	1.10 (0.48)	1.41 (0.44)	3.24 (0.42)	3.05 (0.42)	3.99 (0.45)
	12	-12	2.06 (0.48)	2.60 (0.45)	4.65 (0.43)	4.65 (0.43)	5.75 (0.46)
		-18	2.51 (0.47)	2.91 (0.45)	4.81 (0.43)	4.65 (0.44)	5.60 (0.46)
	24	-12	ND	0.55 (0.48)	2.75 (0.46)	2.78 (0.47)	3.89 (0.50)
		-18	0.14 (0.52)	0.84 (0.48)	2.95 (0.46)	2.86 (0.46)	3.86 (0.49)
	52	-12	1.42 (0.78)	2.77 (0.60)	5.34 (0.57)	5.43 (0.62)	6.54 (0.80)
		-18	1.62 (0.78)	3.02 (0.59)	5.60 (0.58)	5.70 (0.61)	6.80 (0.79)
B	0	-12	ND	1.19 (0.32)	3.09 (0.34)	3.84 (0.36)	3.67 (0.46)
		-18	0.39 (0.41)	1.53 (0.31)	2.84 (0.34)	3.51 (0.37)	3.71 (0.49)
	4	-12	ND	0.86 (0.27)	2.25 (0.26)	2.80 (0.28)	2.69 (0.36)
		-18	0.25 (0.35)	1.25 (0.26)	2.35 (0.26)	2.69 (0.29)	2.48 (0.38)
	8	-12	ND	0.50 (0.25)	1.36 (0.26)	1.67 (0.28)	1.57 (0.36)
		-18	0.13 (0.23)	0.93 (0.25)	1.78 (0.25)	1.77 (0.29)	1.14 (0.37)
	12	-12	0.11 (0.32)	0.21 (0.26)	0.63 (0.30)	0.76 (0.28)	0.70 (0.42)
		-18	0.04 (0.32)	0.67 (0.26)	1.30 (0.29)	1.02 (0.33)	0.10 (0.42)
	24	-12	0.50 (0.40)	0.14 (0.33)	0.17 (0.41)	0.41 (0.42)	0.85 (0.53)
		-18	ND	0.60 (0.33)	1.26 (0.40)	0.97 (0.42)	0.13 (0.54)
	52	-12	0.87 (0.64)	ND	0.03 (0.53)	0.22 (0.53)	0.43 (0.66)
		-18	ND	0.49 (0.50)	1.09 (0.50)	0.85 (0.52)	0.36 (0.66)
C	0	-12	ND	ND	0.48 (0.30)	0.89 (0.34)	1.26 (0.47)
		-18	ND	ND	0.20 (0.30)	0.66 (0.34)	1.08 (0.47)
	4	-12	ND	ND	0.36 (0.26)	0.75 (0.29)	0.40 (0.99)
		-18	ND	ND	0.22 (0.27)	0.62 (0.29)	0.39 (0.89)
	8	-12	ND	ND	0.23 (0.25)	0.61 (0.27)	0.99 (0.36)
		-18	ND	ND	0.23 (0.25)	0.58 (0.27)	0.89 (0.36)
	12	-12	ND	ND	0.11 (0.25)	0.48 (0.27)	0.87 (0.36)
		-18	ND	ND	0.25 (0.25)	0.54 (0.27)	0.79 (0.36)
	24	-12	ND	ND	ND	0.17 (0.35)	0.61 (0.45)
		-18	ND	ND	0.29 (0.32)	0.41 (0.35)	0.48 (0.46)
	52	-12	ND	ND	ND	ND	0.64 (0.74)
		-18	ND	ND	0.32 (0.54)	ND	ND

Table 10.3.17. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* L* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	17.5 (0.9)	18.2 (0.6)	18.5 (0.6)	18.4 (0.7)	18.3 (1.0)
		-18	17.2 (0.9)	18.1 (0.6)	18.6 (0.6)	18.8 (0.7)	18.8 (1.0)
	4	-12	17.6 (0.7)	17.8 (0.5)	17.8 (0.5)	17.7 (0.5)	17.6 (0.8)
		-18	17.2 (0.7)	17.6 (0.5)	17.8 (0.5)	18.0 (0.5)	18.0 (0.8)
	8	-12	17.7 (0.7)	17.4 (0.5)	17.2 (0.4)	17.1 (0.5)	17.0 (0.7)
		-18	17.2 (0.7)	17.1 (0.5)	17.1 (0.4)	17.2 (0.5)	17.3 (0.7)
	12	-12	17.7 (0.6)	17.1 (0.5)	16.7 (0.5)	16.6 (0.6)	16.5 (0.8)
		-18	17.2 (0.6)	16.7 (0.5)	16.5 (0.5)	16.5 (0.6)	16.6 (0.8)
	24	-12	17.7 (0.8)	16.5 (0.6)	15.9 (0.7)	15.7 (0.8)	15.7 (1.1)
		-18	16.9 (0.8)	15.9 (0.6)	15.3 (0.7)	15.3 (0.8)	15.4 (1.1)
	52	-12	16.4 (1.3)	16.5 (0.9)	16.4 (0.8)	16.0 (1.0)	15.5 (1.4)
		-18	15.0 (1.3)	15.2 (0.9)	15.1 (0.8)	14.8 (1.0)	14.3 (1.4)
1	0	-12	15.8 (0.7)	19.9 (0.6)	20.1 (0.6)	18.8 (0.6)	15.5 (0.7)
		-18	15.6 (0.7)	19.7 (0.6)	19.7 (0.6)	18.4 (0.6)	14.9 (0.7)
	4	-12	13.9 (0.5)	16.1 (0.5)	16.1 (0.5)	15.2 (0.5)	13.2 (0.5)
		-18	13.9 (0.5)	16.0 (0.5)	16.0 (0.5)	15.2 (0.5)	13.1 (0.5)
	8	-12	14.0 (0.5)	14.7 (0.5)	14.7 (0.5)	14.0 (0.5)	12.8 (0.5)
		-18	14.0 (0.5)	14.7 (0.5)	14.8 (0.5)	14.1 (0.5)	13.1 (0.5)
	12	-12	14.6 (0.6)	14.4 (0.5)	14.6 (0.5)	13.9 (0.5)	13.1 (0.6)
		-18	14.6 (0.6)	14.5 (0.5)	14.8 (0.5)	14.3 (0.5)	13.6 (0.6)
	24	-12	14.3 (0.7)	12.8 (0.7)	13.2 (0.7)	12.3 (0.7)	12.3 (0.7)
		-18	14.4 (0.7)	13.1 (0.7)	13.7 (0.7)	13.1 (0.7)	13.4 (0.7)
	52	-12	14.7 (0.9)	14.4 (0.7)	13.2 (0.7)	13.1 (0.7)	13.6 (0.9)
		-18	15.2 (0.9)	14.9 (0.7)	13.9 (0.7)	14.1 (0.7)	15.0 (0.9)
2	0	-12	157 (0.6)	18.7 (0.5)	18.3 (0.6)	16.3 (0.6)	13.6 (0.6)
		-18	15.3 (0.6)	18.2 (0.5)	17.8 (0.6)	15.7 (0.6)	13.0 (0.6)
	4	-12	13.5 (0.5)	14.8 (0.4)	14.4 (0.4)	12.9 (0.4)	10.9 (0.5)
		-18	13.1 (0.5)	14.5 (0.4)	14.1 (0.4)	12.6 (0.4)	10.6 (0.5)
	8	-12	13.2 (0.5)	13.4 (0.4)	13.0 (0.4)	11.8 (0.4)	10.3 (0.5)
		-18	12.8 (0.5)	13.1 (0.5)	12.8 (0.4)	11.8 (0.4)	10.3 (0.5)
	12	-12	13.0 (0.5)	12.5 (0.5)	12.3 (0.5)	11.3 (0.5)	9.8 (0.5)
		-18	12.7 (0.5)	12.4 (0.5)	12.2 (0.5)	11.5 (0.5)	10.1 (0.5)
	24	-12	12.2 (0.6)	10.8 (0.6)	10.9 (0.6)	10.3 (0.6)	8.7 (0.7)
		-18	12.2 (0.6)	10.9 (0.6)	11.2 (0.6)	10.8 (0.6)	9.5 (0.7)
	52	-12	12.4 (0.8)	12.9 (0.7)	11.7 (0.6)	10.9 (0.7)	10.7 (0.8)
		-18	12.8 (0.8)	13.2 (0.7)	12.0 (0.6)	11.3 (0.7)	11.4 (0.8)
3	0	-12	15.0 (0.6)	15.5 (0.5)	15.0 (0.5)	13.6 (0.5)	11.8 (0.7)
		-18	14.6 (0.6)	15.1 (0.5)	14.6 (0.5)	13.3 (0.5)	11.5 (0.7)
	4	-12	13.7 (0.5)	13.9 (0.4)	13.3 (0.4)	12.1 (0.4)	10.5 (0.5)
		-18	13.4 (0.5)	13.6 (0.4)	13.1 (0.4)	11.9 (0.4)	10.3 (0.5)
	8	-12	13.0 (0.5)	12.9 (0.4)	12.2 (0.4)	11.1 (0.4)	9.7 (0.5)
		-18	12.6 (0.5)	12.6 (0.4)	12.1 (0.4)	11.1 (0.4)	9.7 (0.5)
	12	-12	12.6 (0.5)	12.3 (0.4)	11.6 (0.5)	10.6 (0.4)	9.4 (0.5)
		-18	12.3 (0.5)	12.1 (0.4)	11.6 (0.5)	10.6 (0.4)	9.5 (0.5
	24	-12	11.7 (0.6)	11.2 (0.6)	10.6 (0.6)	9.69 (0.6)	8.6 (0.7)
		-18	11.6 (0.6)	11.2 (0.6)	10.7 (0.6)	9.94 (0.6)	9.0 (0.7)
	52	-12	11.8 (0.9)	11.9 (0.7)	11.5 (0.6)	10.5 (0.7)	9.5 (0.9)
		-18	12.0 (0.9)	12.2 (0.7)	11.8 (0.6)	11.0 (0.7)	9.9 (0.9)

Table 10.3.18. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* a* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	32.6 (1.6)	33.2 (1.3)	33.9 (1.2)	34.5 (1.3)	35.1 (1.6)
		-18	33.0 (1.6)	33.9 (1.3)	34.9 (1.2)	35.8 (1.3)	36.7 (1.6)
	4	-12	36.0 (1.3)	36.7 (1.0)	37.4 (0.9)	38.0 (1.0)	38.7 (1.3)
		-18	36.4 (1.3)	37.3 (1.0)	38.1 (0.9)	39.0 (1.0)	39.9 (1.3)
	8	-12	37.8 (1.2)	38.5 (1.0)	39.2 (0.9)	39.9 (1.0)	40.6 (1.2)
		-18	38.1 (1.2)	39.0 (1.0)	39.8 (0.9)	40.6 (1.0)	41.4 (1.2)
	12	-12	38.3 (1.3)	39.0 (1.1)	39.8 (1.0)	40.5 (1.1)	41.2 (1.3)
		-18	38.7 (1.3)	39.5 (1.1)	40.2 (1.0)	41.0 (1.1)	41.7 (1.3)
	24	-12	364 (1.5)	37.3 (1.3)	38.1 (1.2)	38.9 (1.3)	39.7 (1.5)
		-18	37.2 (1.5)	37.8 (1.3)	38.4 (1.2)	39.0 (1.3)	39.6 (1.5)
	52	-12	33.9 (2.2)	35.0 (1.6)	36.0 (1.3)	37.1 (1.6)	38.1 (2.2)
		-18	37.5 (2.2)	37.7 (1.6)	37.8 (1.3)	38.0 (1.6)	38.2 (2.2)
1	0	-12	33.6 (0.9)	33.6 (0.6)	33.5 (0.6)	33.8 (0.6)	34.8 (0.9)
		-18	33.6 (0.9)	34.2 (0.6)	34.6 (0.6)	35.1 (0.6)	35.4 (0.9)
	4	-12	33.3 (0.7)	33.6 (0.5)	33.8 (0.4)	34.1 (0.5)	34.6 (0.7)
		-18	33.4 (0.7)	34.1 (0.5)	34.6 (0.4)	35.0 (0.5)	35.2 (0.7)
	8	-12	33.1 (0.6)	33.6 (0.5)	34.1 (0.4)	34.4 (0.5)	34.5 (0.6)
		-18	33.2 (0.6)	34.0 (0.5)	34.6 (0.4)	34.9 (0.5)	35.1 (0.6)
	12	-12	32.8 (0.7)	33.6 (0.5)	34.4 (0.5)	34.7 (0.5)	34.4 (0.7)
		-18	33.2 (0.7)	33.9 (0.5)	34.5 (0.5)	34.9 (0.5)	35.0 (0.7)
	24	-12	31.9 (0.9)	33.6 (0.7)	35.0 (0.8)	35.3 (0.7)	34.0 (0.9)
		-18	33.1 (0.9)	33.6 (0.7)	34.2 (0.8)	34.7 (0.7)	35.0 (0.9)
	52	-12	30.4 (1.4)	32.4 (1.0)	33.7 (1.0)	34.3 (1.0)	34.4 (1.4)
		-18	32.0 (1.4)	34.0 (1.0)	35.1 (1.0)	35.2 (1.0)	34.0 (1.4)
2	0	-12	33.3 (1.0)	33.0 (0.8)	32.8 (0.8)	33.2 (0.8)	35.4 (1.0)
		-18	32.8 (1.0)	34.1 (0.8)	34.4 (0.8)	35.4 (0.8)	36.2 (1.0)
	4	-12	33.0 (0.7)	33.4 (0.6)	33.7 (0.6)	34.1 (0.6)	35.1 (0.7)
		-18	32.5 (0.7)	34.2 (0.6)	34.9 (0.6)	35.6 (0.6)	35.9 (0.7)
	8	-12	32.7 (0.7)	33.9 (0.5)	34.6 (0.5)	34.9 (0.5)	34.8 (0.7)
		-18	32.4 (0.7)	34.3 (0.5)	35.5 (0.5)	35.8 (0.5)	35.7 (0.7)
	12	-12	32.4 (0.8)	34.3 (0.6)	35.4 (0.7)	35.8 (0.6)	34.6 (0.8)
		-18	32.5 (0.8)	34.3 (0.6)	35.8 (0.7)	36.0 (0.6)	35.7 (0.8)
	24	-12	31.5 (1.0)	35.5 (0.9)	37.5 (1.0)	37.6 (0.9)	34.2 (1.0)
		-18	34.1 (1.0)	34.2 (0.9)	35.3 (1.0)	35.9 (1.0)	37.3 (1.0)
	52	-12	31.7 (1.3)	33.1 (1.0)	33.9 (1.0)	34.7 (1.0)	36.4 (1.3)
		-18	32.2 (1.3)	34.9 (1.0)	35.8 (1.0)	36.1 (1.0)	35.4 (1.3)
3	0	-12	33.1 (0.9)	33.4 (0.7)	33.7 (0.8)	34.3 (0.7)	35.4 (1.0)
		-18	32.8 (0.9)	34.1 (0.7)	34.8 (0.8)	35.5 (0.7)	35.8 (1.0)
	4	-12	32.7 (0.7)	33.4 (0.5)	34.0 (0.5)	34.5 (0.5)	35.1 (0.7)
		-18	32.8 (0.7)	34.0 (0.5)	34.8 (0.5)	35.4 (0.5)	35.8 (0.7)
	8	-12	32.4 (0.7)	33.6 (0.5)	34.4 (0.5)	34.9 (0.5)	35.1 (0.7)
		-18	33.0 (0.7)	34.1 (0.5)	35.0 (0.5)	35.5 (0.5)	36.0 (0.7)
	12	-12	32.3 (0.7)	34.0 (0.6)	35.1 (0.6)	35.5 (0.6)	35.3 (0.7)
		-18	33.5 (0.7)	34.4 (0.6)	35.3 (0.6)	35.8 (0.6)	36.4 (0.7)
	24	-12	32.6 (1.0)	35.5 (0.8)	37.1 (0.9)	37.4 (0.8)	36.3 (1.0)
		-18	35.3 (1.0)	35.6 (0.8)	36.2 (0.9)	36.9 (0.8)	38.3 (1.0)
	52	-12	31.4 (1.3)	33.5 (1.0)	35.0 (1.0)	36.0 (1.0)	37.0 (1.3)
		-18	32.9 (1.3)	35.2 (1.0)	36.3 (1.0)	36.5 (1.0)	35.9 (1.3)

Table 10.3.19. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* b* values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	13.5 (1.1)	15.7 (0.8)	16.8 (0.8)	16.6 (0.8)	16.2 (1.1)
		-18	13.3 (1.1)	15.6 (0.8)	16.9 (0.8)	17.0 (0.8)	16.7 (1.1)
	4	-12	14.8 (0.8)	15.9 (0.6)	16.5 (0.5)	16.5 (0.6)	16.3 (0.8)
		-18	14.5 (0.8)	15.8 (0.6)	16.6 (0.5)	16.7 (0.6)	16.7 (0.8)
	8	-12	15.8 (0.7)	16.1 (0.5)	16.2 (0.5)	16.3 (0.6)	16.3 (0.8)
		-18	15.6 (0.7)	15.9 (0.5)	16.2 (0.5)	16.4 (0.6)	16.6 (0.8)
	12	-12	16.6 (0.8)	15.2 (0.6)	15.8 (0.6)	16.0 (0.7)	16.2 (0.9)
		-18	16.3 (0.8)	15.9 (0.6)	15.8 (0.7)	16.0 (0.7)	16.2 (0.9)
	24	-12	16.9 (1.1)	15.2 (0.9)	14.2 (0.9)	14.5 (0.9)	14.8 (1.2)
		-18	16.7 (1.1)	14.9 (0.9)	13.9 (0.9)	14.1 (0.9)	14.5 (1.2)
	52	-12	13.5 (1.4)	15.2 (1.0)	16.1 (0.9)	16.0 (1.0)	15.6 (1.4)
		-18	13.2 (1.4)	14.6 (1.0)	15.2 (0.9)	14.8 (1.0)	14.1 (1.4)
1	0	-12	15.4 (0.8)	15.7 (0.6)	15.8 (0.6)	15.8 (0.6)	15.9 (0.8)
		-18	14.4 (0.8)	15.5 (0.6)	16.0 (0.6)	15.9 (0.6)	15.3 (0.8)
	4	-12	14.8 (0.7)	15.3 (0.5)	15.4 (0.5)	15.4 (0.5)	15.3 (0.7)
		-18	14.1 (0.7)	15.0 (0.5)	15.4 (0.5)	15.4 (0.5)	15.1 (0.7)
	8	-12	14.2 (0.6)	14.9 (0.5)	15.1 (0.5)	15.1 (0.5)	14.9 (0.6)
		-18	14.0 (0.6)	14.6 (0.5)	14.9 (0.5)	15.0 (0.5)	15.0 (0.6)
	12	-12	13.8 (0.6)	14.6 (0.5)	14.9 (0.5)	14.9 (0.5)	14.5 (0.6)
		-18	13.8 (0.6)	14.2 (0.5)	14.5 (0.5)	14.7 (0.5)	15.0 (0.6)
	24	-12	13.0 (0.8)	14.0 (0.7)	14.5 (0.7)	14.5 (0.7)	14.0 (0.8)
		-18	13.6 (0.8)	13.8 (0.7)	13.9 (0.7)	14.3 (0.7)	15.0 (0.8)
	52	-12	13.8 (1.2)	14.4 (0.9)	14.8 (0.8)	15.0 (0.9)	15.4 (1.2)
		-18	13.9 (1.2)	15.1 (0.9)	15.8 (0.8)	16.0 (0.9)	15.7 (1.2)
2	0	-12	15.9 (0.7)	17.0 (0.6)	17.1 (0.5)	16.7 (0.6)	16.0 (0.7)
		-18	15.1 (0.7)	16.5 (0.6)	16.8 (0.5)	16.6 (0.6)	16.0 (0.7)
	4	-12	14.0 (0.6)	15.1 (0.5)	15.2 (0.5)	14.9 (0.5)	14.2 (0.6)
		-18	13.3 (0.6)	14.6 (0.5)	14.9 (0.5)	14.7 (0.5)	14.2 (0.6)
	8	-12	13.0 (0.5)	14.1 (0.4)	14.3 (0.4)	14.0 (0.4)	13.3 (0.5)
		-18	12.5 (0.5)	13.7 (0.4)	13.9 (0.4)	13.8 (0.5)	13.4 (0.5)
	12	-12	12.6 (0.6)	13.7 (0.5)	13.9 (0.5)	13.6 (0.5)	13.0 (0.6)
		-18	12.2 (0.6)	13.3 (0.5)	13.6 (0.5)	13.5 (0.5)	13.2 (0.6)
	24	-12	11.7 (0.6)	12.8 (0.6)	12.9 (0.6)	12.8 (0.6)	12.4 (0.6)
		-18	11.6 (0.6)	12.5 (0.6)	12.7 (0.6)	12.7 (0.6)	12.6 (0.6)
	52	-12	12.9 (0.9)	13.8 (0.7)	14.0 (0.7)	14.2 (0.7)	14.3 (0.9)
		-18	13.5 (0.9)	14.0 (0.7)	13.9 (0.7)	14.2 (0.7)	14.7 (0.9)
3	0	-12	14.9 (0.4)	14.9 (0.3)	14.8 (0.3)	14.7 (0.3)	14.6 (0.4)
		-18	14.3 (0.4)	14.5 (0.3)	14.7 (0.3)	14.9 (0.3)	15.1 (0.4)
	4	-12	13.8 (0.4)	13.8 (0.3)	13.8 (0.3)	13.7 (0.3)	13.7 (0.4)
		-18	13.2 (0.4)	13.5 (0.3)	13.7 (0.3)	13.9 (0.3)	14.1 (0.4)
	8	-12	13.6 (0.3)	13.7 (0.3)	13.7 (0.3)	13.7 (0.3)	13.8 (0.3)
		-18	13.1 (0.3)	13.4 (0.3)	13.6 (0.3)	13.9 (0.3)	14.2 (0.3)
	12	-12	14.0 (0.4)	14.1 (0.3)	14.2 (0.3)	14.3 (0.3)	14.4 (0.4)
		-18	13.6 (0.4)	13.8 (0.3)	14.1 (0.3)	14.4 (0.3)	14.7 (0.4)
	24	-12	12.5 (0.4)	12.6 (0.3)	12.7 (0.3)	12.9 (0.3)	13.2 (0.4)
		-18	12.2 (0.4)	12.4 (0.3)	12.7 (0.3)	13.0 (0.3)	13.3 (0.4)
	52	-12	13.2 (0.6)	13.5 (0.4)	13.8 (0.3)	14.0 (0.4)	14.2 (0.6)
		-18	13.4 (0.6)	13.6 (0.4)	13.7 (0.3)	13.9 (0.4)	14.0 (0.6)

Table 10.3.20. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* 680 nm and 530 nm spectral ratio values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	5.02 (0.38)	5.67 (0.29)	6.04 (0.32)	6.11 (0.37)	5.98 (0.50)
		-18	4.82 (0.38)	5.51 (0.29)	5.90 (0.32)	6.01 (0.37)	5.91 (0.50)
	4	-12	5.12 (0.30)	5.19 (0.22)	5.15 (0.21)	5.00 (0.26)	4.78 (0.36)
		-18	4.95 (0.30)	5.05 (0.22)	5.04 (0.21)	4.93 (0.26)	4.75 (0.36)
	8	-12	5.21 (0.27)	4.85 (0.20)	4.56 (0.21)	4.32 (0.26)	4.12 (0.36)
		-18	5.06 (0.27)	4.74 (0.20)	4.47 (0.21)	4.27 (0.26)	4.11 (0.36)
	12	-12	5.26 (0.27)	4.68 (0.22)	4.29 (0.25)	4.08 (0.31)	3.98 (0.42)
		-18	5.12 (0.28)	4.58 (0.22)	4.22 (0.25)	4.05 (0.31)	3.99 (0.42)
	24	-12	5.28 (0.36)	4.49 (0.29)	4.05 (0.37)	3.99 (0.41)	4.21 (0.55)
		-18	5.14 (0.36)	4.38 (0.29)	3.99 (0.37)	3.97 (0.41)	4.23 (0.55)
	52	-12	4.58 (0.55)	4.74 (0.39)	4.70 (0.35)	4.45 (0.42)	4.04 (0.58)
		-18	4.00 (0.55)	4.21 (0.39)	4.22 (0.35)	4.02 (0.42)	3.65 (0.58)
1	0	-12	3.71 (0.25)	5.17 (0.22)	5.45 (0.23)	5.08 (0.23)	3.74 (0.26)
		-18	3.66 (0.25)	5.06 (0.22)	5.27 (0.23)	4.83 (0.23)	3.42 (0.26)
	4	-12	3.23 (0.20)	3.90 (0.17)	4.01 (0.17)	3.75 (0.17)	3.01 (0.20)
		-18	3.21 (0.20)	3.83 (0.17)	3.90 (0.17)	3.61 (0.17)	2.87 (0.20)
	8	-12	3.35 (0.19)	3.43 (0.17)	3.45 (0.17)	3.24 (0.17)	2.90 (0.19)
		-18	3.34 (0.19)	3.39 (0.17)	3.40 (0.17)	3.19 (0.17)	2.91 (0.19)
	12	-12	3.64 (0.21)	3.32 (0.19)	3.33 (0.20)	3.12 (0.19)	3.00 (0.28)
		-18	3.65 (0.21)	3.31 (0.19)	3.32 (0.20)	3.16 (0.19)	3.14 (0.22)
	24	-12	3.45 (0.26)	2.72 (0.25)	2.85 (0.25)	2.55 (0.25)	2.51 (0.28)
		-18	3.52 (0.26)	2.77 (0.25)	2.94 (0.25)	2.74 (0.25)	2.94 (0.28)
	52	-12	3.55 (0.33)	3.32 (0.28)	3.04 (0.28)	2.96 (0.29)	3.12 (0.33)
		-18	3.76 (0.33)	3.43 (0.28)	3.15 (0.28)	3.23 (0.29)	3.84 (0.33)
2	0	-12	3.44 (0.20)	4.58 (0.18)	4.64 (0.19)	3.97 (0.19)	2.78 (0.21)
		-18	3.29 (0.20)	4.39 (0.18)	4.42 (0.19)	3.72 (0.19)	2.51 (0.21)
	4	-12	2.86 (0.16)	3.23 (0.14)	3.13 (0.15)	2.72 (0.14)	2.12 (0.17)
		-18	2.72 (0.16)	3.09 (0.14)	2.98 (0.15)	2.56 (0.14)	1.96 (0.17)
	8	-12	2.85 (0.16)	2.72 (0.14)	2.56 (0.14)	2.29 (0.14)	2.05 (0.16)
		-18	2.74 (0.16)	2.62 (0.14)	2.47 (0.14)	2.21 (0.14)	1.99 (0.16)
	12	-12	2.89 (0.17)	2.54 (0.16)	2.39 (0.16)	2.17 (0.16)	2.05 (0.17)
		-18	2.80 (0.17)	2.48 (0.16)	2.36 (0.17)	2.16 (0.16)	2.07 (0.18)
	24	-12	2.60 (0.20)	2.14 (0.20)	2.19 (0.21)	1.94 (0.20)	1.70 (0.21)
		-18	2.57 (0.20)	2.16 (0.20)	2.26 (0.21)	2.06 (0.20)	0.87 (0.21)
	52	-12	2.57 (0.26)	2.57 (0.22)	2.32 (0.21)	2.12 (0.22)	2.07 (0.26)
		-18	2.70 (0.26)	2.72 (0.22)	2.49 (0.21)	2.30 (0.22)	2.28 (0.26)
3	0	-12	2.96 (0.18)	3.23 (0.15)	3.17 (0.15)	2.77 (0.15)	2.15 (0.18)
		-18	2.98 (0.18)	3.13 (0.15)	3.08 (0.15)	2.69 (0.15)	2.09 (0.18)
	4	-12	2.77 (0.13)	2.81 (0.11)	2.68 (0.10)	2.37 (0.11)	1.98 (0.13)
		-18	2.69 (0.13)	2.74 (0.11)	2.61 (0.10)	2.32 (0.11)	1.93 (0.14)
	8	-12	2.68 (0.13)	2.54 (0.10)	2.35 (0.11)	2.12 (0.10)	1.90 (0.13)
		-18	2.61 (0.13)	2.48 (0.10)	2.30 (0.11)	2.08 (0.10)	1.87 (0.13)
	12	-12	2.63 (0.14)	2.38 (0.12)	2.17 (0.13)	1.98 (0.12)	1.86 (0.14)
		-18	2.58 (0.14)	2.34 (0.12)	2.14 (0.13)	1.97 (0.12)	1.85 (0.14)
	24	-12	2.37 (0.18)	2.11 (0.16)	1.98 (0.17)	1.80 (0.16)	1.63 (0.18)
		-18	2.38 (0.18)	2.13 (0.16)	2.01 (0.17)	1.84 (0.16)	1.68 (0.18)
	52	-12	2.16 (0.22)	2.35 (0.17)	2.29 (0.16)	2.02 (0.17)	1.61 (0.22)
		-18	2.30 (0.22)	2.50 (0.17)	2.45 (0.16)	2.18 (0.17)	1.79 (0.22)

Table 10.3.21. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* chroma values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	23.0 (1.2)	24.5 (0.9)	25.1 (0.9)	24.9 (1.0)	24.3 (1.4)
		-18	22.6 (1.2)	24.4 (0.9)	25.3 (0.9)	25.3 (1.0)	25.1 (1.4)
	4	-12	23.4 (1.0)	24.0 (0.7)	24.2 (0.6)	24.2 (0.7)	24.0 (1.0)
		-18	22.9 (1.0)	23.7 (0.7)	24.3 (0.6)	24.5 (0.7)	24.5 (1.0)
	8	-12	23.7 (0.9)	23.5 (0.6)	23.4 (0.6)	23.5 (0.7)	23.6 (0.9)
		-18	23.1 (0.9)	23.1 (0.6)	23.3 (0.6)	23.6 (0.7)	24.0 (0.9)
	12	-12	23.9 (0.9)	23.0 (0.7)	22.7 (0.7)	22.9 (0.7)	23.3 (1.0)
		-18	23.3 (0.9)	22.6 (0.7)	22.5 (0.7)	22.9 (0.7)	23.4 (1.0)
	24	-12	24.1 (1.1)	22.2 (0.9)	21.4 (1.1)	21.7 (1.1)	22.5 (1.5)
		-18	23.3 (1.1)	21.5 (0.9)	20.7 (1.1)	21.2 (1.1)	22.1 (1.5)
	52	-12	21.8 (1.8)	22.7 (1.3)	22.9 (1.1)	22.5 (1.3)	21.8 (1.9)
		-18	20.6 (1.8)	21.3 (1.3)	21.4 (1.1)	20.8 (1.3)	19.9 (1.9)
1	0	-12	22.6 (1.0)	25.9 (0.8)	26.2 (0.9)	25.2 (0.8)	22.8 (1.0)
		-18	21.7 (1.0)	25.6 (0.8)	26.2 (0.9)	24.9 (0.8)	21.7 (1.0)
	4	-12	19.4 (0.8)	22.0 (0.7)	22.2 (0.7)	21.3 (0.7)	19.4 (0.8)
		-18	18.9 (0.8)	21.7 (0.7)	22.0 (0.7)	21.2 (0.7)	19.1 (0.8)
	8	-12	19.1 (0.8)	21.0 (0.7)	21.1 (0.7	20.4 (0.7)	18.9 (0.8)
		-18	18.9 (0.8)	20.8 (0.7)	20.9 (0.7)	20.4 (0.7)	19.2 (0.8)
	12	-12	20.0 (0.8)	21.4 (0.7)	21.4 (0.8)	20.9 (0.7)	19.7 (0.8)
		-18	20.1 (0.8)	21.2 (0.7)	21.1 (0.8)	21.0 (0.7)	20.6 (0.8)
	24	-12	19.1 (1.0)	19.3 (0.9)	18.9 (0.9)	18.8 (0.9)	18.7 (1.0)
		-18	19.7 (1.0)	19.4 (0.9)	18.9 (0.9)	19.3 (0.9)	20.5 (1.0)
	52	-12	20.6 (1.3)	20.5 (1.1)	19.4 (1.1)	19.9 (1.1)	21.0 (1.3)
		-18	21.0 (1.3)	21.3 (1.1)	20.5 (1.1)	21.2 (1.1)	22.2 (1.3)
2	0	-12	22.3 (0.8)	25.8 (0.7)	25.4 (0.7)	23.3 (0.7)	21.2 (0.8)
		-18	21.5 (0.8)	25.1 (0.7)	24.8 (0.7)	22.9 (0.7)	21.0 (0.8)
	4	-12	18.9 (0.7)	21.2 (0.6)	20.8 (0.6)	19.4 (0.6)	17.5 (0.7)
		-18	18.1 (0.7)	20.6 (0.6)	20.4 (0.6)	19.1 (0.6)	17.4 (0.7)
	8	-12	18.3 (0.6)	19.6 (0.5)	19.4 (0.6)	18.5 (0.5)	16.6 (0.6)
		-18	17.7 (0.6)	19.1 (0.5)	19.0 (0.6)	18.3 (0.6)	16.8 (0.6)
	12	-12	18.2 (0.7)	18.8 (0.6)	18.7 (0.6)	18.2 (0.6)	16.3 (0.7)
		-18	17.7 (0.7)	18.4 (0.6)	18.4 (0.6)	18.1 (0.6)	16.6 (0.7)
	24	-12	17.2 (0.8)	16.5 (0.8)	16.5 (0.8)	16.8 (0.8)	15.3 (0.8)
		-18	17.1 (0.8)	16.4 (0.8	16.5 (0.8)	16.9 (0.8)	16.0 (0.8)
	52	-12	17.9 (1.0)	19.1 (0.9)	18.2 (0.9)	17.8 (0.9)	17.9 (1.0)
		-18	18.7 (1.0)	19.4 (0.9)	18.2 (0.9)	18.0 (0.9)	18.9 (1.0)
3	0	-12	21.1 (0.7)	21.6 (0.6)	21.3 (0.6)	20.3 (0.6)	19.0 (0.7)
		-18	20.3 (0.7)	21.1 (0.6)	21.0 (0.6)	20.2 (0.6)	19.2 (0.7)
	4	-12	19.2 (0.6)	19.4 (0.5)	19.0 (0.4)	18.2 (0.5)	17.2 (0.6)
		-18	18.6 (0.6)	19.0 (0.5)	18.8 (0.4)	18.3 (0.5)	17.5 (0.6)
	8	-12	18.8 (0.5)	18.8 (0.4)	18.3 (0.4)	17.7 (0.4)	16.9 (0.5)
		-18	18.2 (0.5)	18.4 (0.4)	18.2 (0.4)	17.7 (0.4)	17.2 (0.5)
	12	-12	19.1 (0.6)	18.8 (0.5)	18.4 (0.5)	17.9 (0.5)	17.2 (0.6)
		-18	18.6 (0.6)	18.5 (0.5)	18.3 (0.5)	18.0 (0.5)	17.5 (0.6)
	24	-12	17.3 (0.7)	16.9 (0.6)	16.5 (0.6)	16.2 (0.6)	15.7 (0.7)
		-18	17.0 (0.7)	16.8 (0.6)	16.6 (0.6)	16.4 (0.6)	16.1 (0.7)
	52	-12	17.4 (0.9)	18.2 (0.6)	18.2 (0.6)	17.7 (0.6)	16.9 (0.9)
		-18	17.7 (0.9)	18.4 (0.6)	18.4 (0.6)	17.9 (0.6)	17.1 (0.9)

Table 10.3.22. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* hue (in degrees) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	37.3 (1.1)	40.5 (0.9)	41.3 (0.9)	41.3 (0.9)	40.7 (1.2)
		-18	38.0 (1.1)	41.0 (0.9)	41.7 (0.9)	41.6 (0.9)	40.8 (1.2)
	4	-12	39.2 (0.9)	41.9 (0.7)	42.5 (0.7)	42.8 (0.7)	42.6 (0.9)
		-18	39.9 (0.9)	42.4 (0.7)	42.8 (0.7)	42.9 (0.8)	42.5 (0.9)
	8	-12	41.1 (0.9)	43.4 (0.7)	43.7 (0.7)	44.3 (0.7)	44.4 (0.9)
		-18	41.8 (0.9)	43.8 (0.7)	44.0 (0.7)	44.3 (0.7)	44.2 (0.9)
	12	-12	42.4 (0.9)	44.2 (0.8)	44.3 (0.8)	45.0 (0.8)	45.3 (1.0)
		-18	43.1 (0.9)	44.7 (0.8)	44.5 (0.8)	44.9 (0.8)	45.0 (1.0)
	24	-12	41.6 (1.2)	42.4 (1.0)	41.8 (1.0)	42.4 (1.0)	42.7 (1.2)
		-18	42.4 (1.2)	42.9 (1.0)	42.0 (1.0)	42.2 (1.0)	42.2 (1.2)
	52	-12	39.8 (1.4)	43.2 (1.1)	44.3 (1.0)	44.9 (1.1)	44.8 (1.4)
		-18	41.9 (1.4)	44.7 (1.1)	45.3 (1.0)	45.3 (1.1)	44.7 (1.4)
1	0	-12	43.2 (1.0)	42.6 (0.7)	42.5 (0.8)	42.9 (1.0)	43.4 (1.3)
		-18	41.8 (1.0)	42.2 (0.7)	42.6 (0.8)	42.8 (1.0)	43.0 (1.3)
	4	-12	43.1 (0.8)	43.5 (0.6)	44.2 (0.6)	45.0 (0.7)	46.0 (0.9)
		-18	42.1 (0.8)	43.1 (0.6)	44.0 (0.6)	44.9 (0.7)	45.7 (0.9)
	8	-12	43.0 (0.7)	44.3 (0.5)	45.6 (0.5)	46.9 (0.7)	48.1 (0.9)
		-18	42.3 (0.7)	43.8 (0.5)	45.2 (0.5)	46.5 (0.7)	47.9 (0.9)
	12	-12	42.9 (0.7)	44.9 (0.6)	46.6 (0.6)	48.0 (0.8)	49.3 (1.0)
		-18	42.6 (0.7)	44.2 (0.6)	45.8 (0.6)	47.5 (0.8)	49.1 (1.1)
	24	-12	42.9 (0.9)	45.4 (0.7)	47.3 (0.9)	48.4 (1.0)	49.1 (1.4)
		-18	43.5 (0.9)	44.5 (0.7)	45.7 (0.9)	47.2 (1.0)	49.1 (1.4)
	52	-12	44.8 (1.5)	45.6 (1.1)	46.8 (1.0)	48.4 (1.1)	50.2 (1.6)
		-18	44.7 (1.5)	45.7 (1.1)	46.5 (1.0)	47.2 (1.1)	47.8 (1.6)
2	0	-12	44.3 (1.0)	44.6 (0.8)	45.4 (0.8)	46.9 (0.9)	48.9 (1.3)
		-18	43.2 (1.0)	44.5 (0.8)	45.9 (0.8)	47.6 (0.9)	49.7 (1.3)
	4	-12	44.3 (0.8)	45.4 (0.6)	46.7 (0.6)	48.5 (0.7)	50.6 (0.9)
		-18	43.4 (0.8)	45.1 (0.6)	46.7 (0.6)	48.7 (0.7)	50.9 (0.9)
	8	-12	44.2 (0.8)	46.1 (0.6)	47.8 (0.6)	49.9 (0.6)	52.2 (0.9)
		-18	43.6 (0.8)	45.6 (0.6)	47.5 (0.6)	49.7 (0.6)	52.1 (0.9)
	12	-12	44.2 (0.8)	46.6 (0.6)	48.8 (0.7)	51.1 (0.7)	53.5 (1.0)
		-18	44.9 (0.8)	46.0 (0.6)	48.1 (0.7)	50.5 (0.7)	53.0 (1.0)
	24	-12	44.4 (0.9)	47.7 (0.7)	50.3 (0.9)	52.7 (1.0)	54.9 (1.4)
		-18	44.9 (0.9)	46.6 (0.7)	48.7 (0.9)	51.3 (1.0)	54.2 (1.37)
	52	-12	46.6 (1.5)	47.7 (1.1)	49.2 (1.0)	51.4 (1.2)	54.0 (1.6)
		-18	46.4 (1.5)	47.7 (1.1)	48.9 (1.0)	50.4 (1.2)	52.1 (1.6)
3	0	-12	45.4 (1.0)	45.0 (0.8)	45.1 (0.9)	47.1 (1.0)	50.3 (1.3)
		-18	44.7 (1.0)	45.1 (0.8)	45.7 (0.9)	48.1 (1.0)	51.5 (1.3)
	4	-12	45.6 (0.9)	46.1 (0.7)	47.0 (0.7)	49.6 (0.8)	53.2 (1.0)
		-18	45.1 (0.9)	46.1 (0.7)	47.4 (0.7	50.3 (0.8)	54.2 (1.0)
	8	-12	45.8 (0.8)	47.1 (0.7)	48.5 (0.7)	51.5 (0.7)	55.4 (1.0)
		-18	45.4 (0.8)	46.9 (0.7)	48.6 (0.7)	52.0 (0.7)	56.2 (1.0)
	12	-12	46.1 (0.8)	47.7 (0.7)	49.4 (0.7)	52.7 (0.8)	56.6 (1.0)
		-18	45.8 (0.8)	47.4 (0.7)	49.3 (0.7)	52.9 (0.8)	57.3 (1.0)
	24	-12	46.7 (0.9)	48.5 (0.8)	50.3 (0.9)	53.2 (1.0)	56.5 (1.3)
		-18	46.7 (0.9)	47.9 (0.8)	49.5 (0.9)	52.7 (1.0)	56.7 (1.3)
	52	-12	48.3 (1.6)	49.5 (1.2)	50.7 (1.2)	53.3 (1.2)	56.0 (1.6)
		-18	47.8 (1.6)	48.8 (1.2)	49.7 (1.2)	51.7 (1.2)	53.8 (1.6)

Table 10.3.23. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* metmyoglobin fraction (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	29.8 (0.9)	30.3 (0.7)	30.8 (0.7)	31.4 (0.7)	31.9 (0.9)
		-18	30.6 (0.9)	30.9 (0.7)	31.3 (0.7)	31.6 (0.7)	32.0 (0.9)
	4	-12	33.4 (0.7)	33.9 (0.6)	34.3 (0.6)	34.8 (0.6)	35.3 (0.7)
		-18	33.8 (0.7)	34.1 (0.6)	34.5 (0.6)	34.8 (0.6)	35.1 (0.7)
	8	-12	34.3 (0.7)	34.7 (0.6)	35.1 (0.5)	35.5 (0.6)	35.9 (0.7)
		-18	34.4 (0.7)	34.7 (0.6)	34.9 (0.5)	35.2 (0.6)	35.5 (0.7)
	12	-12	34.9 (0.7)	34.5 (0.7)	34.8 (0.6)	35.2 (0.7)	35.5 (0.7)
		-18	33.9 (0.7)	34.2 (0.7)	34.5 (0.6)	34.7 (0.7)	35.0 (0.7)
	24	-12	36.1 (0.8)	36.3 (0.7)	36.5 (0.7)	36.6 (0.7)	36.8 (0.8)
		-18	35.5 (0.8)	35.7 (0.7)	35.9 (0.7)	36.0 (0.7)	36.2 (0.8)
	52	-12	36.1 (1.2)	35.8 (0.8)	35.6 (0.7)	35.4 (0.8)	35.1 (1.2)
		-18	35.5 (1.2)	35.5 (0.8)	35.5 (0.7)	35.5 (0.8)	35.4 (1.2)
1	0	-12	38.7 (1.0)	38.1 (0.8)	39.1 (0.8)	40.3 (0.8)	40.7 (0.9)
		-18	38.9 (1.0)	37.9 (0.8)	38.1 (0.8)	39.4 (0.8)	40.9 (0.9)
	4	-12	39.4 (0.7)	39.6 (0.5)	40.2 (0.5)	41.1 (0.5)	41.7 (0.7)
		-18	39.2 (0.7)	39.3 (0.5)	39.6 (0.5)	40.4 (0.5)	41.3 (0.7)
	8	-12	39.9 (0.7)	40.7 (0.5)	41.0 (0.5)	41.6 (0.5)	42.5 (0.7)
		-18	39.5 (0.7)	40.4 (0.5)	40.7 (0.5)	41.1 (0.5)	41.6 (0.7)
	12	-12	40.2 (0.7)	41.6 (0.6)	41.4 (0.6)	41.8 (0.6)	43.1 (0.7)
		-18	39.6 (0.7)	41.1 (0.6)	41.3 (0.6)	41.5 (0.6)	41.8 (0.7)
	24	-12	40.9 (1.0)	42.7 (0.9)	41.6 (1.0)	41.8 (0.9)	44.3 (1.0)
		-18	40.4 (1.0)	42.0 (0.9)	41.3 (1.0)	41.5 (0.9)	42.7 (1.0)
	52	-12	40.5 (1.4)	39.9 (1.2)	38.4 (1.2)	40.3 (1.2)	43.0 (1.4)
		-18	40.2 (1.4)	39.2 (1.2)	37.0 (1.2)	38.6 (1.2)	41.7 (1.4)
2	0	-12	40.2 (1.4)	38.4 (1.2)	39.8 (1.2)	42.9 (1.2)	46.5 (1.4)
		-18	40.4 (1.4)	38.6 (1.2)	39.9 (1.2)	43.1 (1.2)	46.6 (1.4)
	4	-12	40.9 (1.0)	41.2 (0.8)	42.6 (0.8)	44.8 (0.8)	47.2 (1.0)
		-18	40.9 (1.0)	41.2 (0.8)	42.6 (0.8)	44.9 (0.8)	47.2 (1.0)
	8	-12	41.6 (1.0)	43.4 (0.8)	44.7 (0.8)	46.2 (0.8)	47.8 (1.0)
		-18	41.4 (1.0)	43.3 (0.8)	44.6 (0.8)	46.1 (0.8)	47.7 (1.0)
	12	-12	42.4 (1.1)	44.9 (0.9)	45.8 (1.0)	47.0 (0.9)	48.5 (1.1)
		-18	42.1 (1.1)	44.7 (0.9)	45.5 (1.0)	46.8 (0.9)	48.3 (1.1)
	24	-12	45.3 (1.4)	46.8 (1.3)	45.9 (1.4)	47.3 (1.3)	50.7 (1.4)
		-18	44.5 (1.4)	46.1 (1.3)	45.2 (1.4)	46.6 (1.3)	50.1 (1.4)
	52	-12	46.9 (1.8)	45.3 (1.4)	45.8 (1.3)	17.0 (1.4)	48.1 (1.8)
		-18	44.8 (1.8)	43.4 (1.4)	44.1 (1.3)	45.5 (1.4)	46.7 (1.8)
3	0	-12	42.3 (1.2)	42.5 (1.0)	43.4 (0.9)	45.3 (1.0)	47.6 (1.2)
		-18	41.5 (1.2)	42.4 (1.0)	44.0 (0.9)	46.6 (1.0)	49.5 (1.2)
	4	-12	43.6 (1.0)	43.8 (0.8)	44.7 (0.7)	46.6 (0.8)	48.8 (1.0)
		-18	42.7 (1.0)	43.5 (0.8)	45.0 (0.7)	47.5 (0.8)	50.3 (1.0)
	8	-12	44.7 (1.0)	44.9 (0.7)	45.8 (0.7)	47.6 (0.7)	49.8 (1.0)
		-18	43.7 (1.0)	44.4 (0.7)	45.9 (0.7)	48.3 (0.7)	51.0 (1.0)
	12	-12	45.6 (1.0)	45.7 (0.8)	46.6 (0.8)	48.4 (0.8)	50.6 (1.0)
		-18	44.5 (1.0)	45.2 (0.8)	46.5 (0.8)	48.8 (0.8)	51.4 (1.0)
	24	-12	47.4 (1.1)	47.5 (1.0)	48.3 (1.0)	50.0 (1.0)	52.1 (1.2)
		-18	46.2 (1.1)	46.6 (1.0)	47.7 (1.0)	49.7 (1.0)	52.1 (1.2)
	52	-12	47.6 (1.8)	47.3 (1.3)	47.8 (1.2)	49.2 (1.3)	50.9 (1.8)
		-18	45.8 (1.8)	45.7 (1.3)	46.3 (1.2)	47.8 (1.3)	49.7 (1.8)

Table 10.3.24. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* oxymyoglobin fraction (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	30.6 (2.8)	33.8 (2.1)	36.7 (1.9)	39.3 (2.1)	41.8 (2.8)
		-18	28.9 (2.8)	32.7 (2.1)	36.4 (1.9)	39.8 (2.1)	43.0 (2.8)
	4	-12	40.8 (2.1)	42.6 (1.6)	44.4 (1.4)	46.0 (1.6)	47.6 (2.1)
		-18	38.9 (2.1)	41.4 (1.6)	43.8 (1.4)	46.1 (1.6)	48.4 (2.1)
	8	-12	46.9 (2.0)	47.4 (1.5)	48.0 (1.4)	48.7 (1.6)	49.3 (2.0)
		-18	44.8 (2.0)	46.0 (1.5)	47.2 (1.4)	48.5 (1.6)	49.8 (2.0)
	12	-12	49.5 (2.2)	49.0 (1.8)	48.5 (1.7)	48.2 (1.8)	48.0 (2.3)
		-18	47.4 (2.2)	47.4 (1.8)	47.5 (1.7)	47.9 (1.8)	48.3 (2.3)
	24	-12	46.8 (2.9)	43.8 (2.2)	44.1 (2.0)	38.8 (2.2)	36.7 (3.0)
		-18	44.9 (2.9)	42.3 (2.2)	40.1 (2.0)	38.3 (2.2)	36.7 (3.0)
	52	-12	40.2 (3.4)	39.7 (2.4)	39.0 (2.0)	38.1 (2.4)	37.0 (3.4)
		-18	41.2 (3.4)	40.9 (2.4)	40.4 (2.0)	39.6 (2.4)	38.7 (3.4)
1	0	-12	45.1 (2.0)	43.5 (1.5)	43.2 (1.5)	43.4 (1.5)	44.3 (2.0)
		-18	45.9 (2.0)	44.3 (1.5)	44.0 (1.5)	44.1 (1.5)	45.0 (2.0)
	4	-12	45.1 (1.8)	43.2 (1.3)	43.7 (1.3)	44.1 (1.3)	44.2 (1.8)
		-18	45.9 (1.8)	44.0 (1.3)	44.5 (1.3)	44.8 (1.3)	44.8 (1.8)
	8	-12	45.2 (1.6)	42.9 (1.2)	44.2 (1.2)	44.8 (1.2)	44.1 (1.6)
		-18	46.0 (1.6)	43.6 (1.2)	44.9 (1.2)	45.4 (1.2)	44.7 (1.6)
	12	-12	45.2 (1.6)	42.6 (1.2)	44.8 (1.3)	45.4 (1.2)	44.0 (1.6)
		-18	46.1 (1.6)	43.3 (1.2)	45.4 (1.3)	46.0 (1.2)	44.5 (1.6)
	24	-12	45.2 (1.9)	41.7 (1.6)	46.5 (1.7)	47.6 (1.6)	43.5 (1.9)
		-18	46.1 (1.9)	42.4 (1.6)	47.1 (1.7)	48.0 (1.6)	43.8 (1.9)
	52	-12	44.0 (3.5)	40.5 (3.1)	52.2 (3.0)	53.4 (3.1)	41.1 (3.5)
		-18	45.0 (3.5)	41.2 (3.0)	52.6 (3.0)	53.5 (3.1)	40.9 (3.5)
2	0	-12	44.2 (2.2)	43.2 (1.7)	42.2 (1.5)	40.9 (1.7)	39.5 (2.2)
		-18	44.4 (2.2)	43.7 (1.7)	42.9 (1.5)	42.5 (1.7)	42.4 (2.2)
	4	-12	43.4 (1.8)	42.5 (1.3)	41.6 (1.1)	40.6 (1.3)	39.5 (1.8)
		-18	43.6 (1.8)	43.1 (1.3)	42.6 (1.1)	42.3 (1.3)	42.1 (1.8)
	8	-12	42.8 (1.7)	42.0 (1.2)	41.2 (1.0)	40.4 (1.2)	39.7 (1.7)
		-18	42.8 (1.7)	42.6 (1.2)	42.3 (1.0)	42.0 (1.2)	41.7 (1.7)
	12	-12	42.5 (1.7)	41.7 (1.3)	41.0 (1.1)	40.5 (1.3)	40.2 (1.7)
		-18	42.0 (1.7)	42.0 (1.3)	42.0 (1.1)	41.7 (1.3)	41.3 (1.7)
	24	-12	42.4 (2.0)	42.2 (1.6)	42.0 (1.6)	42.1 (1.6)	42.5 (2.0)
		-18	39.7 (2.0)	40.2 (1.6)	40.9 (1.6)	40.9 (1.6)	40.4 (2.0)
	52	-12	38.1 (3.3)	39.6 (2.4)	41.1 (2.0)	42.3 (2.4)	43.5 (3.3)
		-18	40.8 (3.3)	41.6 (2.4)	42.5 (2.0)	43.6 (2.4)	45.0 (3.3)
3	0	-12	40.6 (1.4)	40.5 (1.0)	40.4 (1.0)	40.2 (1.3)	39.9 (1.7)
		-18	42.1 (1.4)	41.2 (1.0)	40.3 (1.0)	39.5 (1.3)	38.9 (1.7)
	4	-12	40.0 (1.3)	40.8 (0.9)	41.1 (0.8)	40.9 (0.9)	40.9 (1.3)
		-18	41.6 (1.3)	41.5 (0.9)	41.1 (0.8)	40.4 (0.9)	40.1 (1.3)
	8	-12	39.5 (1.1)	41.1 (0.8)	41.8 (0.8)	41.6 (0.9)	42.0 (1.2)
		-18	41.0 (1.1)	41.9 (0.8)	41.9 (0.8)	41.3 (0.9)	41.4 (1.2)
	12	-12	38.9 (1.1)	41.3 (0.8)	42.4 (0.8)	42.3 (1.0)	43.0 (1.3)
		-18	40.5 (1.1)	42.2 (0.8)	42.7 (0.8)	42.2 (1.0)	42.6 (1.3)
	24	-12	37.3 (1.3)	42.0 (1.1)	44.1 (1.2)	43.9 (1.5)	45.3 (1.9)
		-18	38.9 (1.3)	43.0 (1.1)	44.7 (1.2)	44.2 (1.5)	45.5 (1.9)
	52	-12	33.4 (2.7)	41.5 (2.2)	44.0 (2.1)	41.6 (2.2)	42.8 (2.8)
		-18	35.1 (2.7)	42.9 (2.2)	45.3 (2.1)	42.9 (2.2)	44.4 (2.8)

Table 10.3.25. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* deoxymyoglobin fraction (%) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp) and across the display period (0-3 days)

Display (d)	Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
			0	2	4	6	8
0	0	-12	40.4 (3.1)	36.7 (2.4)	33.1 (2.1)	29.4 (2.4)	25.8 (3.1)
		-18	41.2 (3.1)	37.0 (2.4)	32.8 (2.1)	28.6 (2.4)	24.4 (3.1)
	4	-12	25.7 (2.4)	23.4 (1.9)	21.2 (1.7)	18.9 (1.9)	16.6 (2.4)
		-18	27.1 (2.4)	24.3 (1.9)	21.5 (1.7)	18.7 (1.9)	15.9 (2.4)
	8	-12	18.7 (2.3)	17.7 (1.8)	16.7 (1.7)	15.8 (1.9)	14.8 (2.3)
		-18	20.7 (2.3)	19.2 (1.8)	17.7 (1.7)	16.2 (1.9)	14.7 (2.3)
	12	-12	16.0 (2.6)	16.3 (2.1)	16.5 (1.9)	16.7 (2.1)	16.9 (2.6)
		-18	18.5 (2.6)	18.2 (2.1)	17.9 (1.9)	17.6 (2.1)	17.3 (2.6)
	24	-12	16.7 (3.3)	19.4 (2.5)	22.1 (2.1)	24.8 (2.5)	27.5 (3.3)
		-18	19.4 (3.3)	21.6 (2.5)	23.8 (2.1)	26.1 (2.5)	28.3 (3.3)
	52	-12	24.0 (3.8)	24.8 (2.7)	25.6 (2.2)	26.4 (2.7)	27.3 (3.8)
		-18	23.5 (3.8)	23.9 (2.7)	24.3 (2.2)	24.7 (2.7)	25.1 (3.8)
1	0	-12	15.4 (1.9)	16.6 (1.4)	16.3 (1.5)	15.3 (1.4)	13.8 (1.9)
		-18	13.9 (1.9)	16.5 (1.4)	16.8 (1.5)	15.5 (1.4)	12.7 (1.9)
	4	-12	15.1 (1.7)	16.7 (1.2)	16.0 (1.3)	14.8 (1.2)	13.5 (1.7)
		-18	14.2 (1.7)	16.5 (1.2)	16.0 (1.3)	14.8 (1.3)	13.3 (1.7)
	8	-12	14.8 (1.5)	16.9 (1.2)	15.8 (1.2)	14.2 (1.2)	13.3 (1.5)
		-18	14.4 (1.5)	16.4 (1.2)	15.3 (1.2)	14.1 (1.2)	13.8 (1.5)
	12	-12	14.6 (1.5)	17.0 (1.2)	15.4 (1.2)	13.7 (1.2)	13.1 (1.5)
		-18	14.6 (1.5)	16.4 (1.2)	14.7 (1.2)	13.5 (1.2)	14.2 (1.5)
	24	-12	14.5 (1.7)	17.3 (1.4)	14.0 (1.5)	11.7 (1.4)	13.1 (1.7)
		-18	14.8 (1.7)	16.6 (1.4)	13.3 (1.5)	11.9 (1.4)	15.2 (1.7)
	52	-12	15.8 (3.2)	17.0 (2.8)	8.8 (2.7)	6.2 (2.8)	15.0 (3.2)
		-18	14.6 (3.2)	17.4 (2.8)	10.4 (2.7)	8.2 (2.8)	16.9 (3.2)
2	0	-12	15.7 (1.7)	16.4 (1.3)	15.7 (1.5)	15.9 (1.6)	15.7 (2.1)
		-18	14.9 (1.7)	15.4 (1.3)	16.6 (1.5)	15.5 (1.6)	10.7 (2.1)
	4	-12	15.2 (1.4)	16.0 (1.0)	15.5 (1.0)	14.9 (1.1)	13.7 (1.5)
		-18	15.5 (1.4)	15.3 (1.0)	15.1 (1.0)	14.0 (1.1)	11.0 (1.5)
	8	-12	14.8 (1.3)	15.4 (1.0)	15.1 (1.0)	13.9 (1.0)	11.8 (1.4)
		-18	16.0 (1.3)	15.2 (1.0)	13.8 (1.0)	12.4 (1.0)	11.2 (1.4)
	12	-12	14.8 (1.3)	14.7 (1.0)	14.4 (1.2)	12.7 (1.2)	10.1 (1.6)
		-18	16.4 (1.3)	15.0 (1.0)	12.5 (1.2)	10.9 (1.2)	11.1 (1.6)
	24	-12	15.9 (1.7)	12.4 (1.4)	11.0 (1.7)	9.1 (1.8)	7.2 (2.3)
		-18	16.2 (1.7)	14.9 (1.4)	10.8 (1.7)	8.2 (1.8)	9.1 (2.3)
	52	-12	13.9 (2.5)	14.1 (1.8)	12.6 (1.7)	11.1 (1.9)	8.9 (2.6)
		-18	14.3 (2.5)	13.9 (1.8)	14.2 (1.7)	12.8 (1.9)	8.5 (2.6)
3	0	-12	14.7 (1.5)	17.2 (1.3)	16.1 (1.4)	18.8 (1.4)	10.0 (1.6)
		-18	14.0 (1.5)	16.4 (1.3)	15.3 (1.4)	18.0 (1.4)	9.2 (1.6)
	4	-12	15.6 (1.1)	15.9 (0.9)	14.0 (1.0)	13.9 (0.9)	9.9 (1.2)
		-18	14.8 (1.1)	15.3 (0.9)	13.4 (1.0	13.4 (0.9)	9.5 (1.2)
	8	-12	16.0 (1.1)	14.6 (0.9)	12.8 (1.0)	10.0 (1.0)	9.4 (1.2)
		-18	15.3 (1.1)	14.1 (0.9)	12.4 (1.0)	9.7 (1.0)	9.3 (1.2)
	12	-12	17.0 (1.2)	12.7 (1.1)	11.6 (1.2)	7.3 (1.2)	9.1 (1.4)
		-18	16.4 (1.2)	12.3 (1.1)	11.4 (1.2)	1.3 (1.2)	9.3 (1.4)
	24	-12	17.5 (1.5)	8.2 (1.5)	11.9 (1.6)	5.4 (1.7)	4.5 (2.0)
		-18	16.9 (1.5)	8.1 (1.5)	12.1 (1.6)	6.0 (1.7)	5.5 (2.1)
	52	-12	18.1 (1.9)	12.0 (1.7)	7.2 (1.7)	10.7 (1.7)	6.7 (2.0)
		-18	17.8 (1.9)	12.2 (1.7)	7.9 (1.7)	11.8 (1.7)	8.4 (2.0)

Table 10.3.26. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* particle size (μm) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	-12	192.5 (9.5)	140.1 (7.8)	138.7 (7.1)	143.3 (7.8)	140.8 (9.5)
	-18	186.9 (9.5)	134.3 (7.8)	132.8 (7.1)	137.2 (7.8)	134.6 (9.5)
4	-12	192.0 (8.7)	139.8 (7.3)	138.6 (6.8)	143.4 (7.3)	141.1 (8.7)
	-18	187.8 (8.7)	134.6 (7.3)	132.5 (6.8)	136.2 (7.3)	133.0 (8.7)
8	-12	191.5 (8.0)	139.5 (6.9)	138.5 (6.5)	143.5 (6.9)	141.4 (8.1)
	-18	188.8 (8.0)	135.0 (6.9)	132.1 (6.5)	135.2 (6.9)	131.4 (8.1)
12	-12	191.0 (7.6)	139.2 (6.7)	138.4 (6.3)	143.6 (6.7)	141.7 (7.6)
	-18	189.8 (7.6)	135.3 (6.7)	131.8 (6.3)	134.3 (6.7	129.7 (7.6)
24	-12	189.6 (7.9)	138.4 (6.8)	138.2 (6.4)	143.9 (6.8)	142.6 (7.9)
	-18	192.8 (7.9)	136.3 (6.8)	130.8 (6.4)	131.3 (6.84	124.8 (7.9)
52	-12	186.2 (14.5)	136.3 (11.0)	137.5 (9.6)	144.7 (11.0)	144.8 (14.5)
	-18	199.6 (14.5)	138.6 (11.0)	128.5 (9.6)	124.5 (11.0)	113.5 (14.5)

Table 10.3.27. Predicted means and their standard error (SEM) for lamb *m. longissimus lumborum* soluble sarcoplasmic protein (mg/g) values for each chilled-then-frozen storage treatment combination and for each frozen storage holding temperatures (Temp)

Frozen Storage (weeks)	Frozen Temperature (°C)	Chilled Storage (weeks)				
		0	2	4	6	8
0	-12	21.3 (0.6)	20.7 (0.5)	20.2 (0.4)	19.6 (0.5)	19.1 (0.6)
	-18	20.7 (0.6)	20.2 (0.5)	19.7 (0.4)	19.2 (0.5)	18.8 (0.6)
4	-12	21.2 (0.6)	20.6 (0.4)	20.1 (0.3)	19.6 (0.4)	19.0 (0.6)
	-18	20.8 (0.6)	20.3 (0.4)	19.8 (0.3)	19.3 (0.4)	18.8 (0.6)
8	-12	21.0 (0.5)	20.5 (0.4)	20.0 (0.3)	19.5 (0.4)	19.0 (0.5)
	-18	20.9 (0.5)	20.3 (0.4)	19.8 (0.3)	19.3 (0.4)	18.9 (0.5)
12	-12	20.9 (0.5)	20.5 (0.4)	20.0 (0.3)	19.5 (0.4)	18.9 (0.5)
	-18	20.9 (0.5)	20.4 (0.4)	19.8 (0.3)	19.4 (0.4)	18.9 (0.5)
24	-12	20.6 (0.5)	20.2 (0.4)	19.8 (0.4)	19.4 (0.4)	18.9 (0.5)
	-18	21.2 (0.5)	20.5 (0.4)	19.9 (0.4)	19.4 (0.4)	19.0 (0.5)
52	-12	20.1 (1.0)	20.0 (0.7)	19.8 (0.7)	19.4 (0.7)	18.9 (1.0)
	-18	21.6 (1.0)	20.7 (0.7)	20.0 (0.7)	19.5 (0.7)	19.1 (1.0)

10.4 Project Linked Master's Thesis

School of Animal and Veterinary Sciences

Identifying Storage Thresholds in Frozen and Chilled Lamb Meat

Cassius E. O. Coombs

Bachelor of Animal and Veterinary Bioscience

University of Sydney

This thesis is submitted to Charles Sturt University as fulfilment of the requirements for the degree of Master of Philosophy.

23 March, 2017

"Academics don't retire… they die" – B.W.B. Holman

"Don't go into academia, son, follow the money. Although the aim of education is to make YOUR hour worth more than the next bloke's" – J.H.B. Coombs (1944-2016)

Coincidentally this thesis is submitted on the date of my parents' 27th wedding anniversary, and the first without my father. This thesis is dedicated primarily to him.

Table of Contents:

List of Figures and Tables present in the manuscript

Certificate of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis.

Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged. I agree that this thesis be accessible for the purpose of study and research in accordance with the normal conditions established by the Executive Director, Library Services or nominee, for the care, loan and reproduction of theses.

My family, particularly my mother Alison and late father Jerry, who despite a very rough couple of years never lost their support for me and my work, or my partner or siblings.

To my girlfriend Larissa Gomes dos Reis, whose support for the thesis never wavered, despite completing her own thesis for the entire duration and enduring similar, if not more, struggles and long hours.

List of Publications:

Published

Coombs, C. E. O., Holman, B. W. B., Friend, M. A. & Hopkins, D. L. (2017a). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science*, 84-94.

Coombs, C. E. O., Holman, B. W. B., Collins, D., Friend, M. A. & Hopkins, D. L. (2017b). Effects of chilled-then-frozen storage (up to 52 weeks) on the lamb *M. longissimus lumborum* quality and safety parameters. *Meat Science*, 134, 86-97.

Submitted

Coombs, C. E. O., Holman, B. W. B., Ponnampalam, E. N., Morris, S., Friend, M. A. & Hopkins, D. L. Effects of chilled and frozen storage conditions on the lamb m. longissimus lumborum fatty acid and lipid oxidation parameters. *Meat Science*.

Coombs, C. E. O., Holman, B. W. B., Collins, D., Kerr, M. J., Friend, M. A. & Hopkins, D. L. Effects of chilled-then-frozen storage (up to 52 weeks) on lamb *m. longissimus lumborum* protein oxidation and degradation indicators. *Meat Science.*

External to the thesis (supplementary papers)

Holman, B. W. B., Mao, Y., Coombs, C. E. O., van de Ven, R. J. & Hopkins, D. L. (2016). Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. *Meat Science*, 121, 104-106.

Holman, B. W. B., van de Ven, R. J., Mao, Y., Coombs, C. E. O. & Hopkins, D. L. (2017)., Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science*, 127, 57-62.

Conference papers

Coombs, C. E. O., Holman, B. W. B., van de Ven, R. J., Friend, M. A. & Hopkins, D. L. (2016). Effect of eight weeks chilled or frozen storage on consumer-defined sensory quality traits of lamb. *Proceedings: 31st Biennial Conference of the Australian Society of Animal Production, Adelaide, Australia,* ref. no. 1172.

Coombs, C. E. O., Holman, B. W. B., Collins, D., Friend, M. A. & Hopkins, D. L. (2016). Examining the relationship between colorimetric measurements and microbial loading of beef meat. *Proceedings: 31st Biennial Conference of the Australian Society of Animal Production, Adelaide, Australia,* ref. no. 1244.

Coombs, C. E. O., Holman, B. W. B., van de Ven, R. J., Friend, M. A. & Hopkins, D. L. (2016). Effect of chilled storage (up to 8 weeks) on lamb meat quality traits. *Proceedings: 62nd ICoMST. Bangkok Thailand* (p. 23).

Coombs, C. E. O., Holman, B. W. B., van de Ven, R. J., Friend, M. A. & Hopkins, D. L. (2016). Comparing chilled and frozen storage on

lamb sensory quality parameters. *Proceedings: 62nd ICoMST, Bangkok, Thailand* (p.37).

Coombs, C. E. O., Holman, B. W. B., Collins, D., Friend, M. A. & Hopkins, D. L. (2016). Using beef meat colorimetrics to predict microbial loading following chilled-then-frozen storage. *Proceedings: 62nd ICoMST. Bangkok Thailand* (p. 67).

Holman, B. W. B., Coombs, C. E. O., van de Ven, R. J. & Hopkins, D. L. (2017). Effect of frozen storage (up to 24 weeks) at different temperatures on beef loin eating quality. *Proceedings: 63rd ICoMST. Cork Ireland.*

Other presentations:

HDR Seminar June 2015 at CSU Wagga – Identifying Storage Thresholds in Frozen and Chilled Red Meat – A Proposal

Endorsement of Candidature at CSU Wagga December 2015 – Identifying Storage Thresholds in Frozen and Chilled Red Meat: A Proposal

ASAP Symposium at University of Sydney, Camden Campus (February 2016) – Effect of chilled storage (up to 8 weeks) on lamb meat quality traits

HDR Seminar June 2016 at CSU Wagga – Examining the relationship between colorimetric measurements and microbial loading of beef meat – this poster won a Special Acknowledgement by the Executive Dean

LambEx Young Guns (Albury, NSW) finalist July 2016 - Identifying Storage Thresholds in Frozen and Chilled Lamb

List of Abbreviations present in the manuscript

AMSA: American Meat Science Association

asreml: Analysis software: Restricted maximum likelihood

a_w: water activity

CFU: colony forming units

CIE: Commission International l'Eclairage

CL: cooking loss

CoA: coenzyme A

DHA: docosahexaenoic acid

DMb: deoxymyoglobin

EPA: eicosapentaenoic acid

FAME: fatty acid methyl ester

FAP: fatty acid profile

Fig.: Figure

GC: gas chromatography

H_2O_2: hydrogen peroxide

H_2SO_4: sulfuric acid

IMF: intramuscular fat

KOH: potassium hydroxide

LAB: lactic acid bacteria

LL: *m. longissimus lumborum*

log$_{10}$: logarithmic scale (base 10)

MDA: malondialdehyde

MLA: Meat and Livestock Australia

Mb: myoglobin

MC: moisture content

MMb: metmyoglobin

MRS: de Man, Rogosa & Sharpe (agar)

MUFA: monounsaturated fatty acid

mV: millivolts

n: number (experimental unit)

N: newton

N: normality equivalent concentration (equivalent per litre)

NaCl: sodium chloride

NADH: nicotinamide adenine dinucleotide hydride (reduced form of NAD)

OMb: oxymyoglobin

ORP: oxidation-reduction potential

pHu: ultimate pH

PUFA: polyunsaturated fatty acid

R630/580: Reflectance ratio (Reflectance at 630 nm divided by reflectance at 580 nm)

RIPA: radioimmunoprecipitation assay

SC: synthetic complete

SD: standard deviation

SE: standard error

SF: shear force

SFA: saturated fatty acid

SL: sarcomere length

STAA: streptomycin-thallous acetate-actidione

TBARS: 2-thiobarbituric acid reactive substances

U: amount of peroxidase (in nanomoles) to reduce 1 micromole of hydrogen peroxide (expressed per gram of meat at 37 °C)

UFA: unsaturated fatty acid (MUFA + PUFA)

VFA: volatile fatty acid

VRBG: vile red bile glucose

WHC: water holding capacity

ω3: omega-3 fatty acid (final carbon-carbon double bond 3rd from methyl side)

ω6: omega-6 fatty acid (final carbon-carbon double bond 6th from methyl side)

ABSTRACT

The experiment on which this thesis is based tested the effects of chilled, frozen and chilled-then-frozen storage of lamb at various durations. It was hypothesised that prolonged chilled storage (up to 8 weeks) would lead to rancidity, compromised sensory and nutritional qualities and spoilage due to proliferation of specific microbes. Likewise, it was hypothesised that following chilled storage, frozen storage as per industry practice would preserve quality regardless of duration. Following prescribed chilled-then-frozen durations however, it was hypothesised that meat frozen for longer durations (up to 52 weeks) would exhibit a faster loss of quality upon retail display post-thawing.

A total of 360 lamb *m. longissimus lumborum* (LL) were collected from a collaborating export abattoir and allocated to storage durations of 0, 2, 4, 6 and 8 weeks chilled storage and 0, 4, 8, 12, 24 and 52 weeks frozen storage ($n = 360$). Frozen samples were held at two different frozen storage temperatures (-12 and -18 °C). Upon completion of each chilled-then-frozen

storage duration treatment, samples were sub-sampled for instrumental analyses of meat quality and safety traits and one sub-section was placed under simulated retail display conditions for 3 days (approximately 72 hours) where instrumental colour measures were taken daily.

Results from this experiment indicated that chilled-then-frozen storage influenced sensory quality by decreasing shear force and colour stability and altering lipid oxidation. Nutritional quality, based upon proportions of health-claimable fatty acids, and food safety, were maintained following maximum chilled and frozen storage periods in this experiment, although spoilage microbes (lactic acid bacteria and *Brochothrix thermosphacta*) were close to proliferating above spoilage thresholds following 8 weeks chilled-only storage. Following this experiment, storage thresholds (chilled, frozen and chilled-then-frozen) could be formed based upon previously determined consumer thresholds for selected meat quality and safety traits.

General Introduction

Red meat (beef and lamb; for the purpose of this thesis, with particular focus on the lamb sector) is a valuable international commodity, with Australia exporting a majority of its red meat to overseas nations including Russia, Papua New Guinea, South Africa, China, Japan, South Korea, the USA and the Middle East. A decline in live animal export in recent years has led to the extensive use of preserved meat product in **chilled** (-1 to 5 °C) or **frozen** (below -10 °C) forms being exported to these and other nations through means of air or sea freight. Fifty-six percent of Australian lamb is exported overseas and the lamb export industry is valued at $1.78 billion (MLA, 2016). Chilled export predominates due to market access, with more than 50% of Australian lamb being exported to the Middle East and the United States of America (MLA, 2016), where chilled product is generally preferred in comparison to frozen. China is an emerging market, and is the

fastest growing export market for Australian lamb (MLA, 2016), providing opportunity for frozen meat trade.

Australian export meat needs to maintain its high standing within the international community – based on sensory (eating and visual) and nutritional (lipid and protein) qualities, as well as food safety (low microbial loading, free from spoilage). Coupled with this is the rising cost of transportation, with an opportunity to slow this transportation (slow steaming) offering the scope to optimise fuel use and allow for bulk shipments of Australian product to overseas markets while still ensuring quality and safety of red meat over long-term storage of one year or more using frozen storage temperatures. Frozen storage temperatures are traditionally -18 °C (0 °F) for international export based upon regulations (Food Science Australia, 2005; USDA, 2013), although -12 °C has been mentioned as the minimum temperature for acceptable safety of meat entering the European Union (UK P & I Club, 2006). The lack of testing of frozen storage temperature and its effects on quality and safety of red meat have led to the use in this study of two temperatures (-12 and -18 °C).

Prior research, examined in the literature review (**Chapter one**), has determined that anaerobic chilled storage or 'wet ageing' of red meat in vacuum packs, can improve its qualities, particularly tenderness and flavour (Wood *et al.*, 1999; Colle *et al.*, 2015), which are considered the primary drivers of lamb quality (Muela *et al.*, 2012; MLA, 2015). Additionally, provided that freezing rate is sufficiently fast and storage temperature remains consistent, the freezing process and frozen storage duration will not lead to quality deterioration (Muela *et al.*, 2012; Bueno *et al.*, 2013). This

has also been proven to occur when meat is frozen following chilled storage, however, the frozen storage durations in this situation have not exceeded 2-3 months (Shanks *et al.*, 2002; Lagerstedt *et al.*, 2008; Vieira *et al.*, 2009; Kim *et al.*, 2011; Kim *et al.*, 2013). What is missing is any effect of longer frozen storage periods which are commonly used in industry. Such extended storage helps maintain market access of Australian lamb worldwide by evening out its supply. However, sensory quality ramifications of extended frozen storage periods beyond one year have been identified, particularly following thawing and aerobic chilled storage post-thawing (Muela *et al.*, 2015; Muela *et al.*, 2016). Frozen storage durations of 2 years or longer have been proposed by Winger (1984), with minimal quality deterioration or compromise of safety, provided chilled storage prior to freezing is kept to a minimum.

It is apparent based on the literature that there is a dearth of studies looking at chilled ageing followed by long-term freezing (for the purpose of this thesis, termed **chilled-then-frozen storage**), and this could be the missing link to long-term preservation at low costs to improve trade links and facilitate market access of Australian red meat products. There is also an absence of storage thresholds for either chilled or frozen product in the literature, and the establishment of these to ensure meat exported is safe and of optimal nutritional and eating quality, is paramount to fulfilling this paucity. The majority of recent studies on long-term preservation (> 9 months) have used only frozen storage (Fernandes *et al.*, 2013; Muela *et al.*, 2015; Muela *et al.*, 2016), or have included packaging technologies manipulating the atmospheric gas composition, freezing and thawing

pressure alterations and the addition of anti-freeze proteins or brine/salt to meat (Zhou *et al.*, 2010; Leygonie *et al.*, 2012). As these pose consumer health and safety risks at varying degrees, and increase the cost of meat processing, the study described in this thesis used vacuum packaging to restrict oxygen, followed by chilled ageing and then freezing under these anaerobic conditions. Storage temperatures (chilled and frozen) are controlled and recorded to prevent the quality and safety risks associated with accidental freezing and thawing of meat, and in doing so pose a safe, healthy and cost effective solution to long-term preservation of Australian lamb.

The following research questions were posed:

1. What are the current practices within global red meat industries and future potential options regarding long-term preservation of red meat?

2. How does short- and long-term preservation, particularly chilling and freezing combinations, affect the quality of lamb?

3. How does short- and long-term preservation affect the nutritional value of lamb?

4. Can short- and long-term preservation of lamb compromise food safety beyond currently established market thresholds?

5. Where do these quality and safety thresholds exist for chilled-then-frozen lamb meat and can current market thresholds be extended based on this research?

For this purpose, a comprehensive study focusing on several aspects of meat quality is essential to more thoroughly understand the effects of long-term chilled-then-frozen storage. The experiment on which this thesis is based was designed to contribute to overcoming an absence of scientifically supported storage thresholds. With this information, the international lamb trade can be improved beyond its current boundaries, with the purpose of increasing the safe storage duration of a high-quality product to allow for longer exportation distances at slower speeds to save costs and provide product when demanded instead of being restricted to seasonal availability.

Chapter one comprehensively reviews the literature relating to the topic on both beef and lamb, and identifies the key paucities which justify why this study was undertaken. The review encapsulates the effects of chilled, frozen and the combination of chilled and frozen storage on the meat quality (tenderness, juiciness, flavour and colour) and food safety (microbial profile) of beef and lamb.

Chapter two evaluates the effect of chilled-then-frozen storage at different storage durations on the meat quality and safety traits of lamb *m. longissimus lumborum* (LL). The hypothesis was that the quality traits related to tenderness (shear force) will be improved by chilled storage and then preserved by subsequent frozen storage, and that meat will remain microbiologically safe for up to one year frozen, and up to eight weeks chilled provided temperature is sufficiently controlled. Colour parameters will allow for post-thawing display thresholds to be developed across a range of chilled-then-frozen storage durations, with colour being the main quality factor limiting meat purchasing decisions.

Chapter three evaluates the effects of chilled-then-frozen storage on the parameters of lipid oxidation, and the fatty acid profile, in lamb LL, as this is what primarily affects flavour (taste and aroma) and contributes to sensory rejection of the product. It was anticipated that lipid peroxidation biomarkers would increase with chilled and frozen storage, albeit at different rates, though remain below the consumer rejection threshold levels for the duration of the experiment. Additionally, health-claimable fatty acids were measured to determine the effect of chilled-then-frozen storage upon the nutritional value of lamb.

Chapter four presents a holistic summary of the findings of the experiment, drawing general conclusions from **Chapters two and three**, and outlining the paucities identified from previous literature findings in **Chapter one**. This chapter covers the implications of the findings, with practical application, and also identifies new paucities from this research for future investigation, integrating the previous chapters to provide the reader with sufficient information on the outcomes of the research detailed in this thesis.

A completed list of references for the thesis is provided following the conclusion of **Chapter four**, comprising literature cited in **Chapters one through four**.

Chapter 1. Literature review (based on publication in Meat Science)

1.1 Introduction

Beef and lamb, collectively termed as red meat, are valuable sources of high-quality protein (Williams, 2007), and their preservation throughout export as anoxic vacuum-packaged product in either chilled or frozen form and, at sufficiently cold temperatures, is fundamental for successful international trade (Bell & Garout, 1994; Deards *et al.*, 2014; Eustace *et al.*, 2014). However, over long-term chilled and frozen storage durations, meat structure and biochemistry undergoes significant changes, particularly in the case of accidentally frozen or thawed meat due to poor temperature control (Bell & Garout, 1994), contributing to losses in quality and perceived value

(Leygonie *et al.*, 2012; Eustace *et al.*, 2014). The impact of long-term chilled storage for export purposes has been investigated previously in lamb (Sumner & Jenson, 2011; Kiermeier *et al.*, 2013) and beef (Bell & Garout, 1994; Small *et al.*, 2012). Furthermore, the process of frozen storage has been reviewed (Leygonie *et al.*, 2012), while storage durations have been extended for more than one year in some studies (Muela *et al.*, 2015; 2016). Despite these advances, improvement of preservation technologies within the current export cold chain remains an issue, especially at the industrial level, with potential advances including superchilling, ionising radiation, biopreservation and high hydrostatic pressure (Zhou *et al.*, 2010). Therefore, the scope of this review is to compare and contrast the effects of chilled and frozen storage, comprehensively review their combination, and examine differences in storage duration and temperature upon meat quality and spoilage parameters, defined as tenderness, juiciness, flavour, colour and microbial profile, respectively.

1.2 Chilled storage effects on meat quality and spoilage parameters

Chilled storage (-1.5 to 5 °C) is routinely used post-slaughter, whether in the form of whole carcase hanging (pre-boning) or vacuum-packaged primals (post-boning). Chilled storage generally takes place from between 24 hours to several weeks *post-mortem* and often varies in duration and temperature based upon species, market and processor nuances. Chilled storage preserves meat from microbial spoilage through low temperatures and in an anaerobic environment, while it can also improve meat quality

through proteolysis before, during and after *rigor mortis* (Hopkins & Thompson, 2002). It also allows the meat to be held for longer periods aiding market distribution and smoothing the supply pattern to consumers.

1.2.1. Tenderness

Tenderness is defined as the ease of mastication by a consumer and has been identified as the primary driver influencing perceived quality, particularly in the case of beef (Miller *et al.*, 2001). It is most reliably and easily measured instrumentally using shear force (SF) as a proxy for consumer sensory panels which cannot always be undertaken (Hopkins *et al.*, 2006; Hopkins *et al.*, 2013), although SF in and of itself does not provide a complete tenderness profile (Honikel, 1998; Hopkins *et al.*, 2013). Such variation can be explained in part by sarcomere length, connective tissue content and myofibrillar proteolysis (Koohmaraie *et al.*, 2002; Starkey *et al.*, 2015).

The tenderising process begins in meat following *rigor mortis*, which can occur between 3 to 24 hours *post-mortem* depending on the carcase (Koohmaraie *et al.*, 2002). Calcium release from the sarcoplasmic reticulum and mitochondria leads to the activation of calcium-dependent proteases, known as the calpains (1 and 2) (Dransfield, 1994; Hopkins & Thompson, 2002). Also implicated in *post-mortem* proteolysis are the cathepsin enzymes (Ouali, 1992), proteasomes (Huff-Lonergan *et al.*, 2010) and caspases (Kemp & Parr, 2012), although their exact role, if any, in tenderisation is still a matter of debate, whereas there is ample evidence for the role of the calpains regardless of species, breed or muscle type (Hopkins

& Thompson, 2002). Continued chilled storage results in calpains progressively degrading myofibrillar proteins (titin, nebulin, desmin and troponin-T) in a process known as proteolysis (Koohmaraie *et al.*, 2002; Huff-Lonergan *et al.*, 2010). As proteolysis occurs, the meat becomes tenderer and SF decreases (Hopkins & Thompson, 2002). Electrical stimulation and higher rigor temperatures can also inhibit cold shortening, resulting in longer sarcomeres and therefore improved tenderness of beef and lamb (Smulders *et al.*, 1990; Hopkins & Thompson, 2002) in the absence of chilled storage (Starkey *et al.*, 2015).

Despite these positive effects on tenderness from chilled storage, other studies reported that prolonged chilled storage durations do not pose any additional improvement on SF or sensory tenderness (Table 1.1). From this, chilled storage duration thresholds for optimal tenderness can be recommended; namely 7 to 10 days in lamb at 1-4 °C (Dransfield, 1994; Starkey *et al.*, 2015) and 2 to 3 weeks in beef at 1-2 °C (Dransfield, 1994; Shanks *et al.*, 2002). Optimal beef tenderness may, however, not be reached until a longer chilled storage duration (12 weeks at -1 °C) according to a recent study (Hughes *et al.*, 2015), and as a result this chilled storage threshold should be re-examined with particular focus on chilled storage temperature. It has been suggested in other recent studies that tenderisation continues for longer durations in beef at low temperatures (e.g. 0 °C) (Colle *et al.*, 2015) and optimal SF of lamb is attained at shorter durations of 8 days when chilled storage temperatures are 3-7 °C compared to 14 days at -1.5 °C (Choe *et al.*, 2016) due to higher calpain-1 activity at these temperatures (Camou *et al.*, 2007).

27

1.2.2. Fluid losses and juiciness

Juiciness refers to the amount of fluid released from meat, in addition to the salivation stimulated upon mastication. A partial insight into juiciness can be made through measuring water holding capacity (WHC) as a percentage of liquid losses during storage (purge or drip) and cooking. However, the relationship between WHC and sensory juiciness is inconsistent across chilled storage durations due to the many factors influencing mouthfeel (Honikel & Hamm, 1994; Winger & Hagyard, 1994; Honikel, 1998; Kim *et al.*, 2014) including the content of intramuscular fat (IMF) (Savell & Cross, 1988; Hopkins *et al.*, 2006). However, Hopkins *et al.* (2006) reported an improvement in overall acceptability rather than juiciness alone as cooking loss decreased, while juiciness could be estimated by increased fat on salivary flow (Winger & Hagyard, 1994); therefore WHC and IMF are not suitable proxies for sensory juiciness (Honikel & Hamm, 1994).

The effects of chilled storage upon juiciness parameters are shown in Table 1.1, from which it is evident that chilled storage rarely inferred a negative effect upon sensory juiciness (Lagerstedt *et al.*, 2008; Vieira *et al.*, 2009; Colle *et al.*, 2015), despite decreases in WHC resulting from increased chilled storage duration. In particular, purge losses significantly increased at shorter durations of 0 to 2 weeks (Lagertstedt *et al.*, 2008; Vieira *et al.*, 2009) and cooking losses from 3 to 5 weeks (Shanks *et al.*, 2002; Colle *et al.*, 2015) in chilled beef, compared to fresh meat, dependent on the muscle (Colle *et al.*, 2015). Shanks *et al.* (2002) suggested a mechanism for these results; namely damage to cell membranes and

proteolysis prompting increased purge and cooking losses, while the lack of clear effects on sensory juiciness can be explained by confounding with improvements in tenderness (Watson *et al.*, 2008), denoted as halo effects (Shorthose & Harris, 1991). Furthermore, juiciness perception may have been affected by the melting of meat fat due to cooking, which may have accounted for the lack of effect on sensory juiciness despite liquid losses (Vieira *et al.*, 2009).

1.2.3. Flavour

Flavour is a sensation of the mouth provoked by fats and other precursors such as sugars and amino acids from within a meat product which impacts upon consumer taste (Khan *et al.*, 2015). In lamb, flavour is often denoted as the most important quality trait (Muela *et al.*, 2012; MLA, 2015). The process of chilled storage tends to increase flavour with storage duration with the oxidation of volatile fatty acids (VFAs) and amino acids (Wood *et al.*, 1999). However, once this duration and associated oxidation has breached an acceptability threshold, it can infer rancidity via its association with a rancid flavour by consumers (Campo *et al.*, 2006; Bueno *et al.*, 2013; Corbin *et al.*, 2015). A key marker for lipid oxidation has been

identified as malondialdehyde (MDA), measured using the 2-thiobarbituric acid reactive substances (TBARS) method (Tarladgis *et al.*, 1960).

Despite the widespread use of TBARS, its relationship with sensory perception of rancidity is still quite low (Greene & Cumuze, 1981), while thresholds based on MDA content correlated with consumer acceptance and rejection often vary between studies. For instance, in one study beef could be considered "unacceptable" based on TBARS (Campo *et al.*, 2006) while in another at the same storage duration, TBARS was not measured, but flavour perception by consumers continued to improve with further chilled storage (Hughes *et al.*, 2015). This variation likely occurred due to differences in packaging (McMillin, 2008) and chilled storage temperature (Jeremiah & Gibson, 2001; Zhou *et al.*, 2010), while MDA measurement methods also commonly vary between studies, providing variation at similar chilled storage durations. Furthermore, a large spectrum of chilled storage duration thresholds have been derived based upon an absence of confinement odour for up to 12 weeks in lamb at -0.5 to -2.4 °C (Sumner & Jenson, 2011) and up to 24 weeks in beef at -0.5°C (Small *et al.*, 2012) and on consumer acceptability for up to 12 weeks in lamb at -0.3 °C (Kiermeier *et al.*, 2013) and for up to 26 weeks in beef at -0.5 to -1 °C (Small *et al.*, 2012; Hughes *et al.*, 2015). No significant differences in flavour or excessive TBARS were found following 9 weeks storage of beef at 0 °C (Colle *et al.*, 2015), though in other studies, TBARS values were higher at these durations due to methodological differences (Ponnampalam *et al.*, 2014a).

Confinement odour occurs due to oxygen restriction in vacuum-packaged meat and is a major limiting factor, even though it does not indicate spoilage (Reis *et al.*, 2016) and, if meat is unspoiled, the odour usually dissipates within 30 minutes of opening of the vacuum pack (Spooncer, 1988). Hughes *et al.* (2015) reported flavour to be more preferable in beef stored chilled at -1 °C for 20 weeks compared to 2 weeks. The evaluation of TBARS concurrent with consumer flavour acceptance is scarce in the literature; as a result the need for further research has been suggested in a recent study (Ponnampalam *et al.*, 2014a). In doing so, TBARS (based upon a standardised measurement) could potentially be established as a proxy for off-odour and flavour formation from prolonged chilled storage.

The quantification of fatty acids to provide similar predictive information to TBARS would also prove valuable, particularly the changes in the fatty acid profile relating to storage (Fisher *et al.*, 2000), due to the effects of the profile on meat flavour (Calkins & Hodgen, 2007) and susceptibility to lipid oxidation (Cifuni *et al.*, 2000). Of particular interest is the volatile aldehyde hexanal, which is linked to a fatty, grassy odour and a lack of consumer acceptability (Calkins & Hodgen, 2007; Callejas-Cárdenas *et al.*, 2014). In addition, increased IMF content (Hopkins *et al.*, 2006; Corbin *et al.*, 2015) and lack of anaerobic microbial proliferation (Bell & Garout, 1994; Kiermeier *et al.*, 2013) are strongly related to improved meat flavour.

The contents of both MDA and hexanal can decrease as chilled storage duration increases due to the formation of secondary lipid oxidation

products; as a result further measures should be taken in addition to these in order to form consumer acceptability thresholds and prevent confusion associated with prior studies.

1.2.4. Colour

Meat colour is an important parameter at the retail level due to its perceived indication of freshness and quality to consumers (Risvik, 1994). Subjective definitions of colour vary, although consumer preference for bright cherry-red beef and brick-red lamb has been reported (Mancini & Hunt, 2005; AMSA, 2012). The use of objective colorimetric measures is common, with research recording Commission International l'Eclairage (CIE) colour scale values: L* denoting lightness to darkness, a* denoting redness to green and b* denoting yellowness to blue (CIE, 1978). Of these, a* was found to be most influential in both lamb (Khliji *et al.*, 2010) and beef (Holman *et al.*, 2017) consumer appraisal

Oxidative changes to myoglobin (Mb) occur as chilled storage increases, which is reflected in instrumental colour measurement variation (L*, a*, b*) over anaerobic chilled storage durations due to the formation of deoxymyoglobin (DMb) with increased storage duration contributing to a purple colour and oxymyoglobin (OMb) formation upon package opening giving a bright red colour (Mancini & Hunt, 2005). Prolonged chilled storage in an aerobic environment (e.g. retail display) leads to increased oxidation of Mb to metmyoglobin (MMb) which contributes to a brown colour. Similarly, increased chilled storage temperatures can also decrease

colour stability (from -1 to 2 °C - Rosenvold & Wiklund, 2011; from -1.5 to 7 °C – Choe *et al.*, 2016).

Increased MMb formation has been shown to negatively affect consumer scoring, with acceptability thresholds set for beef at 40-50% MMb (Kropf *et al.*, 1986; Jeremiah & Gibson, 2001) and 23% MMb for lamb (Khliji *et al.*, 2010). However, the proportion of MMb in these studies was calculated via instrumental colorimetric measurements using the percentage of light reflected at 630 nm divided by the percentage of light reflected at 580 nm (R630/580) (Jacob *et al.*, 2007; AMSA, 2012). As such, R630/580 does not directly measure MMb and instead is used as a proxy of Mb oxidation and therefore an indication of colour deterioration or 'brownness' (AMSA, 2012). A standard protocol for Mb quantification in its different forms has been developed using the isobestic point or absorbance at 525 nm, following its extraction from haem pigment (AMSA, 2012).

Using a visual panel, beef stored chilled and anaerobically was found to be acceptable for up to 24 weeks following a 20 minute bloom period; longer chilled storage durations resulted in unacceptable scores (Small *et al.*, 2012). In lamb, colour acceptability decreased following 3 weeks chilled storage plus 3 to 14 days of retail display when compared to fresh meat (Callejas-Cárdenas *et al.*, 2014). When based upon MMb content, beef was ruled unacceptable following 8 weeks chilled storage plus 6 to 8 days of retail display (Liu *et al.*, 1996). Differing results reported in the literature (Table 1.1) appear to occur due to differences in methodological measurement of colour acceptability (MMb, visual panel or CIE values),

storage temperature, muscle type and oxygen exposure, all of which have been shown to influence colour (Mancini & Hunt, 2005).

It can be concluded that increased anaerobic chilled storage duration results in more rapid discolouration (decreased a* and R630/580) upon exposure to oxygen, which has been found to occur concurrent to lipid oxidation (Ponnampalam *et al.*, 2001; Kim *et al.*, 2011; Callejas-Cárdenas *et al.*, 2014; Colle *et al.*, 2015). It has been determined that Mb and lipid oxidation can both act as precursors for each other (Faustman *et al.*, 2010).

1.2.5. Microbial profile

The microbial profile, particularly spoilage bacteria including lactic acid bacteria (LAB), *Enterobacteriaceae sp.*, *Brochothrix thermosphacta* and psychrotolerant *Clostridium sp. (*e.g. *C. perfringens*, have been found to increase as chilled storage duration increases (Gram *et al.*, 2002; Gribble *et al.*, 2014). These species primarily originate from abattoir contamination (Dainty & Mackey, 1992), and with the exception of LAB and clostridia are limited to proliferate only with oxygen availability; other risk factors involved include chilled storage temperature, pH, water activity (a_w) and packaging conditions (Egan *et al.*, 1988; Gram *et al.*, 2002; Mills *et al.*, 2014). Often, the result of microbial proliferation and the increase in spoilage levels is the development of off-flavours described as cheesy and dairy (Egan *et al.*, 1988), discolouration (Li *et al.*, 2015) and reduced product safety (Gram *et al.*, 2002; Mills *et al.*, 2014).

Anaerobic chilled storage using vacuum packaging generally results in proliferation of LAB, which has a spoilage potential of 10^7 colony forming

units (CFU) per cm^2 and results in off-odour detection (Sumner & Jenson, 2011; Small *et al.*, 2012). LAB proliferation leads to sour flavours and greening or discolouration from the production of hydrogen sulphide reacting with Mb to form sulphmyoglobin (Egan *et al.*, 1988; Mills *et al.*, 2014). Prolonged anaerobic chilled storage also increases availability of water from the meat substrate (increased a_w) (Mills *et al.*, 2014) and increases pH due to muscle tissue breakdown, particularly of lamb meat stored for 6 weeks or more (Moore & Gill, 1987; Kim *et al.*, 2011; Kiermeier *et al.*, 2013) and beef meat stored for 12 weeks or more (Hughes *et al.*, 2015). Both of these traits (a_w and pH) can provide insight into microbial spoilage potential, such that if a_w exceeds 0.93 (Egan *et al.*, 1988) and pH exceeds 5.8 (Gribble *et al.*, 2014) this will result in more rapid proliferation of specific spoilage microbes. However, storage temperature has the greatest impact on microbial loading of chilled-stored meat (Bell & Garout, 1994; Giannuzzi *et al.*, 1998) and ideally should be between 0 and -1.5 °C, although in practice this is not always achieved (Bell & Garout, 1994; Eustace *et al.*, 2014; Mills *et al.*, 2014).

Chilled storage duration thresholds based on the spoilage thresholds for LAB (10^7 CFU/cm^2) and aerobic spoilage bacteria (10^5-10^7 CFU/cm^2) have been proposed in the literature, though these differ based on species and nation (Bell & Garout, 1994; Hinton *et al.*, 1998; Gribble *et al.*, 2014). Microbial proliferation spoilage thresholds are often reported as their maximum level of proliferation (Gribble *et al.*, 2014), and as a result the loading alone is often insufficient to rule on the occurrence of sensory spoilage. Variations in safe storage durations can be attributed to high pH,

temperature fluctuations, improper packaging, high a_w and muscle glycogen reserves (Dainty & Mackey, 1992; Gribble *et al.*, 2014), the latter especially as it provides a favourable substrate for microbes prior to their metabolism of amino acids (Gill & Newton, 1981; Newton & Gill, 1981). Temperature logging is only included spasmodically in chilled storage studies, and temperature abuse (> 5 °C) and accidental freezing (< -2 °C) can lead to more rapid microbial spoilage and product rejection by consumers (Bell & Garout, 1994; Eustace *et al.*, 2014). However, psychrotolerant *Clostridium* sp. causing pack blowing (hydrogen and carbon dioxide gas production) within 2 to 6 weeks can proliferate below 0 °C and as low as -5 °C and also present a cheesy odour upon pack opening and meat greening (Broda *et al.*, 1996; Adam *et al.*, 2010). This highlights the need for good supply chain management - especially at processor and transportation levels (Clemens *et al.*, 2010).

Table 1.1. Summary of the effects of vacuum-packed chilled storage on the shear force, tenderness, fluid levels, flavour and colour of beef and lamb.

Species	Duration	Temperature	Effects on selected traits	Source
			Shear force and tenderness	
Beef	35 days	2°C	Peak tenderisation (SF around 39 N) reached around 2 to 3 weeks	Shanks *et al.* (2002)
Beef	14 days	4°C	Gradual decrease in SF between 2, 7 and 14 days (66 to 56 to 42 N)	Lagerstedt *et al.* (2008)
Beef	3 to 10 days	4°C	Decreased SF (73 to 50 N) and improved tenderness (2.8 to 3.6)	Vieira *et al.* (2009)
Lamb	7 weeks	-1.5 to 2°C	Decreased SF due to chilled storage though but not temperature (to 56 N)	Rosenvold & Wiklund (2011)
Beef	20 weeks	-1°C	Tenderness improved between 2 and 12 weeks (2.0 to 2.8) – not 12 and 20	Hughes *et al.* (2015)
Lamb	2 weeks	3-4°C	SF improved between 1 and 7 days (36 to 25 N) – not 7 and 14	Starkey *et al.* (2015)
Beef	63 days	0°C	Continued SF decrease over 63 days chilled storage (33.5 to 22.2 N)	Colle *et al.* (2015)
			Fluids	
Beef	35 days	2°C	Cooking losses do not increase prior to 35 days storage (23% to 26%)	Shanks *et al.* (2002)
Beef	14 days	4°C	Peak liquid losses at 7 days (16.3% to 24.6%)	Lagerstedt *et al.* (2008)
Beef	10 days	4°C	No effect on juiciness between 3 and 10 days (score 2.6 and 2.7)	Vieira *et al.* (2009)
Lamb	7 weeks	-1.5 to 2°C	No difference in purge losses due to temperature after 7 weeks (4.2 to 4.9%)	Rosenvold & Wiklund (2011)
Beef	63 days	0°C	Cook loss increases between 14 and 21 days (24.5 to 27.9%)	Colle *et al.* (2015)
			Flavour	
Beef	13 days	1°C	More species-specific flavour after 13 days, decreased with 4 days display (TBARS > 2.28)	Campo *et al.* (2006)
Lamb	5 days	0-4°C	More species-specific flavour after 6 days (sensory score 3.16)	Hopkins *et al.* (2006)
Lamb	90 days	0 to -2.5°C	85 days storage acceptable (-0.5°C), unacceptable at 0°C after 90 days	Sumner & Jenson (2011)
Beef	30 weeks	-0.5°C	24 weeks storage retaining acceptable flavour	Small *et al.* (2012)
			Colour	

Beef	56 days	4°C	56 days storage led to acceptable colour (MMb < 50%) for 6 days display	Liu *et al.* (1996)
Beef	24 weeks	-1.5 to 5°C	Reduced 24 week storage temperature (-1.5°C) improves a*(from 8 to 11)	Jeremiah & Gibson (2001)
Lamb	4 weeks	0°C	4 weeks vacuum storage results in more rapid a*deterioration over 6 days	Ponnampalam *et al.* (2001)
Beef	10 days	4°C	Decreased a* and L* between 3 and 10 days	Vieira *et al.* (2009)
Lamb	7 weeks	-1.5 to 2°C	Decreased a* and increased L* at increased chill temperature	Rosenvold & Wiklund (2011)
Beef	30 weeks	-0.5°C	"Fresh" appearance until 26 weeks – "marginal" at 28 and 30 weeks	Small *et al.* (2012)

All sensory panel scores for different traits given on a scale from 0 to 5. All shear force values converted to newtons (N) by the equation 1 kgF = 9.80665 N. SF = shear force; TBARS = 2-thiobarbituric reactive substances (mg malondialdehyde per kg meat); MMb = metmyoglobin; a* = colorimetric measure of redness.

1.3. Frozen storage effects on meat quality and spoilage parameters

The objective of frozen storage is to preserve quality and, through the use of comparatively longer safe storage durations, even out the supply variations (Shanks *et al.*, 2002). Several studies have investigated the effects of frozen storage temperature and freezing rate on meat quality, however frozen storage duration appears to be the crucial factor in terms of maintaining meat quality and preventing spoilage for export purposes (Leygonie *et al.*, 2012). For example, the routinely applied export storage temperature (-18 °C) has been found to maintain acceptable quality and prevent spoilage in lamb for more than one year (Fernandes *et al.*, 2013; Muela *et al.*, 2015; 2016). In fact, no further improvements in sensory quality or storage duration have been noted when frozen storage temperatures are lower than -18 °C (Hagyard *et al.*, 1993; Farouk *et al.*, 2003; Estévez, 2011). Frozen storage temperatures above -18 °C have been rarely reported in literature, though based on sensory quality, -15 °C yielded longer acceptable frozen storage durations than -10 °C and -5 °C, although temperature variation was not reported in this study (Hagyard *et al.*, 1993). One study reported that, provided frozen storage temperature remained below -10 °C and freezing rate was controlled, lamb could be stored safely without deleterious effects on quality for two years or longer (Winger, 1984). This was thought to be due to sufficiently low a_w (0.9) preventing microbial growth and low temperatures preventing oxidative flavour changes.

1.3.1. Tenderness

When meat is frozen, the rate of proteolysis is halted due to suppression of calpain activity, but not destruction of calpains (Koohmaraie, 1992; Dransfield, 1994). Furthermore, proteolysis will be restored upon thawing, due to the re-activation of the calcium-dependent proteases (Dransfield, 1994), and normally at a faster rate than prior to freezing (Crouse & Koohmaraie, 1990). This proteolysis rate increase can be attributed to the inhibition of calpastatin by frozen storage allowing for more rapid tenderisation upon thawing (Crouse & Koohmaraie, 1990; Koohmaraie, 1992). For this reason, results for the effect of frozen storage on tenderness and SF (as shown in Table 1.2) are often dependent on when (whether cooked from frozen or thawed) and how (by instrumental SF or a sensory panel) tenderness is measured (Holman *et al.*, 2016a). Honikel (1998) mentioned that cooking from frozen is ideal for SF determination, despite a majority of studies reporting cooking from thawed (Holman *et al.*, 2016a).

Trained sensory panels often present conflicting results when reporting tenderness differences between fresh and frozen meat (Table 1.2) – for instance, Lagerstedt *et al.* (2008) reported decreased tenderness in beef frozen (24 hours) post-chill (7 days at 4 °C) compared to chilled beef, though other studies on lamb have reported no difference between fresh (unaged) and frozen meat (Muela *et al.*, 2012; Bueno *et al.*, 2013; Fernandes *et al.*, 2013; Muela *et al.*, 2016), although for the most part, untrained sensory panels have been unable to distinguish between fresh and frozen

meat (Lagerstedt *et al.*, 2008; Muela *et al.*, 2012) unless the frozen storage duration was excessively long, although storage post-thaw may have had some effect on tissue damage (Muela *et al.*, 2016). Results suggest that tenderness and SF were more affected by chilled storage prior to (Vieira *et al.*, 2009) or following freezing (Muela *et al.*, 2015) than frozen storage itself.

Two recent studies on lamb which did not apply chilled storage before freezing observed the lack of any effect from 8-9 months frozen storage on SF, although at longer durations SF was significantly higher than their fresh (unaged) counterparts (Fernandes *et al.*, 2013; Muela *et al.*, 2015). Neither study, however, was able to associate SF increases with reduced tenderness scores (Fernandes *et al.*, 2013; Muela *et al.*, 2016), Slaughter day variation has been shown to confound SF results due to handling, weather and stress factors (Warner *et al.*, 2010). Display post-thawing (3 to 6 days) resulted in decreased SF (Muela *et al.*, 2015), and a lack of change in sensory tenderness compared to unfrozen meat (Muela *et al.*, 2016). These are thought to be the basis of the observed results in the aforementioned studies. Thawing and storage at chilled temperatures post-thaw could potentially initiate proteolysis and reduce SF (Crouse & Koohmaraie, 1990). The adoption of a protocol of cooking frozen meat without thawing first for SF (Honikel, 1998; Hopkins & Thompson, 2001) and particle size analysis to measure protein degradation (Starkey *et al.*, 2015) could provide insight into the true effect of freezing on the proteolysis and tenderness of meat.

Frozen storage temperatures below -18 °C have been linked to decreased myofibrillar protein solubility (Farouk *et al.*, 2003), while slower freezing

rates or high freezing temperatures above -10 °C may accelerate protein oxidation, measurable as carbonyl content (Xiong, 2000; Huff-Lonergan *et al.*, 2010) and cause excessive damage to muscle cell ultrastructure, decreasing myofibrillar protein solubility (Petrović *et al.*, 1993). Protein oxidation correlates with tougher meat due to the formation of cross-linkages (Xia *et al.*, 2009) inhibiting proteolysis due to the inactivation of calpain-1 (Rowe *et al.*, 2004). Another study associated lamb frozen at slower rates with decreased sensory quality (Muela *et al.*, 2012). Protein solubility and carbonyl content measurements can explain variations in SF and sensory tenderness associated with frozen storage, particularly when SF results are unexpected (Estévez, 2011).

1.3.2. Fluid losses and juiciness

The process of frozen storage generally results in diminished WHC compared to fresh meat and chilled-stored meat due to liquid losses upon thawing (Añón & Calvelo, 1980; Winger & Hagyard, 1994; Vieira *et al.*, 2009; Kim *et al.*, 2015) resulting from disruption to muscle fibres during the freezing process (Rahelić *et al.*, 1985). WHC has also decreased concurrently with frozen storage duration due to thawing losses (Farouk *et al.*, 2003; Vieira *et al*., 2009; Muela *et al.*, 2010; Fernandes *et al.*, 2013). Rapid freezing has been reported to result in more, smaller and intracellular ice crystals, which therefore contribute to reduced thaw losses (Petrović *et al.*, 1993; Zhou *et al.*, 2010), although frozen storage temperatures have shown little to no effect on WHC (Farouk *et al.*, 2003).

Despite the effects on WHC, few studies have reported a reduction in sensory juiciness due to the process of frozen storage (Lagerstedt *et al.*, 2008; Bueno *et al.*, 2013), thus highlighting the lack of support for WHC as an indicator for juiciness (Winger & Hagyard, 1994). Meanwhile, alterations in a_w and consequent losses of the odorant furaneol due to freezing have been mentioned to potentially reduce juiciness (Bueno *et al.*, 2013), although other studies have found little to no effect of the freezing process, rate, duration or storage temperature on juiciness (Vieira *et al.*, 2009; Muela *et al.*, 2012).

1.3.3. Flavour

The effects of frozen storage on flavour parameters is not entirely clear – this is supposed to be due to a lack of flavour detection measures for frozen meat (Leygonie *et al.*, 2012). This paucity was investigated for frozen lamb, and in spite of the lack of storage effects on sensory flavour, the absence of furaneol was found to be a marker of frozen-stored meat (Bueno *et al.*, 2013). The general consensus is that if meat is frozen at or below -15 °C, thawed and cooked immediately upon thawing, its flavour will be acceptable regardless of frozen storage duration (Winger, 1984; see also Table 1.2). In fact, lamb frozen for 15 months could be displayed for 3 days post-thawing and still be considered acceptable in sensory quality; however this was not true for lamb kept frozen for 21 months (Muela *et al.*, 2016). Frozen storage temperature posed a greater effect on sensory flavour than storage duration (Hagyard *et al.*, 1993), even though a prior study found lamb to be of acceptable quality following 2 years frozen storage at -10 °C (Winger, 1984), although these results could have been affected by

variations in temperature, chilled storage prior to freezing and sensory panel protocol and demographics. These factors and lack of reported methodology from earlier sensory panels restricts the interpretation of these results.

Following nine months frozen storage, TBARS levels peaked (Muela *et al.*, 2010) and then continued to increase post-thawing when on retail display over 6 days (Muela *et al.*, 2015). Despite these high levels of MDA, an oxidised perception of flavour was not detected in corresponding sensory panel studies following these durations (Muela *et al.*, 2012; 2016). Decreases in TBARS following longer frozen storage and retail display durations can be explained by the formation of secondary products of lipid oxidation (Leygonie *et al.*, 2012; Muela *et al.*, 2015), particularly as consumer acceptability decreases with frozen storage duration (Muela *et al.*, 2016). Further investigation into these secondary products comprising aldehydes, ketones and hydrocarbons (Bueno *et al.*, 2013; Khan *et al.*, 2015), along with a re-examination of the TBARS rancidity threshold, are necessary for a more complete understanding of frozen storage effects on meat flavour.

1.3.4. Colour

Freezing has proven to affect colour stability of beef – this is evident in the literature and is manifested as a decrease in instrumental colour parameters (L*, a*, b*) as frozen storage durations increase (Table 1.2; Farouk & Swan, 1998; Ben Abdallah *et al.*, 1999; Vieira *et al.*, 2009). Meanwhile, lamb stored frozen up to 6 months exhibited increased a* and b*, though when placed under retail display both values decreased and L*

increased, and negative effects on a* and b* were more severe when frozen storage duration was greater (Muela *et al.*, 2010; Muela *et al.*, 2015). The basis for this colour deterioration could be the inherent muscle fibre damage contributions and thus, thaw losses and ultimate leaching of MMb reducing enzymes (NADH cytochrome and β-hydroxyacyl CoA-dehydrogenase). This is supported by results which demonstrated that MMb is greater in frozen meat compared to fresh (unaged) meat (Farouk & Swan, 1998) or beef aged for 9-11 days at 5 °C (Ben Abdallah *et al.*, 1999). The effect on L* was noteworthy when freezing rates were faster due to the reduced thaw losses and decreased light reflectance potential (Farouk *et al.*, 2003; Muela *et al.*, 2010). Similarly, decreases in a* were apparent in both beef and lamb with display duration (Table 1.2), likely because of the increased susceptibility of Mb to oxidation as a result of the globin moiety denaturation due to freezing (Calvelo, 1981). Bloom time and colour measurement schedule can influence CIE values and these are not always reported in literature (Tapp *et al.*, 2011), which, along with temperature of the meat at measurement (Honikel, 1998), can compromise any interpretation of reported effects of frozen storage on instrumental colour.

Long-term frozen storage duration can produce varied effects on colour parameters. For instance, an increase in beef L* was reported following 6-12 months frozen storage compared to fresh meat or meat frozen for 3 months and this was thought to be due to increased protein denaturation, or even lipid oxidation, at longer storage durations (Farouk *et al.*, 2003). Although the CIE values were not tested across retail display in this study, little change in L* was found in lamb following long-term frozen storage

(Fernandes *et al.*, 2013) and when followed by 6 days retail display (Muela *et al.*, 2015), although both studies reported increased b*, but not a* on day 0 when frozen storage duration exceeded 9 months. The ramifications of this are highlighted by research that identified a positive relationship between consumer acceptability of beef colour and L* values (Holman *et al.*, 2016b), whereas in lamb this association with consumer acceptance was not evident (Khliji *et al.*, 2010). Following 6 days display of frozen-thawed lamb, b* increased and a* decreased (Muela *et al.*, 2015); this can be related to similar increases in TBARS with display duration and the lipid oxidation level being associated with yellowness of the red meat via contributions to Mb oxidation (Faustman *et al.*, 2010; Estévez, 2011).

1.3.5. Microbial profile

Generally, frozen storage (below -5 °C) inhibits microbial proliferation (Adam *et al.*, 2010), although similarly to what is observed for protease enzymes, microbial activity will resume and often accelerate upon thawing (Lowry & Gill, 1985; Löndahl & Nilaaon, 1993; Vieira *et al.*, 2009). A reason for this is the decrease in a_w during frozen storage (Nollet, 2012). Increases to a_w upon thawing and, consequently, values above 0.94 can result in the proliferation of spoilage microbes (Egan *et al.*, 1988; Nollet, 2012). In terms of storage duration, one study on beef (Hinton *et al.*, 1998) and two on lamb (Winger, 1984; Fernandes *et al.*, 2013) reported that meat could be stored frozen for one year and remain safe (unspoiled) based on domestically and internationally recognised bacterial enumeration thresholds.

Despite these findings, it is apparent that there are a lack of studies investigating microbial profiles and a_w of frozen meat following retail display, although reports exist of more rapid spoilage of frozen-thawed meat compared to fresh meat (Vieira *et al.*, 2009), due to the reactivation of microbial activity upon thawing coupled with cellular damage resulting from frozen storage (Farouk *et al.*, 2003; Leygonie *et al.*, 2012). Also, frozen storage temperatures have not been tested for their effects on bacterial proliferation potential and a_w, despite -18 °C being reported and applied as the maximum frozen storage temperature for growth inhibition (Ben Abdallah *et al.*, 1999; Fernandes *et al.*, 2013; Eustace *et al.*, 2014).

Table 1.2. Summary of the effects of frozen storage on the shear force, tenderness, fluid levels, flavour and colour of beef and lamb.

Species	Duration	Temperature	Effects on selected traits	Source
			Shear force and tenderness	
Lamb	42 days	-20°C	SF improved up to 8 days and maintained in callipyge lamb (59 to 34 N)	Duckett *et al.* (1998b)
Beef	12 months	-18 to -75°C	SF decreased when stored frozen (particularly 9 months – 70 to 57 N)	Farouk *et al.* (2003)
Beef	90 days	-20 to -80°C	SF decreased after 75 days storage (73 to 66 N), no effect on sensory	Vieira *et al.* (2009)
Lamb	6 months	-18°C	No difference in preference or tenderness between fresh and frozen meat	Muela *et al.* (2012)
Lamb	10 months	-18°C	No effect on tenderness (3.1 to 2.7)	Bueno *et al.* (2013)
Lamb	12 months	-18°C	No effect on tenderness after 8 months – SF increased between 4 and 12 months (48 to 78 N)	Fernandes *et al.* (2013)
Lamb	21 months	-18°C	No difference in preference between fresh and frozen meat Storage beyond 9 months increased SF (68 to 91 N)	Muela *et al.* (2015)
Lamb	21 months	-18°C	Increased storage duration to 9 months improved tenderness (2.9 to 3.5)	Muela *et al.* (2016)
			Fluids	
Lamb	12 months	-18°C	Purge loss increased between 20 and 42 days (9.2 to 12.9%), no effects on cook loss or juiciness	Duckett *et al.* (1998b)
Beef	42 days	-20°C	Decreased WHC over 12 months storage (69.9 to 59.1%)	Farouk *et al.* (2003)
Beef	12 months	-18 to -75°C	Decreased WHC over 90 days storage (64.1 to 51.1%), no effect on juiciness	Vieira *et al.* (2009)
Lamb	90 days	-20 to -80°C	1 and 10 months storage decreased juiciness (2.9 to 2.3)	Bueno *et al.* (2013)
Lamb	10 months	-18°C	Increased cook loss until 8 months storage (13 to 26%)	Fernandes *et al.* (2013)
			Flavour	
Lamb	1 year	-15 to -35°C	No rancid flavours detected following up to 1 year storage	Hagyard *et al.* (1993)
Lamb	42 days	-20°C	No effect on flavour	Duckett *et al.* (1998b)
Beef	90 days	-20 to -80°C	No effect on flavour	Vieira *et al.* (2009)
Lamb	10 months	-18°C	No effect on flavour	Bueno *et al.* (2013)
Lamb	21 months	-18°C	TBARS below 0.2 for more than 21 months if meat not displayed	Muela *et al.* (2015)
			Colour	

Beef	3 days	-18°C	Increased MMb (24.8% compared to 3 days chilled) due to thaw losses	Ben Abdallah *et al.* (1999)
Beef	90 days	-20 to -80°C	All CIE parameters (L*, a*, b*) decreased with storage duration	Vieira *et al.* (2009)
Lamb	6 months	-18°C	Increased b* and decreased a* with display due to freezing	Muela *et al.* (2010)
Lamb	12 months	-18°C	Rapid discolouration (a*) following storage durations of 9 months or more	Muela *et al.* (2015)
Lamb	12 months	-18°C	Frozen meat appeared more discoloured at all times – particularly after 9 months	Muela *et al.* (2016)

All sensory panel scores for different traits given on a scale from 0 to 5. All shear force values converted to newtons (N) by the equation 1 kgF = 9.80665 N. SF = shear force; WHC = water holding capacity; TBARS = 2-thiobarbituric reactive substances (mg malondialdehyde per kg meat); MMb = metmyoglobin; a* = colorimetric measure of redness.

1.4. Effects of combining chilled and frozen storage on meat quality and spoilage parameters

To date, the majority of studies combining chilled and frozen storage have investigated either altered storage temperatures (Hagyard *et al.*, 1993; Bell & Garout, 1994) or frozen-then-chilled storage (Crouse & Koohmaraie, 1990; Duckett *et al.,* 1998a; Duckett *et al.*, 1998b; Muela *et al.*, 2015). By contrast, few studies have examined the effects of chilled-then-frozen storage – being the process which benefits from the advantages of chilled storage (Table 1.1) which are then preserved using frozen storage until utilisation (Table 1.2). Common to these studies were the comparisons between chilled only storage treatments followed by frozen storage (Shanks *et al.*, 2002; Lagerstedt *et al.*, 2008) or the use of total storage durations that were less than four months (Vieira *et al.*, 2009; Kim *et al.*, 2011; Kim *et al.*, 2013; Kim *et al.*, 2015; Choe *et al.*, 2016). From these, it is possible that several studies which have examined frozen storage for much longer durations (Table 1.2) may not have gained the maximum benefits of chilled storage – for example, using storage durations of only 10 days at 4°C for beef (Vieira *et al.*, 2009). Recent studies on lamb have found chilled storage (2-3 weeks at -1.5 °C)-then-frozen storage (6-7 weeks) to yield similar quality to 9 weeks chilled storage without the associated increases in oxidation and to be superior to 9 weeks frozen storage (Kim *et al.*, 2011; 2013). This highlights the knowledge gap identified in this review, namely whether chilled-then-frozen storage can be extended beyond its current parameters (Table 1.3) with minimal negative effects on quality or spoilage. In particular, the extension of the frozen storage component is warranted

given the general lack of detrimental effects resulting from frozen storage duration (Table 1.2) based on consumer and instrumental quality thresholds.

1.4.1. Tenderness

The effects of both chilled-then-frozen and frozen-then-chilled storage treatments on SF and sensory tenderness are summarised in Table 1.3. The use of frozen-then-chilled storage promoted a greater effect on SF in beef compared to beef stored chilled only for the same period (Crouse & Koohmaraie, 1990). Interestingly for lamb, this phenomenon only occurred when the callipyge gene was present (Duckett *et al.*, 1998a, b). Prolonging frozen storage duration between 9 and 21 months was found to result in significant SF decreases in lamb (Muela *et al.*, 2015), as well as improved sensory tenderness (Muela *et al.*, 2016) when these parameters were measured following 4-6 days chilled storage in modified atmospheric packaging post-thaw. This did not occur in frozen-only (not chilled prior) meat, in fact SF increased (Muela *et al.*, 2015). This could have resulted from a reduction in calpastatin activity due to frozen storage and a corresponding increase in proteolysis rate via calpains during chilled storage post-freezing (Crouse & Koohmaraie, 1990; Dransfield, 1994; Duckett *et al.*, 1998a, b), and this effect is reflected in SF results comparing chilled and frozen storage combinations (Kim *et al.*, 2011; 2013) regardless of whether frozen or chilled storage was applied before the other (Table 1.3).

Previously, frozen storage has been suggested as a method for preserving tenderness following chilled storage, although studies often did not report frozen storage duration or any effects of the frozen storage

(Dransfield, 1994). Shanks *et al.* (2002) investigated chilled-then-frozen storage in beef and reported decreased SF to arise from chilled-then-frozen storage compared to chilled storage alone, albeit when the chilled storage durations were less than 3 weeks at 2 °C. These results suggest that frozen storage may have improved tenderness due to its physical disruption to muscle cells via intracellular ice crystal formation, however these effects may have been confounded by the frozen meat being thawed for 24 hours prior to cooking and therefore providing additional chilled storage (Shanks *et al.*, 2002) and thus opportunity for proteolysis and thus tenderisation. Instead, the meat should have been cooked from frozen to overcome this potential confounding effect (Honikel 1998). Similar results were found for thawing prior to cooking when chilled storage (4 °C) was 7 days, but not 14 days (Lagerstedt *et al.*, 2008). However, a reduced SF effect resulting from freezing and thawing of beef chilled for 4 weeks (-1.5 °C) was not explained by desmin degradation, instead attributed to structural weakening of myofibrils from the thawing process (Kim *et al.*, 2015).

Lamb stored chilled (3 weeks at -1.5 °C)-then-frozen (6 weeks) had comparable similar SF results to lamb stored chilled only for 9 weeks (Kim *et al.*, 2011). Further research found chilled (2 weeks at -1.5 °C)-then-frozen (7 weeks) storage of lamb to also present comparable results (Kim *et al.*, 2013). Similarly based on SF, Choe *et al.* (2016) found that 2 weeks chilled storage at -1.5 °C could be shortened to 8 days if chilled storage temperatures were increased to 3 or 7 °C; with no SF decrease occurring due to freezing following this duration. Peak tenderness of lamb was attained at 7 to 10 days at 1-4 °C (Dransfield, 1994; Starkey *et al.*, 2015),

consequently, once this is achieved, frozen storage would pose no further effect on tenderisation. Similarly for beef, peak tenderness is reported to occur following 2 to 3 weeks chilled storage at 1-2 °C (Morgan *et al.*, 1991; Dransfield, 1994), meaning that frozen storage following longer chilled storage durations will not reduce SF (Shanks *et al.*, 2002; Farouk *et al.*, 2009a).

These conflicting results suggest that the effect of chilled-then-frozen storage upon proteolysis and SF requires further investigation, similar to that of the work of Starkey *et al.* (2015). Furthermore, results investigating sensory tenderness of chilled-then-frozen meat have been scarce and inconsistent, likely due to consumer panel and cooking method variations (Lagerstedt *et al.*, 2008; Vieira *et al.*, 2009; Wiklund *et al.*, 2009a; Wiklund *et al.*, 2009b). These factors should be investigated in relation to meat stored chilled-then-frozen to provide better understanding and management.

1.4.2. Fluid losses and juiciness

The effects of chilled-then-frozen storage on juiciness and WHC are varied, though there is a general trend toward a reduction in both (Table 1.3). Particular attention is to be paid towards the effect of chilled and frozen storage periods upon cooking losses, as two studies on beef found no effect of frozen storage when it was preceded by chilled storage of 21 days or longer (Shanks *et al.*, 2002; Farouk *et al.*, 2009a). One later study in fact found cook losses from chilled beef (4 weeks) to be decreased by freezing (2 weeks) after the chilled storage period (Kim *et al.*, 2015). When comparing chilled (2-3 weeks)-then-frozen (6-7 weeks) storage of lamb, no

effect on cooking losses was found; instead, rigor temperature and electrical stimulation were mentioned as factors (Kim *et al.*, 2013).

Purge losses are increased by increased durations of both chilled and frozen storage, though more so by frozen storage due to thaw loss (Vieira *et al.*, 2009). Kim *et al.* (2015) found that while freezing and thawing increased purge losses, chilling increased cook losses.

Regarding sensory juiciness, one study found a decrease following chilled (7 days)-then-frozen (24 hours) storage (Lagerstedt *et al.*, 2008), while another did not find effects following chilled (3-10 days)-then-frozen (30-90 days) storage (Vieira *et al.*, 2009). The lack of studies examining sensory juiciness following chilled-then-frozen storage highlights the need for a more comprehensive study.

1.4.3. Flavour

Results from chilled-then-frozen storage studies have reported some negative effects on flavour compared to chilled storage alone. For example, when chilled storage duration of beef prior to freezing is one week consumer flavour scores have decreased (Table 1.3; Lagerstedt *et al.*, 2008; Wiklund *et al.*, 2009b) and when long-term frozen storage temperatures of lamb are above -15 °C before the temperatures are then lessened, increased flavour intensity has been reported (Hagyard *et al.*, 1993). Other studies have reported no effect on flavour of chilled (10 days at 4 °C)-then-frozen (90 days at -20 or -80 °C) beef (Vieira *et al.*, 2009) or frozen (42 days at -20°C)-then-chilled (14 days at 2 °C) lamb (Duckett *et al.*, 1998b). Effects upon TBARS can provide insight into these results as the chilled storage

component (duration and temperature) is the main factor leading to lipid oxidation (Kim *et al.*, 2011), and consequently, flavour perception as rancid (Campo *et al.*, 2006). Excessively high frozen storage temperatures or accidental thawing of frozen meat may increase the oxidative flavour (Hagyard *et al.*, 1993; Bell & Garout, 1994), and along with prior chilled storage likely had greater effect on negative flavour development than any frozen storage duration, and thus decreased meat shelf life (Winger, 1984).

Further studies may also examine accidental freezing of chilled product, or the use of chilled-then-frozen storage, and its effects on flavour, as there have been accidental freezing in chilled export product (Leygonie *et al.*, 2012). Another potential area for further study is the relationship between TBARS and sensory flavour for chilled-then-frozen meat (Table 1.3). Decreases in flavour scores of chilled-then-frozen meat have not always resulted in a lack of consumer acceptability or preference difference between such meat and unfrozen meat (Lagerstedt *et al.*, 2008; Muela *et al.*, 2016).

1.4.4. Colour

Studies reporting on colorimetric measures for meat stored chilled-then-frozen are scarce, and even more so are those investigating colour stability over retail display. Beef stored chilled (4 °C, 10 days)-then-frozen (-20/-80 °C, 90 days) was found to have the majority of its colour deterioration caused by the frozen storage phase rather than the chilled storage phase (Vieira *et al.*, 2009; Kim *et al.*, 2015). Another study meanwhile found that L* and a* increased in beef due to chilled storage (3 weeks at -1.5 °C) prior

to frozen storage (6 weeks) (Farouk *et al.*, 2009b); similar results were found for lamb L* and b* following chilled (2-3 weeks at -1.5 °C)-then frozen (6-7 weeks at -18 °C) storage remaining higher than those for frozen stored (9 weeks at -18 °C) meat (Kim *et al.*, 2011). Chilled (2-4 weeks at -1.5 °C)-then-frozen (5-7 weeks at -18 °C) storage was found to preserve lamb redness (a*) better over 5-8 days display compared to chilled meat (9 weeks at -1.5 °C) (Wiklund *et al.*, 2009a; Kim *et al.*, 2011; 2013). Similar results were found for colour stability, measured by a lower hue angle with display duration compared to chilled only meat, as chilled storage duration depletes metmyoglobin reducing activity and enzyme respiratory activity (Kim *et al.*, 2011; 2013). This was not seen in beef (Kim *et al.*, 2015), although the beef was only chilled for 4 weeks rather than 9 weeks. However, chilled (3-4 weeks)-then-frozen (2 weeks) beef presented a hue angle more similar to that of frozen (2 weeks) compared to chilled beef (4 weeks at -1.5 °C) (Kim *et al.*, 2015). These results are consistent with results from chilled and frozen storage effects upon colour stability, wherein chilled storage increases the potential for lipid and Mb oxidation, although it also improves colour bloom on exposure to oxygen, compared to fresh or frozen meat. The lack of studies reporting on colour stability of chilled-then-frozen meat, however, prompts the need for further investigation.

1.4.5. Microbial profile

Vieira *et al.* (2009) reported that *Enterobacteriaceae sp.* were not detected in beef stored chilled (10 days at 4 °C)-then-frozen (90 days), although psychrotrophic bacteria proliferated regardless of frozen storage temperature (-20°C or -80 °C), though more so during the chilled storage

period. However, all microbial loadings were measured as being below a threshold for frozen beef safety set at 10^5 CFU/cm^2 (Hinton *et al.*, 1998). Increased pH due to chilled storage may have facilitated microbial proliferation (Moore & Gill, 1987; Kim *et al.*, 2011), though no change occurred during the frozen storage period (Kim *et al.*, 2013). Bacterial proliferation is halted in frozen meat provided temperatures remain sufficiently low (Winger, 1984; Bell & Garout, 1994; Eustace *et al.*, 2014).

The microbial profile has not been thoroughly investigated in chilled-then-frozen meat, particularly in relation to long-term frozen storage following chilled storage. This is alarming as it constitutes a potential failure in the primary measure of food safety (Wood *et al.*, 1999), which could undermine the safe export and long-term storage of product, should microbial loading be above safety thresholds (Bell & Garout, 1994; Hinton *et al.*, 1998; Mills *et al.*, 2014). However, previous studies have shown that frozen storage for 2 years or more at -10 °C or below has been ruled safe provided no chilled storage was used prior (Winger, 1984), and that chilled stored lamb (12 weeks below -0.5 °C; Sumner & Jenson, 2011) and beef (24 weeks below -0.5 °C; Small *et al.*, 2012) have also been ruled safe depending on storage temperature. The combination of these findings may provide a basis for the use of safe chilled-then-frozen storage of red meat for export purposes, particularly given the associated benefits on meat quality (Table 1.3), but further study is merited for verification.

Table 1.3. Summary of the effects of combined chilled and frozen storage on the shear force, tenderness, fluid levels, flavour and colour of beef and lamb.

Species	Chill	Freeze	First	Effects on selected traits	Source
Beef	7 days at 2°C	27 days at -30°C	Freezing	SF decreased further when freezing used first (56 to 50 N) Chilling had greater effect on SF (74 to 53 N)	Crouse & Koohmaraie (1990)
Lamb	24 days at 2°C	42 days at -20°C	Freezing	Frozen callipyge lamb had lower SF (38 to 27 N) SF lower after 12 days chilling if callipyge lamb frozen prior Freezing had no effect on normal lamb SF (around 27 N)	Duckett *et al.* (1998a)
Beef	35 days at 2°C	2 months at -16°C	Chilling	When chilling was less than 21 days, SF decreased and cook losses increased (22.3 to 25.2%) due to freezing At 35 days, SF 38 N chilled, 30 N chilled-then-frozen	Shanks *et al.* (2002)
Beef	14 days at 4°C 7 days at 4°C	2 months at -20°C 24 hours at -20°C	Chilling	When chilling was 0-7 days, freezing reduced SF (61 to 51 N) When chilling was 0-7 days, freezing reduced WHC (79.5 to 74.3%) No preference for chilled-then-frozen compared to chilled, though this method decreased tenderness (2.9 to 2.2), juiciness (2.3 to 1.7) and flavour (2.0 to 1.7)	Lagerstedt *et al.* (2008)
Beef	10 days at 4°C	90 days at -20°C/-80°C	Chilling	When chilling was 3 days, freezing improved tenderness (2.8 to 3.4) Similarly SF (73 to 52 N at 3 days; 52 to 47 N at 10 d) Freezing increased cook losses (18.1 to 23.5%) No effect of either on flavour (2.4 to 2.9) or juiciness (2.4 to 2.7) Freezing increased TBARS (0.89 to 1.93 mg MDA/kg) No effect of frozen storage temperature on any parameters	Vieira *et al.* (2009)
Lamb	2-3 weeks at -1.5°C	6-7 weeks at -18°C	Chilling	Chilling decreased SF more than freezing (28 N and 48 N, respectively, after 9 weeks) Chilling increased TBARS more than freezing (all below 0.05 mg MDA/kg)	Kim *et al.* (2011)

Lamb	2-3 weeks at -1.5°C	6-7 weeks at -18°C	Chilling	Chilling decreased SF (50 to 28 N) from 0 to 2 weeks Lack of influence of longer chilled storage (2-9 weeks) on SF Freezing increased drip losses Chilling (2-3 weeks)-then-freezing (6-7 weeks) retained a* better than 9 weeks either chilling or freezing	Kim *et al.* (2013)
Beef	3-4 weeks at -1.5°C	2 weeks at -18°C	Chilling	Chilled-then-frozen storage decreased SF (58 N) compared to chilled only (74 N) or frozen only (93 N) Freezing increased purge losses more so than chilling Longer chilled storage increased cook losses Frozen meat had lower L* and a* values than chilled meat Chilled-then-frozen meat had intermediate L* and a* values Hue angle higher in frozen meat if chilled prior to freezing	Kim *et al.* (2015)
Lamb	6 days at 2-4°C	21 months at -18°C	Freezing	Freezing increased SF after 15 months (66 to 88 N) Decreased SF due to chilling post-thaw (91 to 40 N after 21 months) SF decreased more on display (3-6 days) if freezing duration longer	Muela *et al.* (2015)
Lamb	8 days at 3°C/7°C 14 days at -1.5°C	1 week at -18°C	Chilling	Neither chilling temperature nor freezing affected SF (around 40 N) Water loses unaffected by freezing (6.6 to 7.6%) – though higher chilling temperatures increased combined water losses (7.6 to 10.7%) Cook loss improved by increased chill temperatures (27.4 compared to 31.2%) Freezing post-chill decreased L* and b*, though higher chill temperatures better preserved it over 7 days display Lower chill temperature improved a* over display, though chilled-then-frozen a* was lower than chilled only	Choe *et al.* (2016)
Lamb	4 days at 2-4°C	21 months at -18°C	Freezing	Sensory panel used Acceptable up to and including 15 months frozen storage (3.4 to 3.1)	Muela *et al.* (2016)

				Acceptability lower at 21 months (2.4)

All sensory panel scores for different traits given on a scale from 0 to 5. All shear force values converted to newtons (N) by the equation 1 kgF = 9.80665 N. SF = shear force; WHC = water holding capacity; TBARS = 2-thiobarbituric reactive substances (mg malondialdehyde per kg meat); MMb = metmyoglobin; a* = colorimetric measure of redness.

1.5. Conclusion

From this review, it is clear that there are few studies focusing on combined chilled and frozen storage practices, particularly regarding long-term chilled-then-frozen storage beyond 3-4 months total duration. Effects on quality parameters have mostly related to tenderness, of which encouraging results have been found, indicating the potential benefits of this combined storage approach for export purposes. However, it is apparent that there is a lack of reliable instrumental proxies for measuring other sensory quality traits, namely juiciness and flavour. Additionally, instrumental colour and microbial profile investigations have been scarce in chilled-then-frozen storage studies. As these investigations are highly important for consumer approval and safety, respectively, the need for a large-scale, comprehensive investigation of the quality traits identified herein over long-term chilled-then-frozen storage durations is apparent. Such a study could build on existing knowledge and enhance our understanding of chilled and frozen storage of red meat and develop quality and safety thresholds, assist in the delivery of a high quality meat product between nations and therefore improve global market access of meat, thus addressing the paucities present within this review.

Aims and objectives of this research

As limitations of current research were clearly stated in the literature review, it can be seen that the chilled-then-frozen storage combination poses an alternative to conventional chilled or frozen storage; however, further work is required on extending the storage duration beyond 3-4 months.

The aim of the proposed research was to use chilled storage to improve lamb meat quality in the short term (up to eight weeks), and determine a threshold upon which no further benefits are attained and quality deterioration has begun to occur. Similarly, the proposed research aimed to use frozen storage following chilled storage to allow for preservation of quality over long-term durations up to one year; where again storage thresholds based on consumer-derived quality and safety thresholds can be developed. Shear force (tenderness), display colour, microbial loading, health-claimable fatty acids and lipid oxidation are key traits which have been previously explored in literature and are known to change based on storage. This proposed study provides a comprehensive, large-scale investigation of the meat quality and food safety parameters previously absent from literature. It is within the bounds of this study that this safe, easy, cost-effective practice can be used to preserve export lamb for long periods of time, and can be applied in a similar way to beef meat.

Chapter 2: Effect of chilled-then-frozen storage (up to 52 weeks) on lamb *m. longissimus lumborum* quality and safety parameters (based on publication in Meat Science)

Abstract

This study evaluated the effect of chilled followed by frozen storage on lamb quality and safety parameters. Experimental ($n = 360$) *m. longissimus lumborum* (LL) were randomly sampled from the boning room of a commercial Australian abattoir, at 24 h *post-mortem,* and assigned to five chilled storage periods (0, 2, 4, 6 and 8 weeks) and six subsequent frozen storage periods (0, 4, 8, 12, 24 and 52 weeks). Upon completion of each storage treatment combination, corresponding LL were sub-sectioned and analysed for colour stability (0, 1, 2 and 3 days), shear force, fluid losses (purge, thaw and cooking losses), intramuscular fat content, sarcomere length, water activity and microbial load (lactic acid bacteria, *Enterobacteriaceae sp.*, *Brochothrix thermosphacta*, *Clostridium perfringens* and *Escherichia coli*). LL stored chilled for 2-4 weeks prior to freezing up to one year presented superior results for shear force (below 40 N; $P < 0.001$), display colour (increased a* and R630/580; $P < 0.001$) and low levels of spoilage microbes ($P < 0.001$) compared to longer durations, correlating with good eating quality and safety following more than one year of frozen storage. Furthermore, frozen storage decreased the load of *B. thermosphacta* ($P < 0.001$) and *Enterobacteriaceae sp.* ($P < 0.05$) and slowed the rate of proliferation of lactic acid bacteria.

2.1. Introduction

Chilled or frozen storage are routinely used to preserve lamb meat over extended periods (Zhou *et al.*, 2010; Leygonie *et al.*, 2012; Bellés *et al.*, 2017), however the sequential combination of these approaches may prove a better alternative (Coombs *et al.* (2017a); Chapter 1). For example, past research has found chilled-then-frozen storage resulted in improved tenderness (as shear force, SF), for lamb stored chilled (2-3 weeks)-then-frozen (6-7 weeks) compared to that kept frozen-only for 9 weeks (Kim *et al.*, 2011). Furthermore, chilled-then-frozen lamb had equivalent colour stability to that kept chilled-only for the same duration (Kim *et al.*, 2011). Other studies have examined different chilled storage temperatures and chilling rates prior to storage and observed only limited variation in meat quality across the subsequent relatively short frozen storage period (Kim *et al.*, 2013; Choe *et al.*, 2016). These studies did not examine chilled-then-frozen storage across long-term periods – a practice important to lamb processors who use freezing to smooth supply. Lamb kept under frozen-only storage for such 'long term' periods (9 months to 2 years) resulted in only minimal effects on eating quality (Winger, 1984; Muela *et al.*, 2010; Fernandes *et al.*, 2013; Muela *et al.*, 2015). From this, it may be hypothesised that frozen storage could preserve the improvement in lamb eating quality inferred during the chilled storage period.

In examination of the effects of frozen storage on lamb preservation, frozen storage temperature effects have received minimal attention, though the general consensus was that any temperature below -15 °C would not pose any detrimental effect on meat sensory quality (Hagyard *et al.*, 1993; Farouk *et al.*, 2003; Vieira *et al.*, 2009). One study reported that storing meat at temperatures as high as -10 °C could still result in good quality (Winger, 1984), despite most studies using the internationally recognised temperature of -18 °C (Eustace *et al.*, 2014; Coombs *et al.* (2017a); Chapter 1). Storing meat at warmer frozen storage temperatures, combined with increased frozen storage periods, could allow for bulk shipments of frozen product at slower ship speeds, with additional cost benefit from an increased storage temperature (Sumner, 2016).

Also apparent from Coombs *et al.* (2017a) (Chapter 1) was the relative scarcity of chilled-then-frozen storage studies that considered microbial analyses. This is an important oversight, as specific microbial loadings can advance the spoilage of meat and compromise product safety. Studies which examined microbial profiles of lamb kept under chilled-only (Sumner & Jenson, 2011; Kiermeier *et al.*, 2013) or frozen-only storage periods (Fernandes *et al.*, 2013) reported that microbial proliferation is a function of storage temperature and duration. This is relevant to spoilage and pathogenic bacteria common for anaerobically stored meat, such as; lactic acid bacteria (LAB), *Enterobacteriaceae sp.*, *Brochothrix thermosphacta*, *Clostridium perfringens* and *Escherichia coli* (Borch *et al.*, 1996; Mills *et al.*, 2014).

This study therefore aimed to evaluate the effect of chilled-then-frozen storage combinations (maximum 8 weeks and 52 weeks, respectively) on the quality and safety of lamb, and simultaneously tested the independent effects of chilled- and frozen-only storage, the latter with two frozen storage temperatures (-12 and -18 °C).

2.2. Materials and Methods

2.2.1. Sampling, sectioning and purge loss assessment

On a single day, at 24 h *post-mortem*, a total of 360 lamb *m. longissimus lumborum* (LL) were randomly sampled from the boning room of a commercial Australian abattoir. These were assigned to five chilled storage periods (0, 2, 4, 6 and 8 weeks) and six ensuing frozen storage periods (0, 4, 8, 12, 24 and 52 weeks), set at two frozen storage temperatures (-12 and -18 °C). All LL were individually weighed, vacuum-packaged and stored onsite as per commercial practice for the duration of their assigned chilled storage period (mean temperature 0.6 ± 1.8 °C). LL assigned to frozen storage were frozen onsite using a commercial plate freezer and then transported to the Centre for Red Meat and Sheep Development (NSW Department of Primary Industries, Cowra, New South Wales, Australia) where they were allocated to four freezers set as two replicates per frozen storage temperature. Chilled-then-frozen temperature profiles were recorded by temperature loggers and are presented per treatment combination in Table 2.1.

Table 2.1. Mean (± SD) temperature (°C) of each storage treatment.

	Frozen storage (weeks)				
	0	**4**		**8**	
Chilled storage (weeks)		**-12**	**-18**	**-12**	**-18**
0	1.88	-12.73 ± 3.58	-15.88 ± 3.73	-11.43 ± 0.09	-17.59 ± 0.08
2	1.27 ± 2.40	-12.36 ± 3.01	-15.21 ±3.64	-11.31 ± 0.23	-17.64 ± 0.20
4	0.69 ± 1.89	-11.98 ± 2.63	-15.07 ± 3.62	-11.19 ± 0.25	-17.84 ± 0.28
6	0.29 ± 1.89	-11.77 ± 2.83	-15.02 ± 3.86	-11.35 ± 0.34	-18.00 ± 0.17
8	0.59 ± 1.77	-12.83 ± 3.72	-15.99 ± 3.98	-11.49 ± 0.37	-18.01 ± 0.16

Frozen storage (weeks)					
12		**24**		**52**	
-12	**-18**	**-12**	**-18**	**-12**	**-18**
-11.19 ± 0.25	-17.84 ± 0.28	-10.66 ± 0.64	-17.74 ± 0.39	-12.00 ± 0.27	-18.17 ± 0.32
-11.35 ± 0.34	-18.00 ± 0.17	-10.64 ± 0.60	-17.80 ± 0.44	-12.06 ± 0.21	-18.15 ± 0.32
-10.86 ± 0.72	-17.86 ± 0.49	-10.89 ± 0.67	-17.91 ± 0.42	-12.09 ± 0.18	-18.13 ± 0.33
-10.74 ± 0.64	-17.85 ± 0.51	-11.00 ± 0.75	-17.92 ± 0.41	-12.11 ± 0.17	-18.44 ± 0.09

| -10.50 ± 0.63 | -17.78 ± 0.45 | -11.38 ± 0.73 | -17.94 ± 0.39 | -12.09 ±0.17 | -18.19 ± 0.34 |

Chilled storage preceded frozen storage. Each frozen storage treatment is a continuation from the previous treatment.

Upon completion of each storage treatment (chilled-only, frozen-only or chilled-then-frozen), each corresponding LL was removed from its vacuum packaging; excess moisture removed with a paper towel; weighed; and the change in weight between pre- and post-treatment used to calculate purge loss as a percentage (Honikel, 1998). These LL were then sub-sectioned as per Fig. 2.1, with care taken to maintain status (i.e. frozen LL were not allowed to thaw) and aseptic conditions. Portions assigned for colour stability and shear force testing were immediately tested and all other portions stored frozen at -80 °C until evaluation.
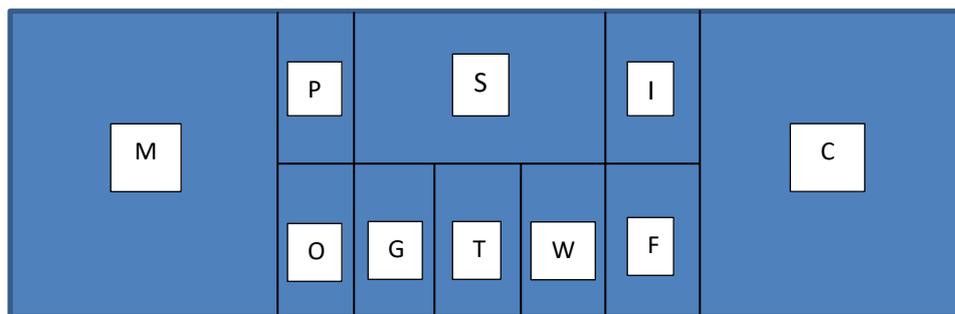
Fig. 2.1. Diagram of experimental lamb LL at sub-sectioning. M: microbial profile (~100 g); P: peroxidase activity; S: shear force (~ 65 g); I: intramuscular fat; C: colour stability; F: fatty acid profile; W: water activity (also includes sarcomere length and ultimate pH); T: TBARS; G: glycogen content; O: oxidation-reduction potential.

2.2.2. Colour stability and thaw loss

Colour stability was evaluated as per Holman *et al.* (2016b), whereby portions used were 3-4 cm thick slices individually placed on Styrofoam trays (13.5 cm x 13.5 cm) so that the muscle fibres were orientated perpendicular to the measured surface. These were then over-wrapped with PVC food film (thickness: 15 µm) and permitted to bloom in

a chiller (mean temperature: 2.8 °C; mean relative humidity: 81.6%) under continuous fluorescent lighting (NEC Tubes 58 W; mean light intensity: 866 lx as per handheld lux meter readings) for approximately 1 h post-slicing prior to the first colour measurement (day 0). This blooming time meant slices from the frozen treatments were thawed before measurement. A spectrophotometer (MiniScan Model 45/0-L, Series No. 7237, HunterLab Associates Laboratory, Inc., Hong Kong, China) with 25 mm aperture size and calibrated using Illuminant D-65 and 10° observer settings calibrated on black and white standard tiles was used to measure reflectance at 400-700 nm and CIE colorimetrics (L*, a* and b*; CIE, 1978). Duplicate measures were recorded with care taken to avoid fat and connective tissue, and this was repeated over the following 3 days (0-3). Hue, chroma and the ratio of absorbance at 630 nm and 580 nm were then calculated as per AMSA (2012).

These same portions were used to determine thaw loss; being the percentage change in weight of each slice between day 0 (frozen) and day 1 (thawed).

2.2.3. Cooking loss and shear force

Sub-sections (mean ± SD: 62.6 ± 9.6 g) for shear force (SF) testing were prepared, weighed and cooked for 35 min in a 71 °C water bath from chilled or frozen depending on whether they had been stored chilled or frozen. Immediately after cooking they were immersed in cold water for 30 min to ensure the cooking process was halted. Cooked samples had excess moisture removed with paper towel and were weighed so that cooking loss

(CL) could be calculated as the percentage weight difference between pre- and post-cooking (Honikel, 1998).

Cooked SF portions were refrigerated overnight (4-5 °C) before being sectioned into six cuboidal strips (cross-sectional area: 1 cm x 1 cm) parallel to muscle fibre direction. These were then cut perpendicular to the fibre direction using a Warner-Bratzler vee-shaped blade with 200 mm/min crosshead speed and attached to a Lloyd texture analyser (Model LRX, Lloyd Instruments, Hampshire, United Kingdom) to measure peak force (in newtons, N). Care was taken to avoid connective and fatty tissue, and SF was calculated as the average of six technical replicates (Holman *et al.*, 2015).

2.2.4. Sarcomere length

Sarcomere length (SL) measurements were taken using thin slices (< 1 mm thickness) parallel to the muscle fibres from each sample. These were analysed using a laser light diffraction unit (5 technical replicates) following the method of Bouton *et al.* (1973).

2.2.5. Ultimate pH

Ultimate pH (pHu) was quantified using a pH meter (smartCHEMC-CP, TPS Pty Ltd., Queensland, Australia) with a polypropylene spear-type gel electrode (IJ-44, Ionode^{TM}, Queensland, Australia) calibrated using pH 4.0 and 6.8 standards (at 20 °C), as the average of duplicate measures as per De Brito *et al.* (2016).

2.2.6. Water activity

Water activity (a_w) was measured using a specialised handheld probe (Model HC2-AW: HygroPalm23-AW, Rotronic Instruments Corp., New York, USA) that capped a 14 mm sample cup containing ~1.0 g still frozen and diced samples. This remained in place for approximately 30 min until a_w stabilised and this and temperature values were then recorded. Each sample was measured as technical duplicates with their results averaged.

2.2.7. Moisture content and intramuscular fat

Moisture content and intramuscular fat (IMF) determinations were made using approximately 40.0 g of diced LL freeze-dried at -50 °C (ScanVac CoolSafe™, LaboGene ApS., Lynge, Denmark) and ground in a FOSS sample mill (Model 1095, KnifeTech™, FOSS Pacific, New South Wales, Australia) as per Hopkins *et al.* (2014). Moisture content was determined as the percentage difference between sample weight pre- and post-freeze drying.

IMF was then calculated using the adapted Soxhlet method (Smith *et al.*, 2017), where 3.0 g freeze-dried sample was extracted with 85.0 mL hexane for 80 min within a Soxtec machine and the residual dried for 30 min at 105 °C and weighed. The difference in weight before and after extraction constituted the IMF, which was then expressed as a percentage of fresh (wet) sample weight.

2.2.8. Glycogen content

Glycogen content was determined using 1.0 g sample incubated with 10.0 mL Milli-Q water in boiling water (100 °C) for 5 min, homogenised at 22, 000 rpm (Series X10/25, Ystral™, Germany) and centrifuged (Model

5810R, Eppendorf Pty. Ltd., Hamburg, Germany) at 2, 465 g and 4 °C for 15 minutes as per De Brito et al. (2016). The supernatant was then compared against a glycogen standard using the colorimetric protocol outlined in the Glycogen Assay Kit (no. MAK016, Sigma-Aldrich Pty. Ltd., Missouri, United States of America) technical bulletin (Sigma-Aldrich, 2016), with absorbance at 570 nm measured using a micro-plate reader (FLUOStar OPTIMA^{TM}, BMG Labtechnologies, Victoria, Australia). Results were the average of technical duplicates and were expressed as mmol per kg fresh (wet) weight of meat.

2.2.9. Microbial loading

The analyses for microbial loading were undertaken at a commercial laboratory where, under aseptic conditions, 10.0 g of each sample was individually homogenised with 90.0 mL 0.1% peptone salt solution for 60 s using a laboratory paddle blender. From these, microbial loads, determined as colony forming units (CFU) per g fresh (wet) meat, were tested as:

Brochothrix thermosphacta samples (0.1 mL) were serially diluted using 0.1% peptone salt solution and spread on duplicate streptomycin-thallous acetate-actidione (STAA) plates which were then incubated for 48 ± 4 h at 22-25 °C. Suspect colonies (shiny, off-white and round) were verified using an oxidase test (oxidase negative) before being counted. Results of technical duplicates were averaged.

Clostridium perfringens samples (1.0 mL) were combined with 10.0-15.0 mL synthetic complete (SC) agar with added supplement on a petri dish, mixed well, and incubated at 44-47 °C in a water bath until

solidification. This was then overlaid with 10.0 mL of the same SC agar and streaked with a reference agar. Control blank plates were also made and evaluated. All plates were then incubated anaerobically for 20 ± 2 h at 37 ± 1 °C before the number of black colonies was counted, with successive dilutions employed when necessary (< 150 colonies).

Escherichia coli samples (1.0 mL) were plated onto the centre of individual *E. coli* petrifilm count bases; upon which the petrifilm tops were then replaced and the sample was spread across the plate. These were allowed to solidify before incubation for 20 ± 2 h at 37 ± 2 °C before the number of blue colonies, with evident bubble formations, was counted.

Enterobacteriaceae sp. samples (1.0 mL) were pour plated with violet red bile glucose (VRBG) agar as the medium; once solidified, these were then overlaid with additional VRBG agar and incubated for 21 ± 3 h at 25-27 °C. Presumptive colonies (verified as dark red or purple with 0.5-2 mm diameter) were counted.

Lactic acid bacteria (LAB) samples were decimally diluted (0.1 mL) and spread on MRS agar to be then incubated for 72 ± 2 h at 30 ± 1 °C under anaerobic conditions – facilitated using a Campygen modified atmosphere gas generator. Typical colonies were counted once verified using both catalase (catalase negative) and Gram stain testing (Gram positive cocci, coccobacilli or rods).

2.2.10. Statistical analysis

To fit smooth non-linear effects of chilled and frozen storage periods, the linear mixed model smoothing spline framework was used as

described by Verbyla *et al.* (1999) and was implemented in the statistical package *asreml* (Butler *et al.*, 2009) under R (R Core Team, 2015). The full model for each response incorporated all the terms required to fit a two dimensional smoothing spline for chilled storage period by frozen storage period, as well as accounting for the experimental design.

Fixed effects included were linear chilling period, linear freezing period, temperature and all interactions; random effects were spline chilled and frozen storage periods, as well as their interactions with linear chilled and frozen storage periods, temperature and each other. Remaining terms in the model accounted for design effects and included non-smooth (factor) effects of chilled and frozen storage period, freezer effects and interactions of freezer with chilled and frozen storage periods. Of the measured traits only data for purge required transformation (square root). Additional covariates were added to the above model as fixed linear effects and a random spline effect; these included pHu on all traits, SL on SF, and glycogen content on microbial loading.

For microbial data, random data was imputed as data above or below the upper or lower limits of detection, which was generated as random uniform data on a logarithmic ($\log_{10}$) scale. In this case, the imputed data was given a reduced weight of 0.5 in the analysis.

Following the approach in Verbyla *et al.* (1999), the statistical significance of fixed and random effects were examined separately in *asreml* (Butler *et al.*, 2009) in R (R Core Team, 2015), with a Wald-type test with Kenward-Roger adjustment (Kenward & Roger, 1997) and

likelihood ratio comparisons used to examine significance of temperature-related and spline components, respectively. The level of significance was set at $P < 0.05$.

2.3. Results

2.3.1. Fluid losses

Purge losses of frozen-only and chilled-then-frozen samples were lower than those of chilled-only samples ($P < 0.001$; Fig. 2.2). Within chilled-only and chilled-then-frozen samples, purge losses increased with increasing chilled storage period ($P < 0.001$; Fig. 2.2), though this tended to decrease within chilled-then-frozen samples as the frozen storage period increased ($P = 0.086$; Fig. 2.2). No treatment effects were observed for thaw losses ($P > 0.05$), although it is worth noting that only LL exposed to frozen storage periods exceeding 0 weeks were tested for thaw loss assessment. Cooking loss increased with corresponding increases in chilled-only storage period ($P = 0.013$), with this effect also evident across chilled-then-frozen storage periods wherein frozen-only LL demonstrated no variation in cooking loss regardless of period ($P > 0.05$). Freezer temperature and interaction effects were not significant ($P > 0.05$).
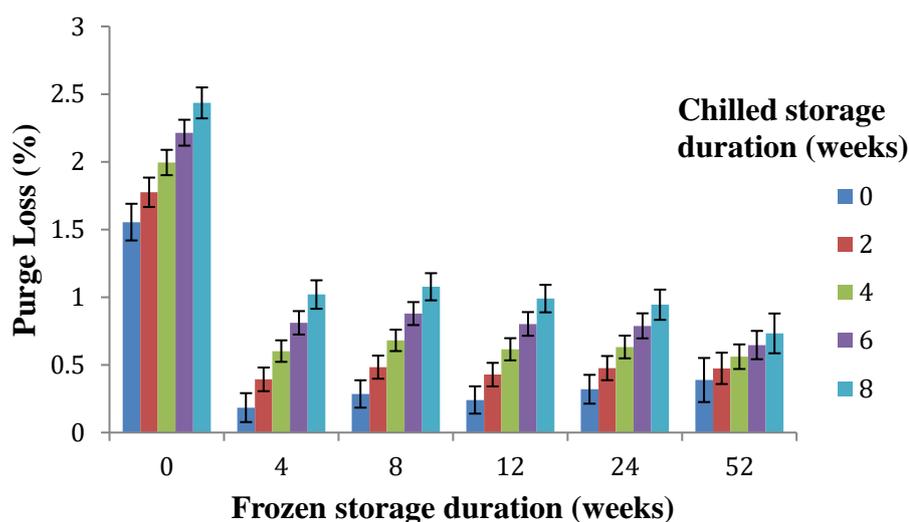
Fig. 2.2. Predicted mean (± SE) purge loss (square root transformed) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. For frozen storage, purge losses were measured on frozen LL without thawing.

2.3.2. Colour stability

Chilled-only storage resulted in decreased colour stability over the display period (3 d) for all CIE traits except b* ($P < 0.05$; Fig. 2.5). The variation and the difference due to chilled-only storage (frozen = 0 weeks) between L* ($P = 0.046$; Fig. 2.3) and hue angle ($P = 0.006$; Fig. 2.8) on day 0 and L* ($P < 0.001$; Fig. 2.3), a* ($P < 0.001$; Fig. 2.4), R630/580 ($P < 0.001$; Fig. 2.6), chroma ($P = 0.003$; Fig. 2.7) and hue angle ($P < 0.001$; Fig. 2.8) on day 3 are illustrated. L* was not influenced by frozen storage period, whether frozen-only or in combination with chilled (chilled-then-frozen; $P > 0.05$; Fig. 2.3), unlike a*, b*, R630/580 and chroma ($P < 0.05$), which all decreased as frozen storage period increased (Figs. 2.4-2.7), or hue angle

80

which increased ($P < 0.01$; Fig. 2.8). This shift was especially obvious following 52 weeks frozen storage (whether frozen-only or chilled-then-frozen). The longer periods of frozen storage (24 and 52 weeks) led to the greatest decline in colour stability and for traits like a* and R630/580 there was a notable effect of freezing compared to chilled meat (Fig. 2.4; Fig. 2.6). No chilled-then-frozen storage period combination or frozen storage temperature effects were observed ($P > 0.05$).
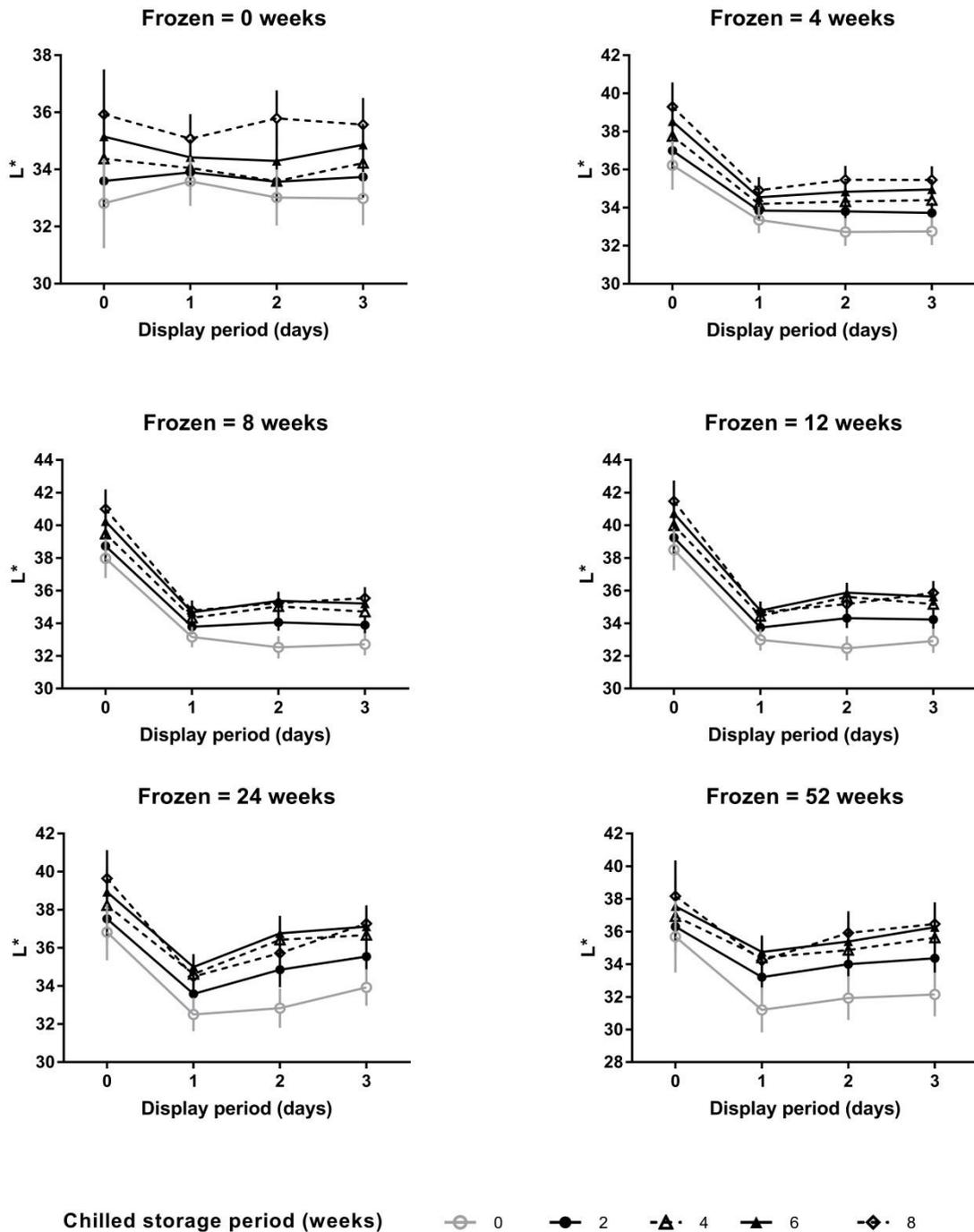
Fig. 2.3. Predicted mean (± SE) lightness score (CIE L*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
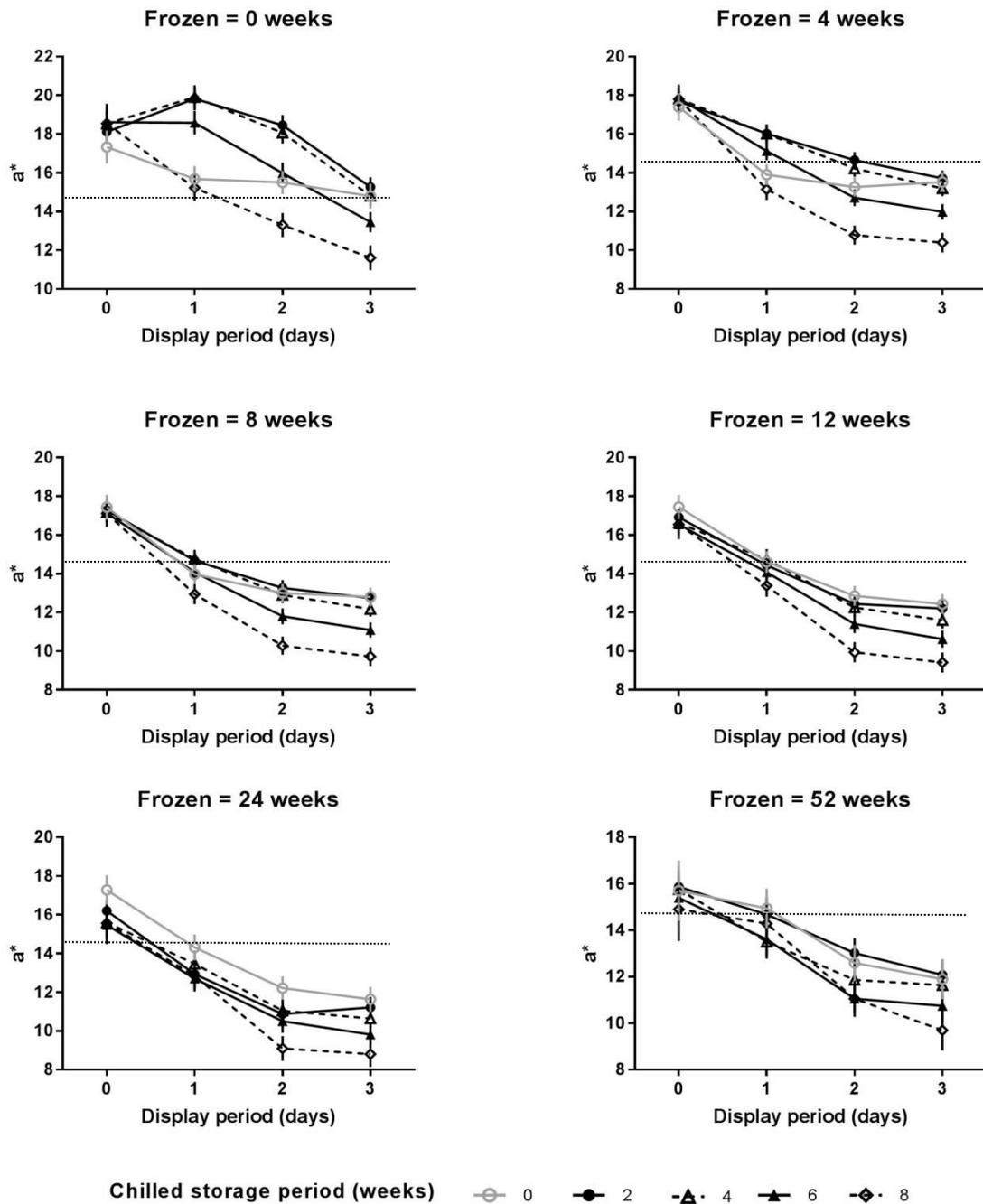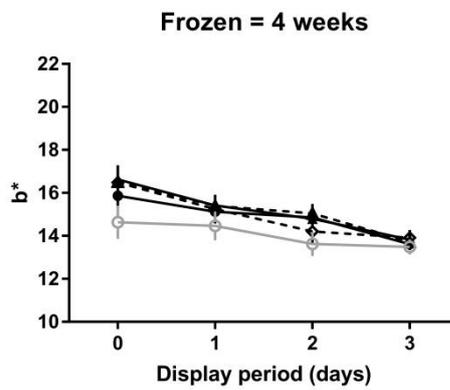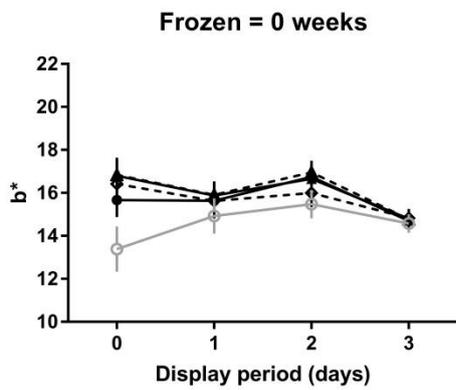
Fig. 2.4. Predicted mean (± SE) redness score (CIE a*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line. The dotted horizontal lines indicate the minimum a* threshold for 50% consumer acceptability (Khliji *et al.*, 2010), with any data below the line indicative of unacceptability.

Frozen = 0 weeks

Frozen = 4 weeks

Frozen = 8 weeks

Frozen = 12 weeks

Frozen = 24 weeks

Frozen = 52 weeks
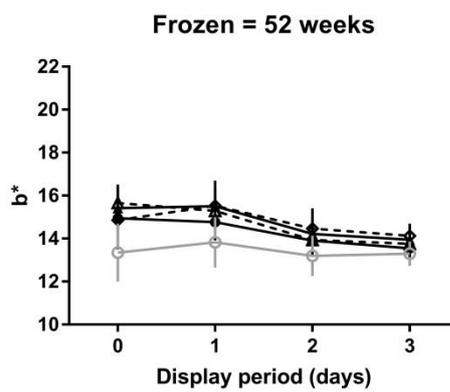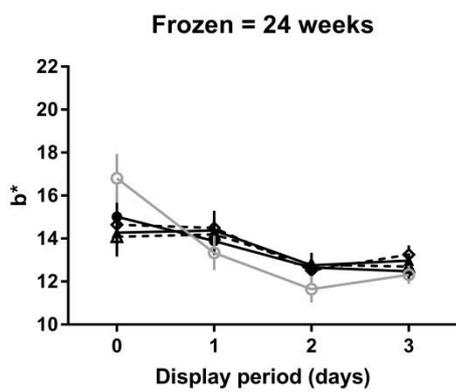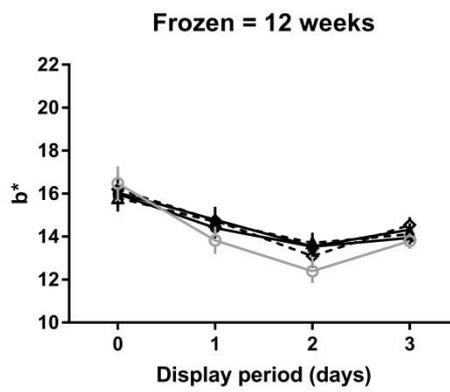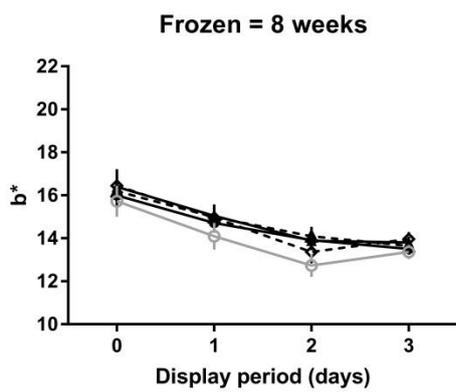
Chilled storage period (weeks) ⊝ 0 ● 2 ▵ 4 ▲ 6 ◇ 8

Fig. 2.5. Predicted mean (± SE) yellowness score (CIE b*) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
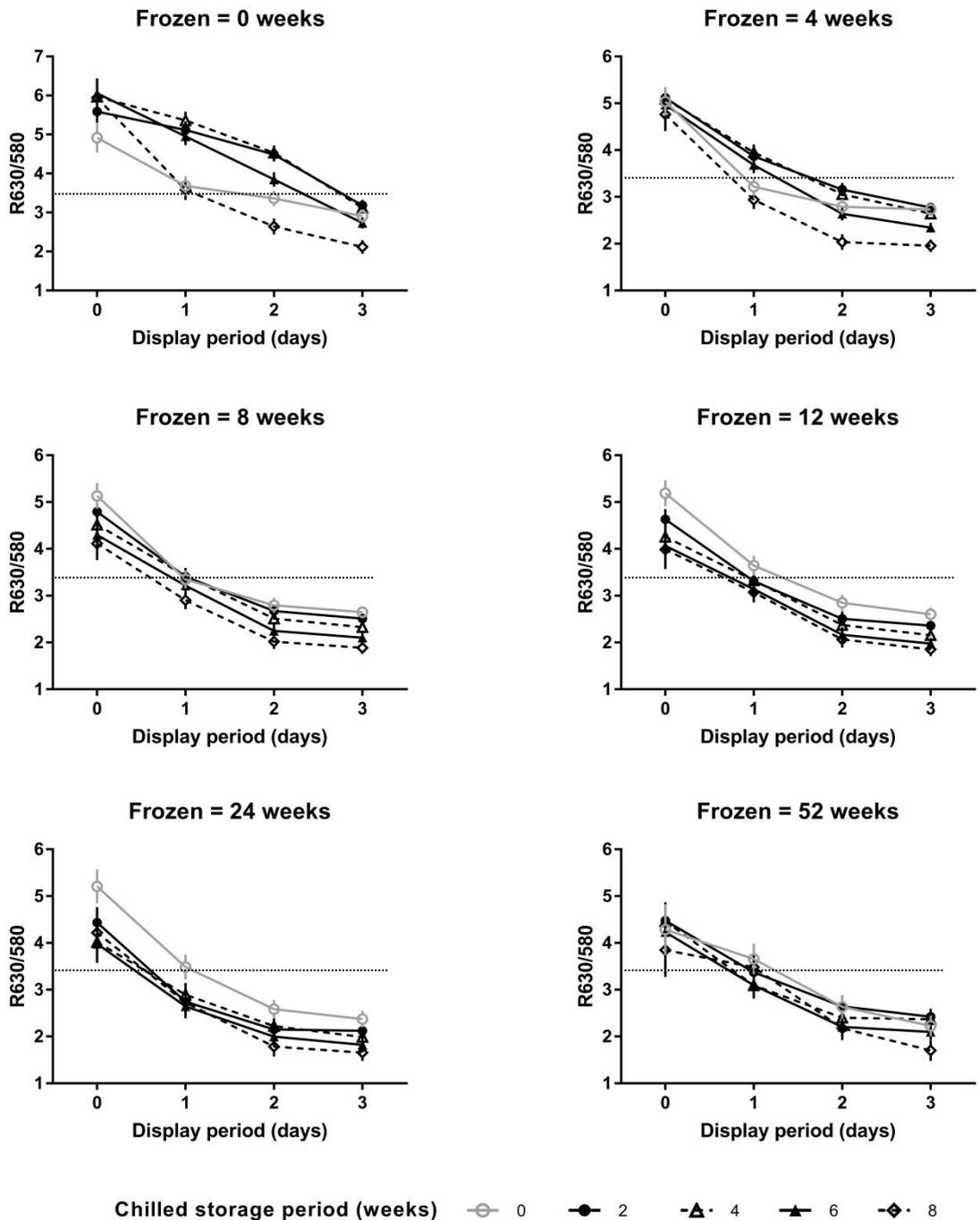

Fig. 2.6. Predicted mean (± SE) reflectance ratio (R630/580) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature

averaged. Frozen-only data has been included as a grey line. The dotted horizontal lines indicate the minimum R630/580 threshold for 50% consumer acceptability (Khliji *et al.*, 2010).
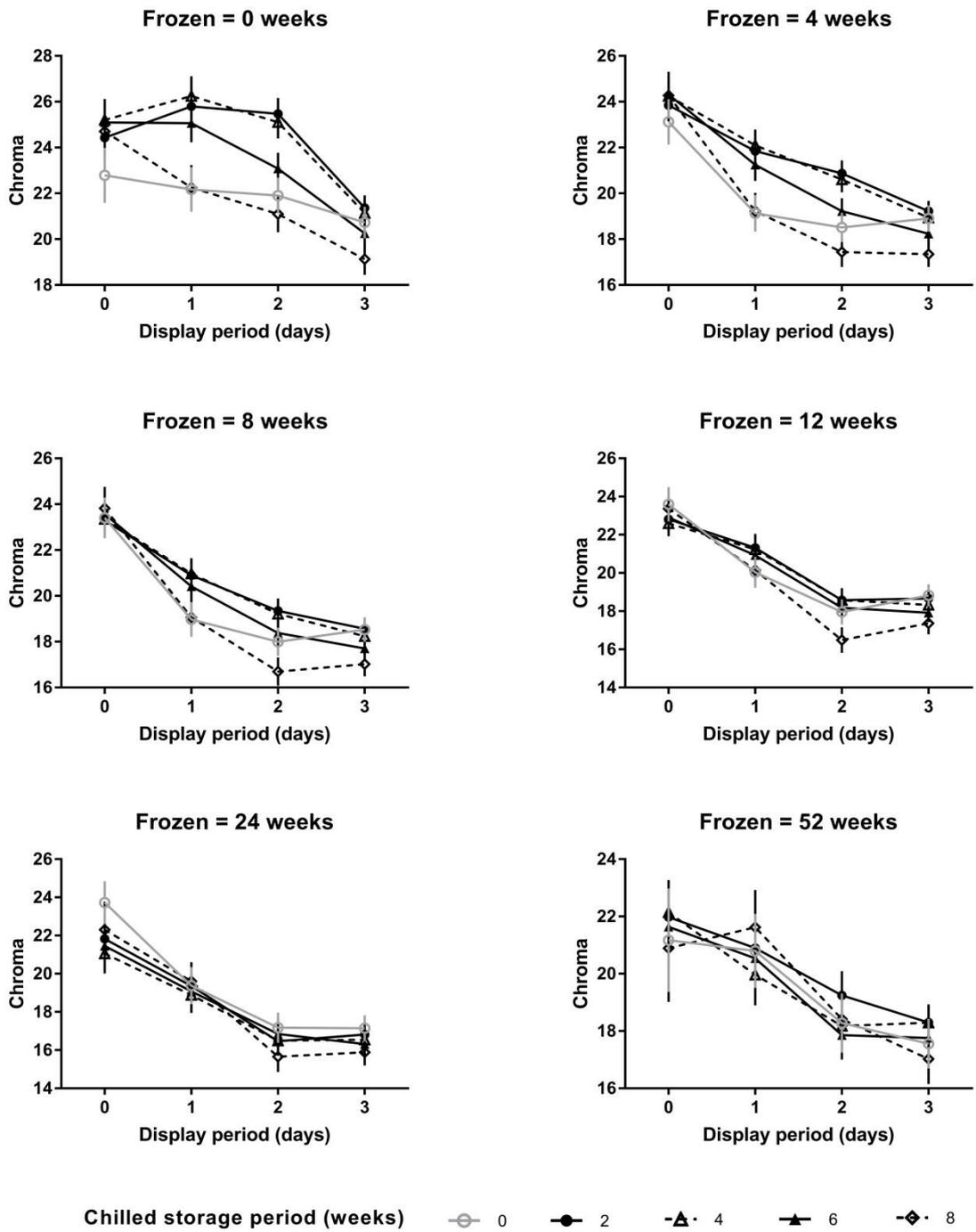
Fig. 2.7. Predicted mean (± SE) colour intensity (chroma) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.
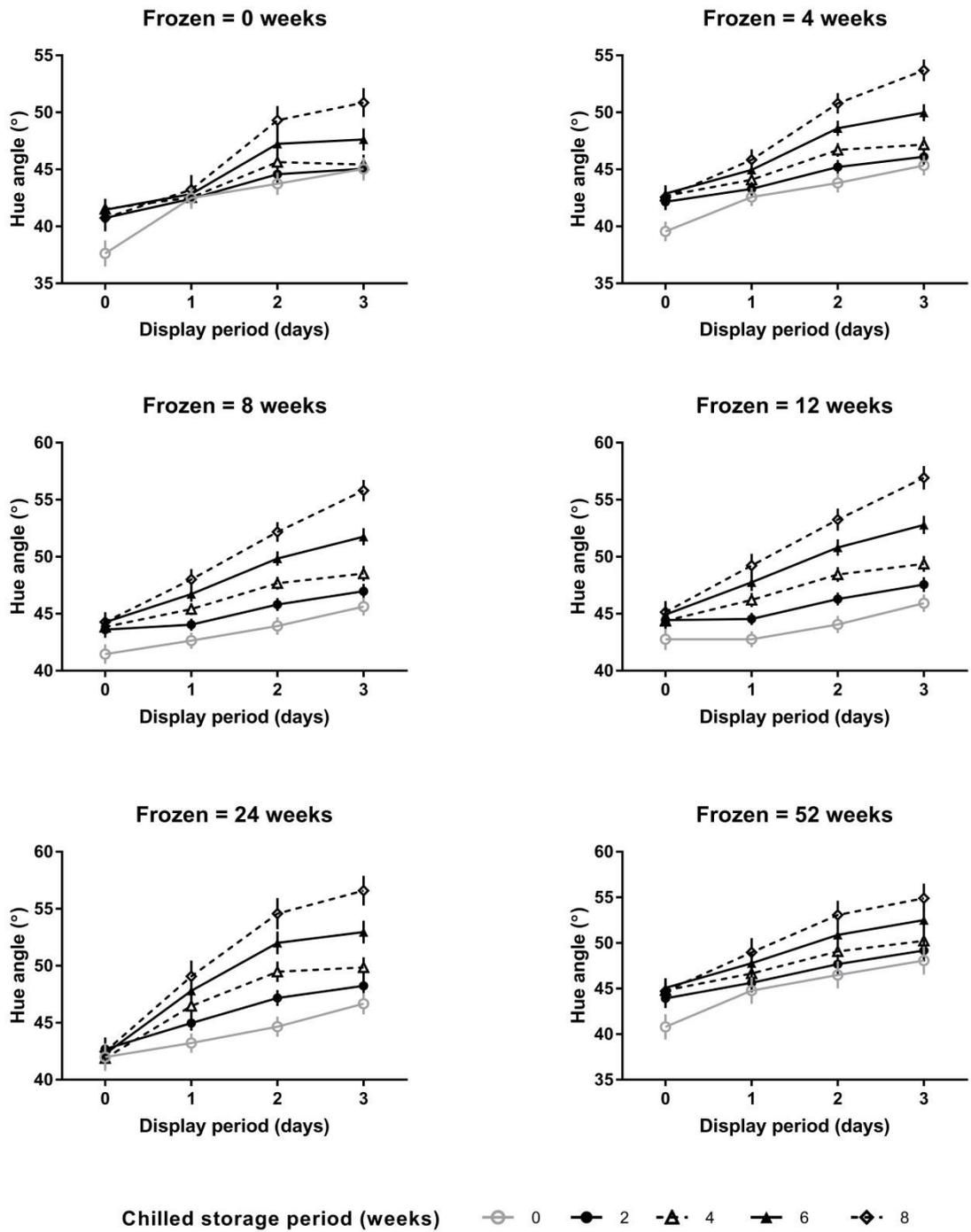
Fig. 2.8. Predicted mean (± SE) hue angle of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

2.3.3. Shear force

Shear force was reduced with increased chilled-only ($P < 0.001$) and frozen-only ($P = 0.028$) storage. There was a noticeable decrease in SF after only 2 weeks of chilled storage, and the relationship between storage period and SF is non-linear (Fig. 2.9) for both chilled and frozen storage, where SF declines were evident between 0 and 8 weeks frozen storage before levelling out at longer storage periods ($P > 0.05$). Chilled-then-frozen storage period combination, pHu and frozen storage temperature did not have any observed effect on SF ($P > 0.05$). There was also no effect ($P > 0.05$) of SL on SF.
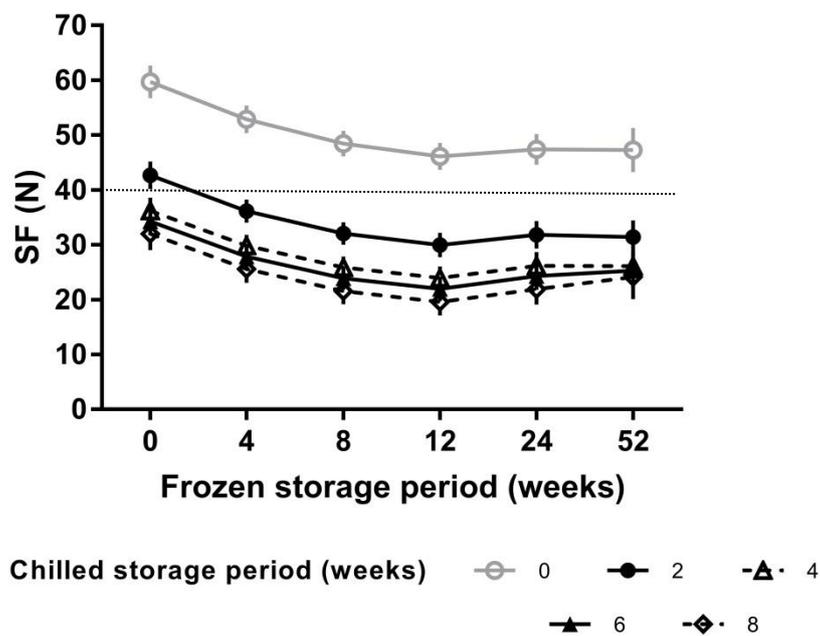

Fig. 2.9. Predicted mean (± SE) shear force (SF) of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been

included as a grey line. The horizontal dotted line indicates the maximum SF threshold for 50% consumer acceptability (Hopkins *et al.*, 2006).

2.3.4 Sarcomere length

No storage type, period or temperature effects were observed for SL (mean ± standard error (SE): 1.71 ± 0.03 µm; $P > 0.05$).

2.3.5. Ultimate pH

No storage type, period or temperature effects were observed for pHu (mean ± SE: 5.70 ± 0.04; range: 5.30 – 6.57; $P < 0.05$). However, pHu was found to exhibit a significant negative relationship with glycogen content, SL, fluid losses and colour on day 0 ($P < 0.001$) and a positive relationship with a* and R630/580 on days 1-3 and chroma on day 1-2 ($P < 0.05$), but this did not change the significance of the fixed terms in the model (e.g. storage type, storage period). The coefficients for pHu effects upon these traits are shown in Table 2.2.

Table 2.2. Interaction of ultimate pH (pHu) with selected meat quality traits. Traits were considered for this table if they were significantly affected by

pHu ($P < 0.05$).

Trait	Coefficient (± SE)	*P*-value
Glycogen	-26.6 (6.65)	< 0.001
SL	-0.14 (0.04)	< 0.001
TL	-6.96 (1.08)	< 0.001
PL	-0.53 (0.12)	< 0.001
CL	-6.78 (1.00)	< 0.001
L* (days 0, 1, 2, 3)	-4.08 (1.47); -5.90 (0.99); -5.66 (0.94); -5.73 (0.91)	0.006; < 0.001; < 0.001; < 0.001
a* (days 0, 1, 2, 3)	-2.20 (0.84); 3.57 (0.55); 4.16 (0.58); 2.61 (0.70)	0.010; < 0.001; 0.003; < 0.001
b* (days 0, 1, 2, 3)	-3.25 (0.78); -0.83 (0.62); -1.43 (0.54); -1.92 (0.44)	< 0.001; 0.208; 0.045; < 0.001
R630/580 (days 0, 1, 2, 3)	0.15 (0.36); 2.83 (0.30); 2.18 (0.22); 1.61 (0.15)	0.681; < 0.001; < 0.001; < 0.001
Chroma (days 0, 1, 2, 3)	-3.65 (1.09); 1.78 (0.68); 1.82 (0.65); -0.01 (0.82)	< 0.001; 0.009; 0.006; 0.995
Hue (days 0, 1, 2, 3)	-0.04 (0.02); -0.13 (0.02); -0.18 (0.02); -0.19 (0.02)	0.041; < 0.001; < 0.001; < 0.001

SL: sarcomere length; TL: thaw loss; PL: purge loss; CL: cooking loss.

2.3.6 Water activity

No effects of chilled or frozen storage period or temperature were observed for a_w (mean $\pm$ SE: 0.94 ± 0.01; $P > 0.05$).

2.3.7 Moisture and intramuscular fat contents

Moisture content (MC) and IMF tended to decrease ($P = 0.090$) and increase ($P = 0.052$), respectively, with increased chilled-only storage period (Table 2.3). This chilled storage period effect was more noticeable in both MC and IMF when LL were frozen for 52 weeks (Table 2.3). Reflecting this trend, moisture content decreased as frozen-only storage period increased ($P < 0.001$), unlike IMF which did not vary ($P > 0.05$) (Table 2.3). IMF did increase due to chilled-then-frozen storage interactions with frozen storage temperature ($P = 0.013$), with IMF higher at -18 °C at shorter chilled-then-frozen periods than -12 °C counterparts, which, in turn, exhibited higher IMF at longer storage periods.

Table 2.3. Predicted means (± SE) for the effect of chilled-then-frozen storage on the proportions of moisture content (MC) and intramuscular fat (IMF). Both are expressed as percentages.

Chilled Storage (weeks)		Frozen Storage (weeks)					
		0	4	8	12	24	52
0	*MC*	53.3 (0.8)[a]	52.8 (0.7)[ab]	52.4 (0.6)[ab]	52.0 (0.7)[b]	51.0 (0.7)[bc]	50.0 (1.3)[c,]
	IMF	4.80 (0.3)	4.78 (0.3)	4.77 (0.3)	4.75 (0.2)	4.69 (0.2)	4.56 (0.5)
2	*MC*	52.7 (0.6)[a]	52.3 (0.5)[a]	51.9 (0.5)[ab]	51.6 (0.4)[ab]	50.4 (0.6)[b]	47.9 (1.0)[c]
	IMF	4.86 (0.2)	4.86 (0.2)	4.86 (0.2)	4.86 (0.2)	4.85 (0.2)	4.83 (0.4)
4	*MC*	52.4 (0.6)[a]	52.1 (0.5)[ab]	51.7 (0.4)[ab]	51.3 (0.5)[ab]	50.3 (0.7)[b]	48.1 (1.0)[c]
	IMF	4.92 (0.2)	4.94 (0.2)	4.95 (0.1)	4.96 (0.1)	5.00 (0.1)	5.10 (0.3)
6	*MC*	52.4 (0.6)[a]	51.8 (0.5)[a]	51.3 (0.5)[ab]	50.9 (0.5)[ab]	49.8 (0.7)[bc]	49.0 (1.0)[c]
	IMF	4.98 (0.2)	5.01 (0.2)	5.04 (0.2)	5.07 (0.2)	5.16 (0.2)	5.37 (0.4)
8	*MC*	52.5 (0.9)[a]	51.7 (0.7)[ab]	51.0 (0.6)[abc]	50.3 (0.7)[bc]	49.2 (0.9)[c]	50.5 (1.3)[bc]
	IMF	5.04 (0.3)	5.09 (0.3)	5.14 (0.3)	5.18 (0.2)	5.32 (0.2)	5.64 (0.5)

abc: Different letters in the same row reflects a significant effect of frozen storage upon MC ($P < 0.05$).

2.3.8. Glycogen content

Glycogen content was unaffected by chilled-only storage periods ($P > 0.05$). Frozen-only storage period did increase glycogen content ($P < 0.001$), particularly at 52 weeks (Fig. 2.10). Chilled-then-frozen storage was influential when frozen storage temperature was -12 °C, resulting in greater glycogen content increases compared to -18 °C – a difference more apparent as chilled storage periods increased ($P = 0.05$).
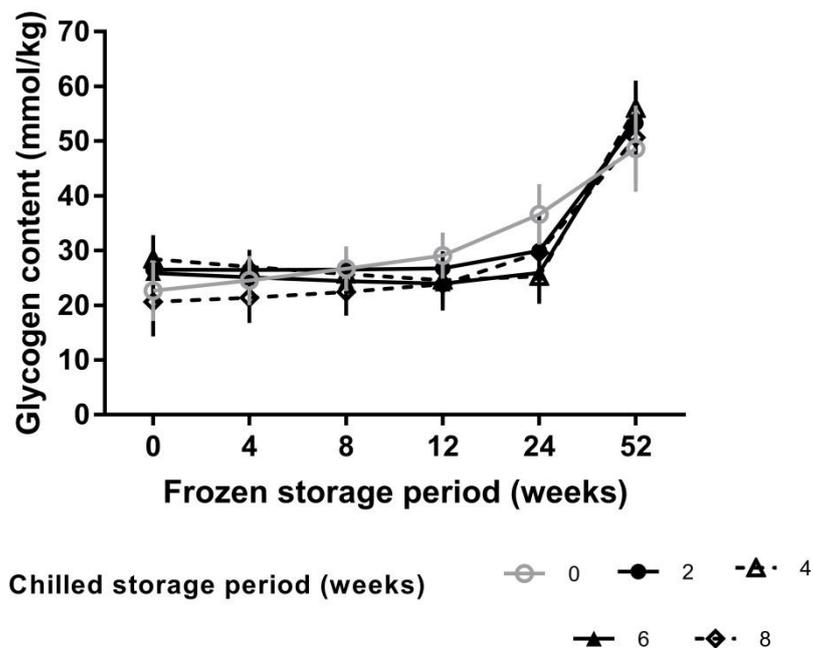

Fig. 2.10. Predicted mean (± SE) glycogen content of chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-only data has been included as a grey line.

2.3.9. Microbial loading

Brochothrix thermosphacta, LAB, and *Enterobacteriaceae sp.* were all detected (limit of detection = 1 $\log_{10}$ CFU/g) across each storage treatment combination. *Clostridium perfringens* and *E. coli* were not detected. Increased chilled-only storage periods resulted in parallel increases to the loading of these detected microbial types ($P < 0.001$; Fig. 2.11)*.* LAB generally increased with increasing frozen-only storage periods ($P < 0.001$), albeit a decrease was evident at 24 weeks frozen storage, but this trend was recovered by 52 weeks (Fig. 2.11a). *B. thermosphacta* increased between 12 and 52 weeks frozen storage when LL were frozen-only ($P < 0.001$; Fig. 2.11b). Furthermore, *B. thermosphacta* was observed to decrease within the chilled-then-frozen storage context ($P = 0.004$; Fig. 2.11b), such that as the period of frozen storage increased, the growth of *B. thermosphacta* was reduced, particularly in LL chilled for between 4-8 weeks. Frozen-only storage had no effect on *Enterobacteriaceae sp.* loadings ($P > 0.05$, Fig. 2.11c). Likewise, frozen storage temperature had no significant effect on microbial load for any tested species ($P > 0.05$). Increased muscle glycogen content prevented microbial proliferation of all three detected species in this study ($P < 0.05$), particularly *Enterobacteriaceae sp.* ($P < 0.001$; Table 2.4).
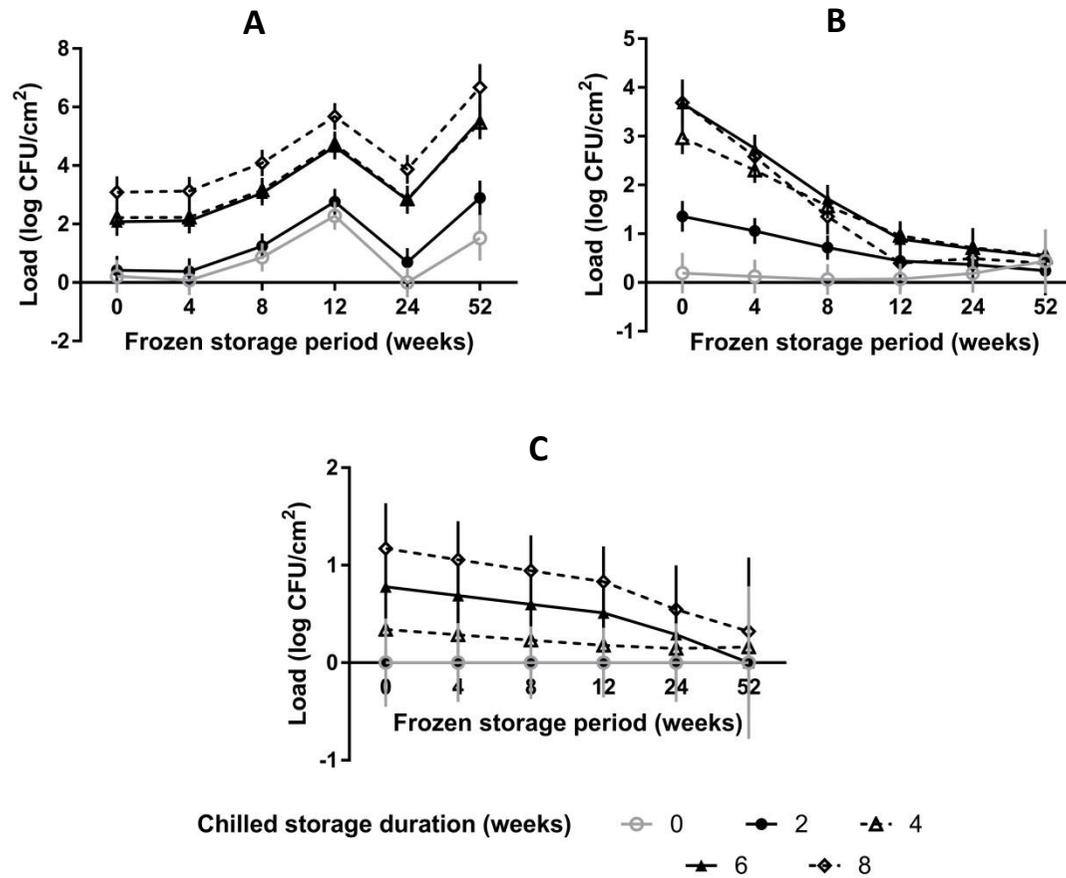
Fig. 2.11. Predicted mean (± SE) microbial loadings of A) *lactic acid bacteria*; B) *Brochothrix thermosphacta*; and C) *Enterobacteriaceae sp.* of

chilled-then-frozen lamb LL stored at two frozen storage temperatures (-12 and -18 °C), with predicted means for each temperature averaged. Frozen-

only data has been included as a grey line. The y-axes are the logarithmic count of each microbial species. CFU = colony forming units. No detections

of *Clostridium perfringens* or *Escherichia coli* were found in any LL.

Table 2.4. Effect of glycogen content upon microbial loading of chilled-then-frozen meat. Microbial species were selected based on loading being above the limit of detection.

Species	Coefficient ($\pm$ SE)	*P*-value
Lactic acid bacteria	-0.01 (0.01)	0.017
Brochothrix thermosphacta	-0.01 (0.01)	0.048
Enterobacteriaceae sp.	-0.03 (0.01)	< 0.001

2.4. Discussion

Both chilled and frozen storage affected the meat quality of lamb LL in this study. The relative absence of any interaction effect from chilled-then-frozen storage on meat quality was interesting. Additionally, frozen storage temperature only had negligible effects on meat quality parameters and no effect on microbial loading.

The use of the warmer frozen storage temperature (-12 °C) compared to the internationally recognised temperature (-18 °C; Eustace *et al.*, 2014) tested the potential to maintain lamb LL quality and safety at different frozen storage temperatures. Differences in quality due to temperature were minimal, and combined with no difference in the load of spoilage microbes suggests -12 °C could be used to provide comparable safe frozen storage to the -18 °C temperature for the maintenance of the quality of conventionally chilled meat. It is also noted that variability in temperature existed at different storage period, however this can be attributed to the position of the temperature logger within samples and within the wider context of the study, this was unlikely to have any affect upon the results. A basis for this observation could be the use of a commercial freezer/freezing rate, which prevented excess damage to muscle ultrastructure (Grujić *et al.*, 1993; Petrović *et al.*, 1993; Ngapo *et al.*, 1999; Fernández *et al.*, 2007) and maintained a uniform ice crystal size (Grujić *et al.*, 1993; Ngapo *et al.*, 1999). This was also evident in the lack of frozen storage period effects upon thaw losses in the present study. Similarly, most prior frozen storage studies have found that freezing method and rate, not storage period or temperature; have resulted in alterations to meat quality

(Muela *et al.*, 2010; Muela *et al.*, 2012; Bueno *et al.*, 2013). This result means that there is potential to reduce the costs associated with maintaining freezers at lower temperatures, and even higher frozen storage temperatures such as -10 °C could be tested for use, provided good temperature control is used, which can fulfil paucities arising from prior studies examining eating quality of lamb stored at -10 °C (Winger, 1984; Hagyard *et al.*, 1993). It would, however, be advantageous to validate this result further through consumer studies focusing on odour and flavour (a complementary sensory study did take place but was limited by its design; Coombs *et al.*, 2016) or through measurement of ice crystal size.

In terms of eating quality type traits, the decreased SF and increased IMF proportion showed the positive effects of both chilled- and frozen-only storage periods. The increased proportion of IMF may be related to the proportional moisture loss from experimental LL, as purge and thaw losses, and the corresponding effect of 'concentrating' IMF levels. This could, in turn, result in improved juiciness and flavour traits (Hopkins *et al.*, 2006; Pannier *et al.*, 2014), but in practice may not be perceived by consumers, whom identify IMF > 4.5% as preferable in terms of juiciness (Pannier *et al.*, 2014); mean values were all higher than this in the present study regardless of treatment. This observation is supported with juiciness found to rarely increase across frozen storage periods (Muela *et al.*, 2012; Fernandes *et al.*, 2013; Muela *et al.*, 2016) and even decrease in some instances (Bueno *et al.*, 2013).

Tenderness increased due to increased chilled storage periods, based on the decreased SF in this study showing consistency with prior studies on

chilled lamb (Bellés *et al.*, 2017). The decrease was most significant following 2 weeks chilled storage, which is consistent with prior studies suggesting tenderisation occurs within 7-10 d from slaughter in lamb (Dransfield, 1994; Thompson *et al.*, 2005; Starkey *et al.*, 2015). Frozen lamb has rarely increased in sensory tenderness (Muela *et al.*, 2012; Bueno *et al.*, 2013; Fernandes *et al.*, 2013; Muela *et al.*, 2016) despite decreases in SF in the short term (Muela *et al.*, 2015). Nevertheless, SF results in the current study remained under the consumer acceptability threshold (40 N) based on the modelling of Hopkins *et al.* (2006) if chilled for more than 4 weeks, irrespective of the period of frozen storage. By contrast the unchilled lamb was not acceptable based on the threshold, and two weeks chilling provided a significant improvement. It is believed that under the extended storage periods, sufficient proteolysis had occurred to tenderise the meat via calpains in the case of chilled storage (Dransfield, 1994; Huff-Lonergan *et al.*, 1996; Hopkins & Thompson, 2002) and tissue damage in the case of frozen storage (Leygonie *et al.*, 2012). It was likely; however, that tenderisation via proteolysis did also occur in frozen-only meat, albeit at a slower rate due to diminished calpain activity at frozen temperatures (Crouse & Koohmaraie, 1990; Dransfield, 1994; Duckett *et al.*, 1998b).

Upon display, both chilled and frozen storage periods increased the rate of discolouration compared to fresh meat, reflected by a decrease in a*, R630/580 and chroma values, which is consistent with prior studies examining chilled (Ponnampalam *et al.*, 2001) and frozen (Muela *et al.*, 2015) storage of lamb. Denaturation of myoglobin from cellular disruption during chilled and frozen storage results in its more rapid oxidation to

metmyoglobin and accumulation on the meat surface upon exposure to oxygen, resulting in a brown colour (Leygonie *et al.*, 2012; Muela *et al.*, 2015) – frozen storage additionally reduces stability due to the metmyoglobin reducing system being compromised which thereby affects the ability of these anaerobically stored samples to bloom upon display (Ledward, 1985). Chilled-only storage did, however, result in higher a* values on day 1 of display after periods of 2-6 weeks compared to zero weeks (Fig. 2.4), which likely presented as a stabilised bloomed red colour due to peak oxygenation from myoglobin to oxymyoglobin, consistent with Ponnampalam *et al.* (2001). This oxygenation effect from the chilled storage was preserved when the lamb was then frozen for 4 weeks, having increased redness compared to frozen-only storage (Fig. 2.4) as per Kim *et al.* (2011), however this effect did not continue with increased frozen storage period.

The presence of spoilage microbial species increased as the chilled storage period lengthened, although all microbial species were below safety thresholds even after 8 weeks; 8 log CFU/g for LAB (Bell, 2001; Kiermeier *et al.*, 2013) and 6 log CFU/g for *B. thermosphacta* and *Enterobacteriaceae sp.* (Gill, 2004; Gribble *et al.*, 2014). *B. thermosphacta* decreased with subsequent frozen storage, particularly following 12 weeks (Fig. 2.11b), which indicates one advantage of freezing. Similarly, proliferation of *Enterobacteriaceae sp.* decreased and proliferation of LAB increased at a slower rate as applied frozen storage periods increased beyond 12 weeks (Fig. 2.11a). Microbial profiles in this study indicate good management practices (initial load < 3 log CFU/g; Gill, 2004), safety (no *E. coli*; Mills et al., 2014) and good temperature control (maintained safety), even though

mean chilled storage temperatures (Table 2.1) were above the ideal temperature for export safety of around -1.5 °C (Bell, 2001; Sumner & Jenson, 2011; Mills *et al.*, 2014; Sumner, 2016). This good management may have contributed to the observation that frozen-stored LL (regardless of prior chilled storage period) exhibited low microbial loading that remained below spoilage thresholds for up to 52 weeks, which is consistent with prior frozen storage studies (Hinton *et al.*, 1998; Fernandes *et al.*, 2013). The implications from this suggest a maintenance of meat quality, as microbial spoilage can result in greening (Egan *et al.*, 1988), discolouration (Li *et al.*, 2015) and off-odours and flavours (Gill, 2004; Gribble *et al.*, 2014; Mills *et al.*, 2014). Even so, the observed discolouration (decreased a* and R630/580, increased hue angle) for 8 weeks chilled storage may be attributable to this microbial proliferation despite safety being assured. Increased glycogen content was associated with decreased microbial proliferation in this study due to it presenting a favourable substrate for spoilage microbes and thus preserving chilled-then-frozen lamb LL from spoilage, where amino acids become consumed by bacteria resulting in off-odours (Coombs *et al.*, (2017); Chapter 1). This result should however be interpreted with caution due to the sharp increase in glycogen content observed between 24 and 52 weeks frozen storage, and the steady decrease in *B. thermosphacta* and *Enterobacteriaceae sp.* at the same period. Meanwhile, glycolysis produces lactic acid as a by-product (Pethick *et al.*, 1995), as does LAB, and the results do not show an inverse relationship between LAB and glycogen at 24 to 52 weeks frozen storage.

Glycogen content, water holding capacity (decreased water losses) and colour stability (increased a* and R630/580 and decreased hue on all days of display) all exhibited strong positive relationships with increased pHu. Higher pHu was associated with a dark red colour, particularly on days 1-2 of display (decreased L* and b*, increased a* and chroma), which is consistent with low glycogen levels at slaughter resulting in lower lactic acid and pH not declining (Pethick *et al.*, 1995). Increased pHu was also associated with shorter sarcomeres, which likely occurred due to pre-rigor shortening (Starkey *et al.*, 2015), although this did not translate into any effect on SF from either pHu or SL, despite prior findings suggesting lower pHu (5.5 – 5.8) to result in increased tenderness (Devine *et al.*, 1993). The wide ranges of pHu and glycogen content encountered in this study may have occurred due to random sampling of LL, and different LL being assigned to different storage period combinations. This may also explain the marked increase of glycogen content in the LL sampled at 52 weeks, given the influence of pre-slaughter factors (Warner *et al.*, 2010), however such an increase as reported in the present study is not biologically plausible. It would be beneficial to validate this observation in future studies through measurement of glycogen content in frozen meat using different techniques and gaining further insight into this mechanism.

In comparison to existing thresholds for meat quality measures and microbial levels we can suggest that the temperatures and periods for chilled storage (Table 2.1) and frozen storage (-12 °C) used in this study could be used in commercial chilled and frozen storage for export and lamb would remain below spoilage thresholds. Meat quality effects were mostly

negligible, although decreased colour stability and increased water losses (purge and thaw) can affect the marketability of chilled and frozen lamb LL at a retail level, particularly when displayed aerobically. Colour acceptability was likely to be the most limiting factor towards adoption of these long-term chilled-then-frozen storage combinations with all experimental LL considered as too discoloured following 3 d display based on R630/580 limits (Khliji *et al.*, 2010), while at 1 d display LL were acceptable up to and including 2/52, 4/12, 6/4 and 8/0 (weeks chilled/frozen combinations). In terms of a* limits from this same study, chilled-only storage for 0-4 weeks would result in acceptable colour for 3 d and 2/52, 4/8, 6/4 and 8/0 (weeks chilled/frozen combinations) would result in acceptable colour for only 1 d.

2.5. Conclusion

It can be concluded that chilled storage prior to conventional frozen storage can improve and preserve the meat qualities of lamb, evident as tenderness (SF) and colour stability parameters. Furthermore, the optimal pre-freeze chilled storage duration was identified as 2 to 4 weeks – this facilitating the least negative effects in terms of promoting microbial spoilage. Chilled storage beyond 4 weeks is not recommended based on the results presented here as there was negligible improvement in shear force and increased development of discolouration (upon aerobic display) and spoilage microbial proliferation. Frozen storage, meanwhile, proved acceptable for up to one year at both -12 and -18 °C regardless of prior chilled storage period with minimal quality deterioration based upon the results of this study, although it would be beneficial if future studies investigated odour/flavour properties, retail storage and display conditions. Additional insight into the effects from similar chilled-then-frozen storage periods on protein and lipid oxidation would complement these recommendations and together could benefit product distribution in terms of guaranteeing product quality and value. Food service markets such as

restaurants or additional processing would be suitable destinations for long-term chilled-then-frozen meat given excessive colour deterioration and thaw losses.

Chapter 3: Effects of chilled-then-frozen storage (up to 52 weeks) on the lamb *m. longissimus lumborum* fatty acid profile and lipid oxidation biomarkers (based on submission to Meat Science)

Abstract

This study investigated the variations to the fatty acid profile and lipid oxidation parameters in lamb *m. longissimus lumborum* (LL) kept under various chilled-then-frozen storage period combinations. These LL ($n = 360$) were randomly sampled at 24 h *post-mortem* from a commercial Australian abattoir and distributed over five chilled storage periods (0, 2, 4, 6 and 8 weeks) followed by six frozen storage periods (0, 4, 8, 12, 24 and 52 weeks) at either -12 °C or -18 °C. All LL were analysed for fatty acid profile and lipid oxidative parameters (TBARS, oxidation-reduction potential and peroxidase activity). Health-claimable polyunsaturated fatty acids (EPA and DHA) were largely unaffected by chilled-then-frozen storage periods ($P > 0.05$; 0.4-0.7 g/ 100 g lipid). This observation implies preservation of nutritional quality and was supported by the TBARS levels not exceeding rancidity thresholds recommended in the literature (2.0 mg/kg meat), despite a general increase in lipid peroxidation markers across these chilled and frozen storage periods ($P < 0.05$), which suggests negligible influences on eating qualities.

Keywords: Lamb; Preservation; Lipid peroxidation; Rancidity; Fatty acids; Nutritional quality; Health-claimable fatty acids.

3.1. Introduction

Lamb is an important international commodity, with countries like Australia and New Zealand being major exporters (MLA, 2016). Participation in the international market, and to benefit from its associated revenue depends on the delivery of high quality meat (Eustace *et al*., 2014). Such lamb has been characterised as having: 1) desirable sensory or eating quality, for traits such as flavour which underpin lamb's desirability to consumers (Muela *et al*., 2012; MLA, 2015); and, 2) nutritional quality, with particular emphasis on health-claimable fatty acids (FA) eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3) because of their association to anti-inflammatory and anti-cancer functions (McAfee *et al*., 2010; Alvarenga *et al*., 2015). Already, effort has been made to improve EPA and DHA content of lamb meat, most commonly through managing animal feeds, and these have contributed to its classification as a source of these healthy FA (Alvarenga *et al*., 2015; Ponnampalam *et al*., 2016), in which a "source" is defined as 30 and 40 mg per 100 g and a "good source" as 60 and 80 mg per 100 g in Australia and New Zealand (FSANZ, 2014) and the European Union (Commission Regulation of European Union, 2010), respectively. In doing so, this may have inadvertently also increased its susceptibility to rancidity and associated detriments to flavour acceptability (Wood *et al*., 2003; Hopkins *et al*., 2014; Alvarenga *et al*., 2015; Ponnampalam *et al*., 2016). All polyunsaturated fatty acids (PUFA) are somewhat susceptible to rancidity, which arises from the prevalence of their double carbon bonds to oxidise (Cifuni *et al*., 2000).

Chilled and frozen storage are routinely used to preserve meat against such oxidation, with recent studies reporting their potential combination (chilled-then-frozen storage) to deliver long-term preservation of lamb meat (Kim *et al*., 2011; Coombs *et al.* (2017a); Chapter 1; Coombs *et al.* (2017b); Chapter 2). These studies, however, did not examine this effect on nutritional quality, specifically whether chilled-then-frozen storage influences lamb fatty acid profile (FAP) or the extent of lipid oxidation in long-term chilled-then-frozen meat (Campo *et al*., 2006; Coombs *et al.* (2017a); Chapter 1).

Lipid oxidation (measured as TBARS) has been found to increase with increased chilled (Campo *et al*., 2006; Colle *et al*., 2015) and frozen storage periods (Muela *et al*., 2010; Muela *et al*., 2015), although TBARS levels were observed to decrease when frozen storage periods exceed 6 months in some cases (Leygonie *et al*., 2012; Muela *et al*., 2015; Alonso *et al*., 2016). In fact, Kim *et al*. (2011) reported TBARS levels of chilled-then-frozen lamb meat to be intermediate of chilled and frozen lamb stored for the same period. This study did not include a fresh meat (0 weeks storage) control or chilled-then-frozen storage periods beyond 9 weeks, which could have added to its value. Furthermore, the study of Kim *et al*. (2011) and others did not investigate peroxidase activity, an important indicator of anti-oxidation in meat tissue due to its catalysis of hydrogen peroxide, thus increasing concurrently with TBARS (Lee *et al*., 1996; Daun *et al*., 2001), nor did they measure oxidation-reduction potential (ORP) that similarly indicates potential for oxidation and which acts as a precursor to the increase in TBARS (Bekhit *et al*., 2013; Alonso *et al*., 2016). Understanding

variations to these would provide insight into lipid oxidation as a result of anaerobic storage (both chilled and frozen).

Based on the literature, it was also apparent that characterisation of the effect of frozen storage temperature upon chilled-then-frozen lamb lipid profile is scarce (Coombs *et al.* (2017a); Chapter 1), although one previous study found flavour intensity towards rancidity to occur more rapidly at frozen storage temperatures of -10 °C compared to -15 °C (Hagyard *et al.*, 1993), but they did not measure FAP or lipid peroxidation markers. This general lack of frozen storage temperature examination in the literature is unfortunate and suggests the generally internationally recognised frozen storage temperature of -18 °C (Food Science Australia, 2005; USDA, 2013) is arbitrary rather than scientifically founded (Coombs *et al.* (2017a); Chapter 1).

This study aimed to evaluate the FAP and biomarkers for lipid peroxidation (including TBARS, peroxidase activity and ORP) for lamb stored under chilled-then-frozen storage period combinations (up to 8 weeks and 52 weeks, respectively) and at two different frozen storage temperatures (-12 and -18 °C).

3.2. Materials and methods

3.2.1. Sample collection and storage treatments

Experimental lamb *m. longissimus lumborum* (LL) were collected (*n* = 360) as per Coombs *et al.* (2017b) (Chapter 2). Following this, LL were assigned to experimental treatment groups which comprised all combinations of five chilled storage periods (0, 2, 4, 6 and 8 weeks), six ensuing frozen storage periods (0, 4, 8, 12, 24 and 52 weeks) and two frozen storage temperatures (-12 and -18 °C). It should be noted that for the analysis, samples allocated to the two nominal groups for chilled-only (0 weeks frozen) storage at -12 and -18 °C were merged to form one group. Vacuum-packaged LL were stored chilled under commercial conditions (export abattoir), with LL assigned to frozen storage first frozen onsite using an industrial plate freezer and then transported to the Centre for Red Meat and Sheep Development (Cowra, New South Wales, Australia) where they were kept in duplicate freezers set at each frozen storage temperature (total: 4 freezers). Once the storage period (chilled-then-frozen) combinations were completed, each LL was sub-sectioned and care was taken to maintain their status (i.e. frozen LL were not permitted to thaw). All subsections not immediately tested were kept at -80 °C.

3.2.2. Fatty acid profile

The quantification of FAP used a protocol adapted from Ponnampalam *et al*. (2014b) and freeze-dried samples sourced as described in Coombs *et al.* (2017b) (Chapter 2). As such, 50.0 mg of homogenised freeze-dried muscle was combined with 500.0 µg tridecanoic acid dissolved

in methanol to act as an internal standard (C13:0, Sigma-Aldrich Pty. Ltd., Castle Hill, New South Wales, Australia) and then hydrolysed with 700.0 µg 10 N potassium hydroxide (KOH, Sigma-Aldrich Pty. Ltd., Castle Hill, New South Wales, Australia) and 5.3 mL methanol through 90 min incubation at 55 °C and agitation every 20 min. Solutions were cooled to room temperature and combined with 600.0 µg sulfuric acid (H_2SO_4, Sigma-Aldrich Pty. Ltd., Castle Hill, New South Wales, Australia) in water and then incubated for 90 min at 55 °C. Solutions were cooled at room temperature, combined with 3.0 mL hexane and 1.0 mL standard sodium chloride solution (NaCl, Sigma-Aldrich Pty. Ltd., Castle Hill, New South Wales, Australia); vortex mixed for 5 min and aliquoted into auto-sampler vials for gas chromatography (GC) analysis.

FAP quantification column specifications were: 60.0 m x 0.25 mm, 70% cyanopropyl polysilphenylene-siloxane with 0.25 µm BPX-70 (SGE). Oven settings were: 30 s at 100 °C before 20 °C temperature increases per minute to 130 °C, at which it was isothermally held for 2 min, then 1 °C temperature increases per minute until 150 °C and isothermal holding for 3 min, then 3 °C temperature increases per minute until 220 °C and isothermal holding for 6 min. FAP were identified against reference standards (no. 47885-U Suplco® 37 Component FAME mix, and no. 47116 Menhaden fish oil, Sigma-Aldrich Pty. Ltd., Castle Hill, New South Wales, Australia) and reported as g 100 g total fatty acid and calculated in summation per FA category to form the FAP (Table 3.1).

3.2.3. Thiobarbituric acid reactive substances

TBARS content determination was adapted from Hopkins *et al.* (2014), where 100.0 mg sample was added to 500.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals, Michigan, United States of America) and homogenised using micro-tube pestles. These were then centrifuged and the supernatant was then analysed as per the OXItek TBARS assay kit technical bulletin (ZeptoMetrix, 2011) and absorbance read at 532 nm on a bench top spectrophotometer. Results were expressed as mg malondialdehyde (MDA) per kg fresh meat.

3.2.4. Oxidation-reduction potential

Sample ORP by was measured through homogenising 1.0 g sample LL with 25.0 mL Milli-Q water using a homogeniser (series X10/25, Ystral^{TM}, Germany) at 22, 000 rpm and then the homogenate was centrifuged (Model 5810R, Eppendorf Pty. Ltd., Hamburg, Germany) for 15 min at 2, 465 g and 4 °C. Supernatant ORP and temperature values were then quantified using a calibrated ORP probe and benchtop monitor (ORP110-GS and HQ440d, Standard ORP Probe, HACH Pacific Ltd., Victoria, Australia) calibrated using ZoBell's ORP standard solution (no. 2316949, HACH Pacific Ltd., Victoria, Australia). Results were the average of technical duplicates and expressed as millivolts (mV) per g fresh meat.

3.2.5. Peroxidase activity

Peroxidase activity was measured using approximately 25.0 mg of sample homogenised with 200.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals, Michigan, United States of America) using micro-tube pestles. These were then centrifuged and the supernatant analysed against peroxide (H_2O_2) standards using the colorimetric protocol detailed by the Peroxidase Activity Assay Kit (no. MAK092, Sigma Aldrich Pty. Ltd., Missouri, United States of America) technical bulletin (Sigma-Aldrich, 2015) using a micro-plate reader (FLUOStar OPTIMA^{TM}, BMG, Labtechnologies, Victoria, Australia) to measure absorbance at 570 nm. Peroxidase activity was expressed as the amount of peroxidase (nmole) that reduces 1.0 µmole H_2O_2 per min at 37 °C per sample (U/g) and was the average of technical duplicates.

3.2.6 Statistical analysis

The response of the measured parameters to the experimental factors (chilled storage period, frozen storage period and frozen storage temperature) was estimated by linear models. A response surface modelling framework was attempted given the quantitative nature of the treatment matrix, though exploration of the data revealed no systematic responses to the treatments. Therefore, the experimental factor levels were taken as

qualitative and a linear model was formed to estimate mean responses at every combination of chilled storage period, frozen storage period and frozen storage temperature. The nonsensical contrast of frozen storage temperature (-12 vs -18 °C) for chilled-only (unfrozen) samples was excluded from the model by construction of a 2 level control factor classifying the samples as frozen or unfrozen. Terms in the linear model were then formed by nesting the main effects and interactions between chilled storage period, frozen storage period and frozen storage temperature within the control factor levels. In the commonly accepted shorthand for linear statistical models, the treatment model was:

Control/(Frozen storage temperature*Chilled storage period*Frozen storage period)

Null hypothesis significance tests for the experimental factors were conducted by analysis of variance derived from the model. Mean responses under each treatment combination and standard errors of the means were also calculated. Pairwise comparison of means after rejection of the relevant null hypothesis was conducted by least significant difference calculated at 5% critical value. All data analysis was conducted in the R environment (R Core Team, 2016). The level of significance in this study was set at $P < 0.05$.

Because of the large number of individual FA evaluated in this study, these were simplified as summative values and ratios (Table 3.1) to be analysed, along with the major individual contributors for each FA type.

3.3. Results

3.1. Fatty acid profile

For the most part, saturated fatty acids (SFA) were found to be predominantly palmitic and stearic acids (Table 3.1). The total levels of SFA did not change throughout the entire storage period ($P > 0.05$; Table 3.2). Palmitic acid concentration decreased as chilled storage period increased from 0 to 2 weeks ($P = 0.03$).

Total monounsaturated fatty acids (MUFA) were primarily comprised of oleic acid (Table 3.1), with no changes in concentration due to increased storage periods ($P > 0.05$; Table 3.2).

In this study, PUFA was the summation of both omega-3 (n-3) and omega-6 (n-6) FA. Major n-3 PUFA contributors were α-linolenic acid (ALA) and docosapentaenoic acid (DPA). Major n-6 PUFA contributors were linoleic acid and arachidonic acid (Table 3.1). Total PUFA tended to increase in concentration across chilled storage periods ($P = 0.05$; Table 3.2), particularly n-6 PUFA between 0 and 8 weeks chilled-only storage ($P = 0.02$).

Table 3.1. Mean fatty acids found in this study based on fresh meat (time zero).

Formula	Name	Content (g/100 g total fatty acid)
C4:0	Butyric acid	0
C6:0	Caproic acid	0
C8:0	Caprylic acid	0
C10:0	Capric acid	0.15
C11:0	Undecylic acid	0
C12:0	Lauric acid	0.11
C13:0	Tridecylic acid	0
C14:0	Myristic acid	2.79
C15:0	Pentadecylic acid	0.33
C16:0	Palmitic acid	25.17
C17:0	Margaric acid	1.08
C18:0	Stearic acid	16.12
C20:0	Arachidic acid	0.64
C21:0	Heneicosylic acid	0.21
C22:0	Behenic acid	0
C23:0	Tricosylic acid	0
C24:0	Lignoceric acid	0
Total SFA		**46.60**
C14:1	Myristoleic acid	0.01
C15:1	Pentadecanoic acid	0
C16:1ω7	Palmitoleic acid	2.06
C17:1ω7	Heptadecanoic acid	0
C18:1iso	Vaccenic acid (ω7)	1.11
C18:1ω9	Oleic acid	41.67
C18:1ω9t	Elaidic acid	1.31
C20:1	Paullinic acid (ω7) or Gondoic acid (ω9)	0.04
C22:1ω9	Erucic acid	0
C24:1ω9	Nervonic acid	0
Total MUFA		**46.21**
C18:2ω6	Linoleic acid	3.72
C18:2ω6t	*trans*-Linoleic acid	0.11
C18:3ω6	γ-Linolenic acid	0.05
C20:2ω6	Eicosadienoic acid	0
C20:3ω6	Dihomo-γ-Linolenic acid	0.08
C20:4ω6	Arachidonic acid	1.07
C22:2ω6	Docosadienoic acid	0
Total n-6 PUFA		*5.04*
C18:3ω3	α-Linolenic acid	1.03
C18:3 ω3t	*trans*-Linolenic acid	0.04
C20:3ω3	Eicosatrienoic acid	0
C20:4ω3	Eicosatetraenoic acid	0
C20:5ω3	Eicosapentaenoic acid (EPA)	0.41
C22:5ω3	Docosapentaenoic acid (DPA)	0.52
C22:6ω3	Docosahexaenoic acid (DHA)	0.14
Total n-3 PUFA		*2.15*

n-3/n-6 ratio	0.43
Total PUFA	**7.19**
Total UFA (MUFA + PUFA)	**53.40**
Total (UFA + SFA)	**100**
EPA + DHA	0.55
IMF (% fresh meat)	4.56

Table 3.2. Effect of chilled-then-frozen storage upon the proportions of polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SFA) fatty acids in lamb *m. longissimus lumborum* (LL).

Chilled storage duration (weeks)	Frozen storage duration (weeks)																	
	0			4			8			12			24			52		
	PUFA	MUFA	SFA	PUFA	MUFA	SFA	PUFA	MUFA	SFA	PUFA	MUFA	SFA	PUFA	MUFA	SFA	PUFA	MUFA	SFA
0	7.19	46.2	46.6	8.06	46.1	46.4	8.72	44.9	46.4	8.07	45.6	46.3	10.0[a]	45.0	45.0	8.43	44.3	47.3
2	7.97	45.6	46.4	7.23	46.5	46.0	7.59	46.5	46.0	7.94	44.8	47.2	7.46[b]	46.6	46.0	7.79	45.3	46.9
4	7.47	45.0	47.5	8.11	46.8	45.6	8.02	46.4	45.6	7.41	45.7	46.9	8.14[ab]	45.8	46.1	7.54	47.0	45.5
6	7.89	45.8	46.3	9.21	45.0	45.8	7.98	43.5	48.5	8.46	44.5	47.1	7.76[\ab]	44.9	47.4	7.65	45.0	47.3
8	8.44	45.2	46.4	7.30	47.9	47.0	8.03	45.0	47.0	7.25	45.2	47.6	6.70[b]	45.2	48.1	8.02	45.6	46.3
SEM	0.60	1.05	0.86	0.84	1.49	1.22	0.84	1.49	1.22	0.84	1.49	1.22	0.84	1.49	1.22	0.84	1.49	1.22

ab: Means with different superscripts are significantly different from other PUFA in the same column due to chilled storage ($P < 0.05$).

Health-claimable n-3 PUFAs (EPA + DHA) were summed and their concentrations at different chilled-then-frozen storage periods are shown in Table 3.3. It is worth noting that, while counted towards total PUFA, EPA and DHA were not the two greatest contributors to total PUFA concentration (Table 3.1). Chilled-only storage maintained the EPA + DHA concentration of fresh meat for 2 weeks, with these FA tending to decrease at longer chilled storage periods ($P = 0.05$), particularly when followed by 24 weeks frozen storage (Table 3.3). Frozen storage period and temperature had no effect on EPA + DHA concentration ($P > 0.05$).

Table 3.3. Effect of chilled-then-frozen storage on the sum of health claimable fatty acids, eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA).

Chilled storage duration (weeks)	Frozen storage duration (weeks)					
	0	**4**	**8**	**12**	**24**	**52**
0	0.550	0.525	0.595	0.520	0.700[a]	0.620
2	0.550	0.410	0.445	0.485	0.380[b]	0.455
4	0.420	0.540	0.520	0.520	0.525[ab]	0.440
6	0.410	0.540	0.465	0.590	0.495[ab]	0.520
8	0.420	0.385	0.500	0.565	0.300[b]	0.570
SEM	0.08	0.12	0.12	0.12	0.12	0.12

ab: Means with different superscripts are significantly different from others in the same column ($P < 0.001$).

3.3.2. TBARS

The TBARS levels increased with increasing chilled-only storage periods ($P < 0.001$), with a peak at 4 weeks chilled-only storage apparent (Fig. 3.1). Frozen-only storage was observed to increase TBARS levels as storage period increased to 8 weeks ($P < 0.001$; Fig. 3.1). Chilled-then-frozen storage interactions also increased TBARS, showing a general increase parallel with total storage period ($P < 0.001$). No other storage treatment combination, including frozen storage temperature, significantly influenced TBARS levels ($P > 0.05$).
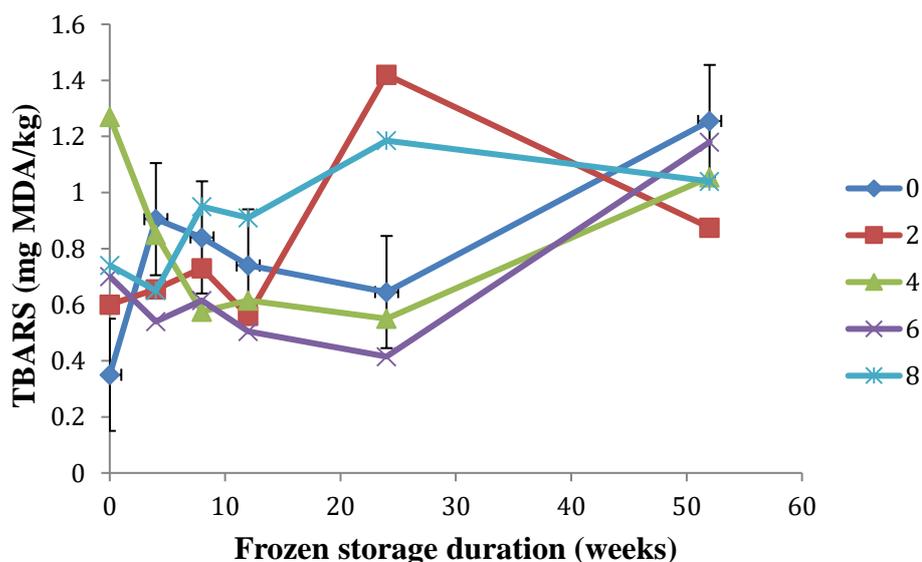
Fig. 3.1. Predicted means ($\pm$ SE) showing the effect of chilled-then-frozen storage period upon TBARS (2-thiobarbituric acid reactive substances) levels (measured as mg malondialdehyde per kg meat) of lamb LL. For simplicity of presentation, standard error has been included on the frozen-only (0 weeks chilled) line.

3.3.3. Oxidation-reduction potential

Chilled-only storage had significant effects on ORP ($P < 0.001$); where there was a decrease noted following 6 weeks storage (Fig. 3.2). ORP trends across increasing frozen-only and chilled-then-frozen storage periods were significant ($P < 0.001$); although it was difficult to ascertain a consistent trend as ORP levels were observed to decrease over frozen-only storage and increase with chilled-then-frozen storage, but only when the preceding chilled storage period was 8 weeks and this was followed by a frozen storage period of 8 to 52 weeks (Fig. 3.2). No frozen storage temperature or other storage effects were significant ($P > 0.05$).
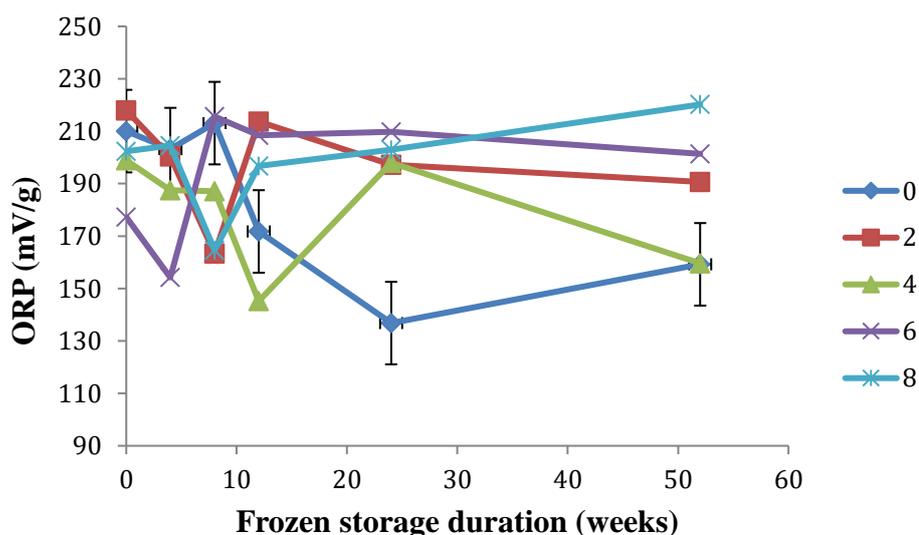

Fig. 3.2. Predicted means ($\pm$ SE) showing the effect of chilled-then-frozen storage period upon oxidation-reduction potential (ORP) of lamb LL. For simplicity of presentation, standard error has been included on the frozen-only (0 weeks chilled) line.

3.3.4. Peroxidase activity

Peroxidase activity was affected by chilled-only storage ($P < 0.001$), though minimal variation between means was found between 0 and 6 weeks storage (Fig. 3.3). However, peroxidase activity increased in a quadratic manner with increasing frozen-only storage ($P < 0.001$), peaking at 8 weeks (Fig. 3.3). Similarly, peroxidase activity increased with increased chilled-then-frozen storage periods ($P < 0.001$), with particular increases due to 52 weeks frozen storage (Fig. 3.3). No frozen storage temperature or other storage effects were significant ($P > 0.05$).
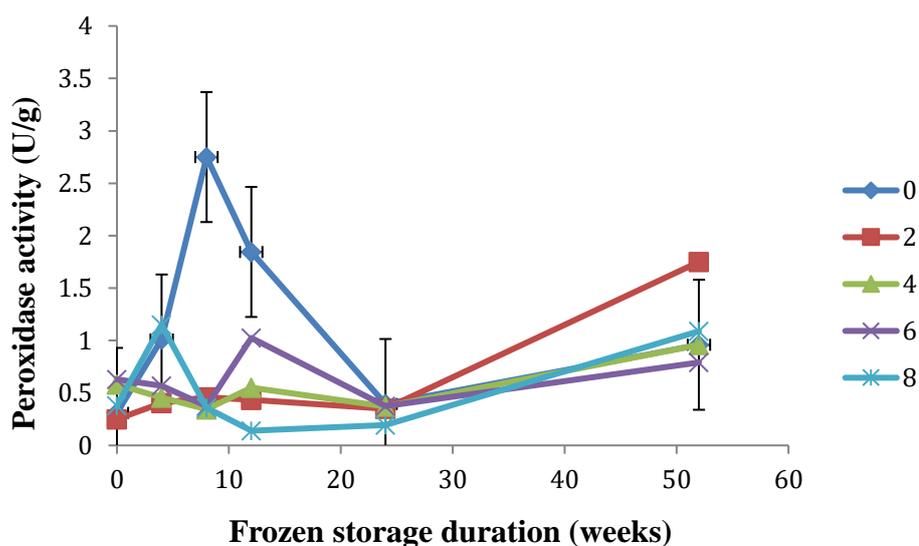

Fig. 3.3. Predicted means ($\pm$ SE) showing the effect of chilled-then-frozen storage period upon peroxidase activity of lamb LL. For simplicity of presentation, standard error has been included on the frozen-only (0 weeks chilled) line.

3.4. Discussion

In this study, chilled, frozen and chilled-then-frozen storage of lamb LL did not affect the FAP, although they did increase biomarkers of lipid peroxidation. Frozen storage temperature had minimal effects on either FAP or lipid peroxidation biomarkers of lamb LL, which provides further evidence for the use of a warmer frozen storage temperature of -12 °C for the preservation of meat quality over long periods; this is corroborated by our previous work in meat quality (Coombs *et al.*, 2017b; Chapter 2).

The summation of FA categories revealed comparative levels to that previously reported for supermarket lamb LL (Enser *et al*., 1996). The only significant point of PUFA increase and diversion from fresh meat FAP occurred at 24 weeks frozen-only storage, where PUFA increased compared to all other storage periods. This was different to the study of Alonso *et al*. (2016), who found that frozen-only storage of pork for 6 months to 2 years resulted in PUFA oxidation to MUFA and SFA. Prior studies on frozen-only storage of lamb LL for 3-6 months have not reported changes in PUFA, MUFA or SFA concentrations (Samouris *et al*., 2011; Popova, 2014). Oxidation of PUFA has been suggested to precede formation of TBARS (Popova & Marinova, 2013), suggesting that lipid oxidation commencing in the chilled storage period continued into frozen storage, or that microbial agents may have contributed to rapid oxidation of frozen-only lamb LL (Coombs *et al.*, 2017b; Chapter 2). Chilled-only storage has been found to increase PUFA concentration previously (Ponnampalam *et al*., 2014b), though decreases are more common and occur concurrent with increased TBARS (Wood *et al*., 1999).

The lack of change in FAP due to chilled and frozen storage similarly did not impact on the nutritional quality of lamb in terms of health-claimable n-3 FA (EPA + DHA) levels. It can therefore be inferred that the nutritional quality was preserved in this study for the entire chilled-then-frozen storage period; however the levels of PUFA, including EPA + DHA, were not sufficient to be considered as a "source" of n-3 PUFA (Alvarenga *et al*., 2015). It is also noteworthy that lamb higher in PUFA through dietary measures (Ponnampalam *et al.*, 2014b) will exhibit more rapid oxidation and will therefore have different nutritional and eating quality thresholds (Cifuni *et al.*, 2000; Hopkins *et al.*, 2014). Although this lamb LL may not be marketable as "healthy" or "source of long-chain omega-3 FA", its nutritional value is unlikely to change from a marketing perspective (Commission Regulation of European Union, 2010; FSANZ, 2014). Previous research has found that EPA decreased in concentration in lamb LL frozen for 3-6 months at -20 °C, though DHA and shorter frozen storage periods were not measured (Popova, 2014).

Chilled-then-frozen storage effects upon lipid oxidation biomarkers (TBARS, ORP and peroxidase activity) were consistent with prior studies which conclude that anaerobic storage of red meat increases lipid peroxidation, though not to the extent of aerobic storage (Kim *et al*., 2011; Bekhit *et al*., 2013; Popova & Marinova, 2013; Coombs *et al.* (2017a); Chapter 1). The observed inconsistencies in all these parameters likely arose from the use of different loins for each storage treatment combination. Meanwhile, the use of anaerobic storage in this study, and the sampling procedure occurring from the frozen state rather than thawed (Muela *et al.*,

2015; Ponnampalam *et al.*, 2017), likely prevented excess lipid oxidation from occurring in long-term chilled and frozen lamb. This lack of excess lipid oxidation is reflected in the TBARS levels not breaching the rancidity threshold of 2.0 mg MDA per kg of meat (Campo *et al.*, 2006) at any chilled-then-frozen storage period. Despite this, TBARS exhibited a general increase due to both chilled and frozen storage in the present study, which is consistent with prior lamb studies finding TBARS to increase due to increased chilled-only storage periods (Berruga *et al.*, 2005; Kim *et al.*, 2011; Samouris *et al.*, 2011; Fernandes *et al.*, 2014). This increase in oxidation occurred more rapidly in chilled-stored meat compared to frozen meat due to the increases of free PUFA and greater instability of lipid free radicals (Igene *et al.*, 1980). Frozen storage, meanwhile, has been found to result in slower, albeit continuing, lipid oxidation (Kanner, 1994), evidenced by increased TBARS following frozen-only storage periods for 3-9 months (Muela *et al.*, 2010; Kim *et al.*, 2011; Samouris *et al.*, 2011; Popova & Marinova, 2013; Muela *et al.*, 2015; Coombs *et al.* (2017a); Chapter 1). Beyond 9 months frozen storage, however, TBARS has been found to decrease or stabilise (Leygonie *et al.*, 2012; Muela *et al.*, 2015), which did not occur in this study.

One potential reason for TBARS stabilisation or decrease with increased frozen storage periods in prior studies is that MDA acted as a precursor to protein oxidation (Xiong, 2000), and this can be reflected by myoglobin oxidation, causing colour changes over a display period (Coombs *et al.*, 2017b; Chapter 2). This area of integrative oxidative processes, particularly the effect of lipid oxidation facilitating myoglobin

oxidation, and *vice versa*, has been extensively reviewed (Kanner, 1994; Min & Ahn, 2005; Faustman *et al*., 2010; Estévez, 2011), although the interaction has not been examined in chilled-then-frozen meat. Greater muscle myoglobin content can increase ORP and thus myoglobin oxidation (Min & Ahn, 2005), while increased peroxidase activity may infer a greater ORP of the muscle involved and therefore increase its susceptibility to increased TBARS (Daun & Åkesson, 2004).

Rancidity (Martínez-Cerezo *et al*., 2005) and confinement odour (Bell & Garout, 1994; Sumner & Jenson, 2011) have been noted in meat stored chilled-only for periods beyond 8 weeks. Although, short-term chilled-only storage periods (< 8 weeks) and associated minor increases in TBARS have been found to improve species-specific flavour (Wood et al., 2003), this may also be linked to increases in medium-chain SFA (6-12 carbon atoms) for sheep meat flavour (Rousset-Akrim *et al*., 1997; Young *et al*., 1997). In the present study, all TBARS levels remained below the rancidity threshold regardless of treatment period, which can be corroborated by an accompanying sensory study finding flavour and overall liking acceptability following 8 weeks chilled-only storage, which in turn was significantly greater than lamb LL stored frozen-only for 8 weeks (Coombs *et al.*, 2016). This threshold could potentially be greater than 2.0 mg/kg for lamb based on prior sensory results showing a majority of consumer odour acceptability at TBARS levels ranging from 2.0 to 4.2 mg/kg (Berruga *et al*., 2005). Regarding frozen-only meat, prior sensory studies have noted acceptability and a lack of difference between lamb meat frozen for one year compared to fresh meat (Hagyard *et al*., 1993;

Ponnampalam *et al*., 2002; Fernandes *et al*., 2013; Muela *et al*., 2016). It is noteworthy that the majority of the aforementioned studies reported lower TBARS values than the present study at corresponding storage periods, which may have occurred due to the lack of a standardised TBARS procedure (Ponnampalam *et al*., 2014a). Meanwhile, resolving discrepancy in the literature between TBARS levels and consumer sensory panels, particularly for lamb, is an additional future investigation (Coombs *et al.* (2017a); Chapter 1).

Based on these results, it is worthwhile to test lipid peroxidation and FAP of lamb LL stored frozen for 2 years or longer as per Winger (1984). At such long frozen storage periods it is unlikely the FAP will change further (Alonso *et al*., 2016), although the health claimable FA did not significantly differ at shorter frozen storage periods. Aerobic display under retail conditions post-thawing should also be tested, as previous studies have reported increases to TBARS (Muela *et al*., 2015), ORP (Kim *et al*., 2002) and rancidity (Muela *et al*., 2016) to result from aerobic storage. Such increases were particularly significant following long-term (> 9 months) frozen storage.

3.5. Conclusion

It can be concluded that chilled-then-frozen storage of lamb LL remained of adequate nutritional quality (health-claimable fatty acids EPA + DHA, and a "source" of n-3 PUFA), meaning that LL can be stored for up to 8 weeks chilled-then-52 weeks frozen, and potentially even longer, and retain these fatty acids. Increases in lipid oxidation were observed due to increased storage periods; however TBARS levels remained below the rancidity threshold regardless of storage period. Furthermore, frozen storage temperatures of -18 °C and -12 °C did not result in significant differences in either lipid oxidation or FAP of chilled-then-frozen lamb. It is recommended that further research be undertaken to investigate the linkages between lipid oxidation and oxidation of myoglobin and other proteins, as well as the relationship between TBARS to rancidity and flavour determination of chilled-then-frozen lamb LL using consumer sensory panels.

Chapter 4: General Discussion and Conclusions

From the experimental chapters of this thesis, it can be concluded that both chilled and frozen storage periods independently affected meat quality and safety traits of lamb. Additionally, the frozen storage temperature posed negligible effects upon meat quality and safety parameters. In particular, shear force, colour stability and microbial loading (**Chapter two**); and lipid oxidation biomarkers (**Chapter three**) were all significantly affected by increased chilled-only storage period. Several of these effects were positive, particularly in the short term, leading to a more tender product (lower shear force for 2-8 weeks compared to 0-weeks; **Chapter two**) and a more nutritional product (slight increase in PUFA concentration for 2-8 weeks compared to 0 weeks; **Chapter three**).

However, chilled-only storage did increase microbial loading, as was seen in **Chapter two**, though at no point during the experimental period did it result in spoilage of meat based on previously defined spoilage thresholds (Mills *et al.*, 2014). Previous studies have also observed that at chilled storage temperatures (particularly below 0 °C), lamb can be stored chilled for at least 8 weeks (and in some cases 12 weeks) with minimal spoilage (microbial load below threshold and absence of confinement odour, greening or off-flavour) (Sumner & Jenson, 2011; Kiermeier *et al.*, 2013).

Similarly, lipid oxidation rancidity thresholds defined in previous research (Berruga *et al.*, 2005; Campo *et al.*, 2006) were not breached (**Chapter three**); rancidity occurring more commonly following aerobic storage (**Chapter one**). This lack of effect occurred in spite of an increase in TBARS with chilled storage period (**Chapter three**).

Display colour was the most affected trait by chilled storage and subsequent frozen storage, as shown by a reduction of redness (a*) and increased browning (increased hue and decreased R630/580) (**Chapter two**) due to myoglobin oxidation, which occurred while lamb LL were kept under both chilled and frozen storage. While prior chilled storage did improve the colour of frozen meat upon display, most experimental LL slices would have been ruled unacceptable at a retail level even after just 1 day (24 hours) post-cutting, based upon previously defined consumer thresholds (Khliji *et al.*, 2010). Additionally, thaw and purge losses from frozen and chilled storage, respectively (**Chapter two**), would cloud the consumer perception of visual quality, freshness and nutritional value. This information in turn leads to the question of how to market this safe, high quality product with a poor visual shelf life with two key markets identified: food service (i.e. restaurants) or vacuum storage of frozen product at display.

Sumner (2016) stated that meat export voyages rarely last more than eight weeks in total from processor to overseas consumer, due to shelf life regulations based upon microbial profiles (Mills *et al.*, 2014). As the paper only examined chilled storage, the frozen storage component as investigated in this thesis could safely follow any of the prescribed chilled storage durations and continue the preservation of quality and safety for up to and,

potentially beyond, one year. In terms of quality, it was found that frozen storage similarly decreased shear force, increased lipid oxidation and promoted increases in lactic acid bacteria, albeit at slower rates than those of chilled storage. In fact, frozen storage in the present study was found to reduce the concentrations of some microbial species that had proliferated during chilled storage, though shelf-life upon thawing was not tested. Winger (1984) stated that frozen storage at -10 °C or lower could preserve meat quality and safety for 2 years or more, provided pre-freeze chilled storage was kept to a minimum. With these in mind, it can be added that 4 weeks chilled storage could constitute a good target for optimal quality prior to frozen storage, as it results in sufficient tenderness as per Hopkins *et al.* (2006), with SF not improved by further chilled storage, which in turn increases microbial load (**Chapter two**).

The examinations of frozen storage temperature (-12 and -18 °C) and chilled-frozen storage interactions in this study were novel. It was found that chilled and frozen storage rarely interacted, instead acted separately upon meat quality. Frozen storage temperature comparisons were re-examined due to the long duration between the present day and the study by Hagyard et al. (1993), which only examined consumer sensory quality at -10 °C and -15 °C. The finding of negligible differences in quality between -12 and -18 °C from the present thesis is significant as it poses an important outcome for the export industry, in that a temperature of -12 °C can be used to preserve quality and safety over frozen storage periods up to one year, in comparison to the conventional international frozen storage temperature of -

18 °C, which can save funds related to freezer operating costs and allow for bulk shipments of product.

It is apparent that from this thesis, several further research questions relating to the topic have arisen:

- Can frozen storage thresholds of lamb be extended for more than one or two years with minimal additional effects on quality or safety (Winger, 1984; Muela *et al.*, 2015)?

- How long is the safe shelf life of thawed product following these chilled-then-frozen storage periods, at which sensory and nutritional quality is maintained?

- Upon thawing, which qualities will be first to deteriorate and why?

- Could frozen storage temperature be increased to -10 °C (Winger, 1984), and provided good control is used, maintain meat quality and safety, which was not possible according to Hagyard *et al.* (1993)?

- What is an appropriate method to quantify meat flavour, a traditionally consumer-based trait, instrumentally?

- Is there an effect on proteins and their oxidation to carbonyls from chilled-then-frozen storage? This has been demonstrated previously in myoglobin (evidenced by colour change) in this study (**Chapter two**) and to be influenced by lipid oxidation (Faustman *et al.*, 2010; Estévez, 2011).

- What governs the variation in thaw losses between samples, even following identical frozen storage periods?

- What is the best method to minimise thaw losses and promote a cherry red muscle colour to improve retail marketability without compromising eating quality or safety?
- Will other species, such as beef and pork, exhibit similar thresholds?
- Will other muscles than the loin exhibit similar thresholds?

Regardless of these outstanding questions, this study was able to determine storage thresholds for chilled-then-frozen lamb LL, based on several different quality and safety parameters. These were:

- Minimum of 4 weeks chilled-only storage for acceptable tenderness based on Australian consumer standards (Hopkins *et al.*, 2006) – this period could be followed by frozen storage of 0-52 weeks and the tenderness preserved (**Chapter two**).
- Maximum of 2 weeks chilled-then-52 weeks frozen, 4 weeks chilled-then-8 weeks frozen, 6 weeks chilled-then-4 weeks frozen, and 8 weeks chilled-only storage to retain acceptable retail colour based on Australian consumer standards (a* and R630/580 as per Khliji *et al.*, 2010) for 1 day post-thaw (**Chapter two**).
- Maximum of 4 weeks chilled-only storage with no ensuing frozen storage period for acceptable redness (a*; Khliji *et al.*, 2010) for 3 days display period (**Chapter two**).
- Lamb LL could be stored for the entire experimental storage period (8 weeks chilled, 52 weeks frozen) and remain safe based on spoilage thresholds for lactic acid bacteria, *Enterobacteriaceae sp.* and *Brochothrix thermosphacta* (Mills *et al.*, 2014), which were all detected in **Chapter two**.

- Lamb LL could be stored for the entire experimental storage period and not undergo significant PUFA oxidation, maintain acceptable nutritional quality by levels of EPA and DHA comparable to fresh meat, and TBARS levels below rancidity thresholds (**Chapter three**).

- Frozen storage reduced concentrations of *Enterobacteriaceae sp.* and *B. thermosphacta*, the latter of which was relatively high following 4-8 weeks chilled storage (**Chapter two**).

It can be concluded that the independent effects of chilled and frozen storage influenced lamb meat quality through mechanisms of proteolysis and oxidation of myoglobin and lipids. Additionally, the microbial profile was affected by both chilled and frozen storage. From this information, several thresholds were formed for the chilled, frozen and chilled-then-frozen storage of lamb LL for export purposes to result in the delivery of a safe, high-quality product. However, further information on proteins and their effect on shear force, post-thaw storage, aerobic and anaerobic product shelf life, and potential extension of the frozen storage period would be required for a more comprehensive insight into long-term preservation of lamb using chilled and frozen storage combinations.

List of References

Adam, K. H., Flint, S. H. & Brightwell, G. (2010). Psychrophilic and psychrotrophic clostridia: sporulation and germination processes and their role in the spoilage of chilled, vacuum-packaged beef, lamb and venison. *International Journal of Food Science and Technology*, 45, 1539-1544.

Alonso, V., Muela, E., Tenas, J., Calanche, J. B., Roncalés, P. & Beltrán, J. A. (2016). Changes in physicochemical properties and fatty acid composition of pork following long-term frozen storage. *European Food Research and Technology*, 242, 2119-2127.

Alvarenga, T. I. R. C., Chen, Y., Furusho-Garcia, I. F., Perez, J. R. O. & Hopkins, D. L. (2016). Manipulation of omega-3 PUFAs in lamb: Phenotypic and genotypic views. *Comperehensive Reviews in Food Science and Food Safety*, 14, 189-204.

AMSA (2012). Meat Color Measurement Guidelines. *In:* Hunt, M. C. & King, A. (Eds.). Chicago, IL: American Meat Science Association.

Añón, M. C. & Calvelo, A. (1980). Freezing rate effects on the drip loss of frozen beef. *Meat Science,* 4**,** 1-14.

Bekhit, A. E.-D. A., Hopkins, D. L., Fahri, F. T. & Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. *Comprehensive Reviews in Food Science and Food Safety,* 12, 565-597.

Bell, R. G. & Garout, A. M. (1994). The effective product life of vacuum-packaged beef imported into Saudi Arabia by sea, as assessed by

chemical, microbiological and organoleptic crietria. *Meat Science*, 36, 381-396.

Bell, R. G. (2001). Meat packaging: Protection, preservation and presentation. In: Hui, Y. H., Nip, W. K., Rogers, R. W. & Young, O. A. (Eds.), *Meat science and applications* (pp. 463-490). New York, USA: Marcel-Decker.

Bellés, M., Alonso, V., Roncáles, P. & Beltrán, J. A. (2017). A review of fresh lamb chilling and preservation. *Small Ruminant Research*, 146, 41-47.

Ben Abdallah, M., Marchello, J. A. & Ahmad, H. A. (1999). Effect of freezing and microbial growth on myoglobin derivatives of beef. *Journal of Agricultural and Food Chemistry,* 47**,** 4093-4099.

Berruga, M. I., Vergara, H. & Gallego, L. (2005). Influence of packaging conditions on microbial and lipid oxidation in lamb meat. *Small Ruminant Research*, 57, 257-264.

Borch, E., Kant-Muermans, M-L & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, 33, 103-120.

Bouton, P. E., Fisher, A. L., Harris, P. V. & Baxter, R. I. (1973). A comparison of the effects of some post-slaughter treatments on the tenderness of beef. *Journal of Food Technology*, 8, 39-49.

Broda, D .M., DeLacy, K. M., Bell, R. G., Braggins, T. J. & Cook, R. L. (1996). Psychrotrophic *Clostridium* spp. associated with 'blown pack' spoilage of chilled vacuum-packed red meats and dog rolls in

gas-impermeable plastic casings. *International Journal of Food Microbiology*, 29, 335-352.

Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V. & Escudero, A. (2013). Effect of freezing method and frozen storage duration on odor-active compounds and sensory perception of lamb. *Food Research International,* 54**,** 772-780.

Butler, D. G., Cullis, B. R., Gilmour, A. R. & Gogel, B. J. (2009). ASReml-R reference manual, version 3. Toowoomba, Australia: Queensland Department of Primary Industries and Fisheries. Retrieved from: http://discoveryfoundation.org.uk/downloads/asreml/release3/asreml -R.pdf

Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science,* 77**,** 63-80.

Callejas-Cárdenas, A. R., Caro, I., Blanco, C., Villalobos-Delgado, L. H., Prieto, N., Bodas, R., Giráldez, F. J. & Mateo, J. (2014). Effect of vacuum ageing on quality changes of lamb steaks from early fattening lambs during aerobic display. *Meat Science,* 98**,** 646-651.

Calvelo, A. (1981). Recent studies on meat freezing. In: Lawrie, R. (Ed.). *Developments in meat science - 2* (pp. 125-158). London: Elsevier Applied Science Publishers.

Camou, J. P., Marchello, J. A., Thompson, V. F., Mares, S. W. & Goll, D. E. (2007). Effect of postmortem storage on activity of µ- and m-calpain in five bovine muscles. *Journal of Animal Science*, 85, 2670-2681.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science,* 72**,** 303-311.

Choe, J. H., Stuart, A. & Kim, Y. H. B. (2016). Effect of different aging temperatures prior to freezing on meat quality attributes of frozen/thawed lamb loins. *Meat Science*, 116, 158-164.

CIE (1978). Recommendations on uniform color spaces - color equations, psychometric color terms (pp. 15 (E-11.13.L)). Paris: Commission Internationale de l'Eclairage (CIE).

Cifuni, G. F., Napolitano, F., Pacelli, C., Riviezzi, A. M. & Girolami, A. (2000). Effect of age at slaughter on carcass traits, fatty acid composition and lipid oxidation of Apulian lambs. *Small Ruminant Research,* 35**,** 65-70.

Clemens, R. M., Adam, K. H. & Brightwell, G. (2010). Contamination levels of *Clostridium estherticum* spores that result in gaseous spoilage of vacuum-packaged chilled beef and lamb meat. *Letters in Applied Microbiology*, 50, 591-596.

Colle, M. J., Richard, R. P., Killinger, K. M., Bolscheid, J. C., Gray, A. R., Locks, W. I., Day, R. N., Cochran, A. S., Nasados, J. A. & Doumit, M. E. (2015). Influence of extended aging on beef quality characteristics and sensory perception of steaks from the *gluteus medius* and *lonigssimus lumborum*. *Meat Science*, 110, 32-39.

Commission Regulation of European Union (2010). Amending regulation (EC) no 1924/2006 of the European parliament and the council with

regard to the list of nutrition claims. *Official Journal of the European Union* (116) (February 2010).

Coombs, C. E. O., Holman, B. W. B., van de Ven, R. J., Friend, M. A. & Hopkins, D. L. (2016). Comparing chilled and frozen storage on lamb sensory quality parameters. *Proceedings: 62nd ICoMST. Bangkok Thailand* (p. 37).

Coombs, C. E. O., Holman, B. W. B., Friend, M. A. & Hopkins, D. L. (2017a). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science*, 125, 84-94.

Coombs, C. E. O., Holman, B. W. B., Collins, D., Friend, M. A. & Hopkins, D. L. (2017b). Effects of chilled-then-frozen storage (up to 52 weeks) on the lamb *M. longissimus lumborum* quality and safety parameters. *Meat Science*, 134, 86-97.

Corbin, C. H., O'Quinn, T. G., Garmyn, A. J., Legako, J. F., Hunt, M. R., Dinh, T. T. N., Rathmann, R. J., Brooks, J. C. & Miller, M. F. (2015). Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. *Meat Science,* 100**,** 24-31.

Crouse, J. D. & Koohmaraie, M. (1990). Effect of freezing of beef on subsequent postmortem aging and shear force. *Journal of Food Science,* 55**,** 573-574.

Dainty, R. H. & Mackey, B. M. (1992). The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *Journal of Applied Bacteriology Symposium Supplement*, 73, 103S-114S.

Daun, C., Johansson, M., Önning, G. & Åkesson, B. (2001). Glutathione peroxidase activity, tissue and soluble selenium content in beef and pork in relation to meat ageing and pig RN phenotype. *Food Chemistry*, 73, 313-319.

Daun, C. & Åkesson, B. (2004). Comparison of glutathione peroxidase activity, and of total and soluble selenium content in two muscles from chicken, turkey, duck, ostrich and lamb. *Food Chemistry*, 85, 295-303.

De Brito, G. F., McGrath, S. R., Holman, B. W. B., Friend, M. A., Fowler, S. M., van de Ven, R. J. & Hopkins, D. L. (2016). The effect of forage type on lamb carcass traits, meat quality and sensory traits. *Meat Science*, 119, 95-101.

Deards, B., Leith, R., Mifsud, C., Murray, C., Martin, P. & Gleeson, T. (2014). *Live export trade assessment*. Department of Agriculture, Canberra.

Devine, C. E., Graafhuis, A., E., Muir, P. D. & Chrystall, B. B. (1993). The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Science*, 35, 63-77.

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science,* 36**,** 105-121.

Duckett, S. K., Klein, T. A., Dodson, M. V. & Snowder, G. D. (1998a). Tenderness of normal and callipyge lamb aged fresh or after freezing. *Meat Science,* 49**,** 19-26.

Duckett, S. K., Klein, T. A., Leckie, R. K., Thorngate, J. H., Busboom, J. R. & Snowder, G. D.(1998b). Effect of freezing on calpastatin activity

and tenderness of callipyge lamb. *Journal of Animal Science,* 76**,** 1869-1874.

Egan, A. F., Eustace, I. J. & Shay, B. J. (1988). Meat packaging – maintaining the quality and prolonging the storage life of chilled beef, pork and lamb. *Meat 88: Proceedings of Industry Day***,** 68-75.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J. & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science*, 42, 443-456.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science,* 89**,** 259-279.

Eustace, I., Gardner, G., Hildebrandt, A., Huynh, L., Jenson, I., Johnston, M., Kaur, M., Langbridge, J., Miles, D., Ross, T., Sumner, J., Tamplin, M., Torcello, P. & Wilkinson, K. (2014). *Shelf Life of Australian Red Meat*, Meat and Livestock Australia, Sydney.

Farouk, M. M. & Swan, J. E. (1998). Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Science,* 49**,** 233-247.

Farouk, M. M., Wieliczko, K. J. & Merts, I. (2003). Ultra-fast freezing and low storage temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Science,* 66**,** 171-179.

Farouk, M., Wiklund, E., Stuart, A. & Dobbie, P. (2009a). Ageing prior to freezing improves waterholding capacity in beef and venison. *Proceedings: 55th ICoMST. Copenhagen Denmark* (pp. 16-21).

Farouk, M., Wiklund, E., Stuart, A. & Dobbie, P. (2009b). Ageing prior to freezing improves the colour of frozen-thawed beef and venison. *Proceedings: 55th ICoMST, Copenhagen Denmark* (pp. 786-790).

Faustman, C., Sun, Q., Mancini, R. & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science,* 86**,** 86-94.

Fernandes, R. D. P. P., Freire, M. T. D. A., Carrer, C. D. C. & Trindade, M. A. (2013). Evaluation of physicochemical, microbiological and sensory stability of frozen stored vacuum-packed lamb meat. *Journal of Integrative Agriculture,* 12**,** 1946-1952.

Fernandes, R. P. P., Freire, M. T. A., de Paula, E. S. M., Kanashiro, A. L. S., Catunda, F. A. P., Rosa, A. F., Balieiro, J. C. C. & Trinidade, M. A. (2014). Stability of lamb loin stored under refrigeration and packed in different modified atmosphere packaging systems. *Meat Science*, 96, 554-561.

Fernández, P. P., Sanz, P. D., Molina-García, A. D., Otero, L., Guignon, B. & Vaudagna, S. R. (2007). Conventional freezing plus high pressure-low temperature treatment: physical properties, microbial quality and storage stability of beef meat. *Meat Science*, 77, 616-625.

Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., Kurt, E., Sinclair, L. A. & Wilkinson, R. G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed $\times$ production systems. *Meat Science,* 55**,** 141-147.

Food Science Australia (2005). Meat Technology Update: The cold chain for cartoned meat exports. *Meat Technology Update*, Newsletter 2/05, available at: http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-2.pdf.

FSANZ (2014). *Food Standards Code – Schedule 4. Nutrition, health and related claims* (July 2014) https://www.foodstandards.gov.au/code/code-revision/Documents/Schedules/62%20-%20Schedule%204%20.pdf.

Giannuzzi, L., Pinotti, A. & Zaritzky, N. (1998). Mathematical modelling of microbial growth in packaged refrigerated beef storted at different temperatures. *International Journal of Food Microbiology*, 39, 101-110.

Gill, C. O. (2004). Spoilage, factors affecting. In: Jensen, W. J., Devine, C. E. & Dikeman, M. (Eds.), *Encyclopedia of meat science* (pp. 1324-1330). Oxford, UK: Elsevier Ltd.

Gill, C. O. & Newton, K. G. (1981). Microbiology of DFD meat. In: Hood, D. E. & Tarrant, P. V. (Eds.), *The problem of dark-cutting in beef* (pp. 305-327). Netherlands: Springer.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B. & Givskov, M. (2002). Food spoilage—interactions between food spoilage bacteria. *International Journal of Food Microbiology,* 78**,** 79-97.

Greene, B. E. & Cumuze, T. H. (1981). Relationship between TBA numbers and inexperienced panelists'assessments of oxidized flavor in cooked beef. *Journal of Food Science,* 47**,** 52-54.

Gribble, A., Mills, J. & Brightwell, G. (2014). The spoilage characteristics of *Brochothrix thermosphacta* and two psychrotolerant *Enterobacteriacae* in vacuum packed lamb and the comparison between high and low pH cuts. *Meat Science,* 97**,** 83-92.

Grujić, R., Petrović, L., Pikula, B. & Amidžić, L. (1993). Definition of the optimum freezing rate—1. Investigation of structure and ultrastructure of beef M. longissimus dorsi frozen at different freezing rates. *Meat Science,* 33**,** 301-318.

Hagyard, C. J., Keiller, A. H., Cummings, T. L. & Chrystall, B. B. (1993). Frozen storage conditions and rancid flavour development in lamb. *Meat Science,* 35**,** 305-312.

Hinton, M., Holder, J. R., Hudson, W. R., Coombs, E., Allen, V. & Corry, J. E. L. (1998). The bacteriological quality of British beef 3. Frozen primal joints. *Meat Science,* 50**,** 403-409.

Holman, B. W. B., Alvarenga, T. I. R. C., van de Ven, R. J. & Hopkins, D. L. (2015). A comparison of technical replicate (cuts) effect on lamb Warner-Bratzler shear force measurement precision. *Meat Science*, 105, 93-95.Holman, B. W. B., Fowler, S. M. & Hopkins, D. L. (2016a). Are shear force methods adequately reported? *Meat Science*, 119, 1-6.

Holman, B. W. B., Mao, Y., Coombs, C. E. O., van de Ven, R. J. & Hopkins, D. L. (2016b). Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. *Meat Science*, 121, 104-106.

Holman, B. W. B., van de Ven, R. J., Mao, Y., Coombs, C. E. O. & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science*, 127, 57-62.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science,* 49**,** 447-457.

Honikel, K. O. & Hamm, R. (1994). Measurement of water-holding capacity and juiciness. In: Pearson, A. M. & Dutson, T. R. (Eds.), *Quality Attributes and their Measurement in Meat, Poultry and Fish Products* (pp. 125-161). Dordrecht: Springer.

Hopkins, D. L. & Thompson, J. M. (2001). The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. *Meat Science*, 57, 1-12.

Hopkins, D. L. & Thompson, J. M. (2002). Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact of tenderisation in beef and sheep meat. *Australian Journal of Agricultural Research*, 53, 149-166.

Hopkins, D. L., Hegarty, R. S., Walker, P. J. & Pethick, D. W. (2006). Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep. *Australian Journal of Experimental Agriculture,* 46**,** 879-884.

Hopkins, D. L., Lamb, T. A., Kerr, M. J. & van de Ven, R. J. (2013). The interrelationship between sensory tenderness and shear force measured by the G2 Tenderometer and a Lloyd texture analyser fitted with a Warner-Bratzler head. *Meat Science*, 93, 838-842.

Hopkins, D. L., Clayton, E. H., Lamb, T. A., van de Ven, R. J., Refshauge, G., Kerr, M. J., Bailes, K., Lewandowski, P. & Ponnampalam, E. N. (2014). The impact of supplementing lambs with algae on growth, meat traits and oxidative status. *Meat Science*, 98, 135-141.

Huff-Lonergan, E. J., Mitsuhashi, T., Beekman, D. D., Parrish Jr, F. C. & Olson, D. G. (1996). Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in post-mortem bovine muscle. *Journal of Animal Science*, 74, 993-1008.

Huff-Lonergan, E., Zhang, W. & Lonergan, S. M. (2010). Biochemistry of postmortem muscle — Lessons on mechanisms of meat tenderization. *Meat Science,* 86**,** 184-195.

Hughes, J. M., McPhail, N. G., Kearney, G., Clarke, F. & Warner, R. D. (2015). Beef *longissimus* eating quality increases up to 20 weeks of storage and is unrelated to meat colour at carcass grading. *Animal Production Science,* 55**,** 174-179.

Igene, J. O., Pearson, A. M., Dugan Jr, L. R. & Price, J. F. (1980). Role of triglycerides and phospholipids on development of rancidity in meat model systems during frozen storage. *Food Chemistry*, 5, 263-276.

Jacob, R. H., D'Antuono, M. F., Smith, G. M., Pethick, D. W. & Warner, R. D. (2007). Effect of lamb age and electrical stimulation on the colour stability of fresh lamb meat. *Australian Journal of Agricultural Research,* 58**,** 374-382.

Jeremiah, L. E. & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and

case-life of retail-ready beef. *Food Research International,* 34**,** 815-826.

Kanner, R. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Science*, 36, 169-189.

Kemp, C. M. & Parr, T. (2012). Advances in apoptotic mediated proteolysis in meat tenderisation. *Meat Science*, 92, 252-259.

Kenward, M. G. & Roger, J. H. (1997). Small sample inference for fixed effect estimators from restricted maximum likelihood. *Biometrics*, 53, 983-997.

Khan, M. I., Jo, C. & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors – A systematic review. *Meat Science*, 110, 278-284.

Khliji, S., Van De Ven, R., Lamb, T. A., Lanza, M. & Hopkins, D. L. (2010). Relationship between consumer ranking of lamb colour and objective measures of colour. *Meat Science,* 85**,** 224-229.

Kiermeier, A., Tamplin, M., May, D., Holds, G., Williams, M. & Dann, A. (2013). Microbial growth, communities and sensory characteristics of vacuum and modified atmosphere packaged lamb shoulders. *Food Microbiology,* 36**,** 305-315.

Kim, Y. H., Min, K. C. & Ahn, D. U. (2002). Color, oxidation-reduction potential, and gas production of irradiated meats from different animal species. *Journal of Food Science*, 67, 1692-1695.

Kim, Y. H. B., Frandsen, M. & Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. *Meat Science,* 88**,** 332-337.

Kim, Y. H. B., Luc, G. & Rosenvold, K. (2013). Pre rigor processing, ageing and freezing on tenderness and colour stability of lamb loins. *Meat Science*, 95, 412-418.

Kim, Y. H. B., Warner, R. D. & Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. *Animal Production Science, 54*, 375-395.

Kim, Y. H. B., Liesse, C., Kemp, R. & Balan, P. (2015). Evaluation of combined effects of ageing period and freezing rate on quality attributes of beef loins. *Meat Science*, 110, 40-45.

Koohmaraie, M. (1992). Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle mu-calpain. *Journal of Animal Science, 70*, 3071-3080.

Koohmaraie, M., Kent, M. P., Shackelford, S. D., Veiseth, E. & Wheeler, T. L. (2002). Meat tenderness and muscle growth: is there any relationship? *Meat Science, 62*, 345-352.

Kropf, D., Hunt, M. & Piske, D. (1996). Color formation and retention in fresh meat. *Proceedings Meat Indust. Res Conference American Meat Institute Washington, DC*, (p. 62).

Lagerstedt, Å., Enfält, L., Johansson, L. & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi. Meat Science, 80*, 457-461.

Ledward, D. A. (1985). Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science*, 15, 149-171.

Lee, S. K., Mei, L. & Decker, E. A. (1996). Lipid oxidation in cooked turkey as affected by added antioxidant enzymes. *Journal of Food Science*, 61, 726-728.

Leygonie, C., Britz, T. J. & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science,* 91**,** 93-98.

Li, S., Zamaratskaia, G., Roos, S., Båth, K., Meijer, J., Borch, E. & Johansson, M. (2015). Inter-relationships between the metrics of instrumental meat color and microbial growth during aerobic storage of beef at 4°C. *Acta Agriculturae Scandinavica, Section A — Animal Science***,** 1-10.

Liu, Q., Scheller, K. K., Arp, S. C., Schaefer, D. M. & Williams, S. N. (1996). Titration of fresh meat color stability and malondialdehyde development with Holstein steers fed vitamin E-supplemented diets. *Journal of Animal Science,* 74**,** 117-126.

Löndahl, G. & Nilaaon, T. (1993). Storage of frozen foods. *Encyclopaedia of food science and nutrition***,** 2732-2735.

Lowry, P. & Gill, C. O. (1985). Microbiology of frozen meat and meat products. *Microbiology of Frozen Foods***,** 109-68.

Mancini, R. A. & Hunt, M. C. (2005). Current research in meat color. *Meat Science,* 71**,** 100-121.

Martínez-Cerezo, S., Sañudo, C., Medel, I. & Olleta, J. L. Breed, slaughter weight and ageing time effects on sensory characteristics. *Meat Science*, 69, 571-578.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M. W., Bonham, M. P. & Fearon, A. M. (2010). Red meat

consumption: An overview of the risks and benefits. *Meat Science*, 84, 1-13.

McMillin, K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Science,* 80**,** 43-65.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science,* 79**,** 3062-3068.

Mills, J., Donnison, A. & Brightwell, G. (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science,* 98**,** 71-80.

Min, B. & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products – a review. *Food Science and Biotechnology*, 14, 152-163.

MLA (2015). *What is MSA sheepmeat?* Meat and Livestock Australia, Sydney, available at: http://www.mla.com.au/globalassets/mla-corporate/blocks/marketing-beef-and-lamb/msa_s1_web.pdf.

MLA (2016). *Fast facts: Australia's sheep industry*. Meat and Livestock Australia, Sydney, available at: https://www.mla.com.au/globalassets/mla-corporate/prices--markets/documents/trends--analysis/fast-facts--maps/mla_sheep-fast-facts-2016.pdf.

Moore, V. J. & Gill, C. O. (1987). The pH and display life of chilled lamb after prolonged storage under vacuum or under CO_2. *New Zealand Journal of Agricultural Research,* 30**,** 449-452.

Morgan, J. B., Miller, R. K., Mendez, F. M., Hale, D. S. & Savell, J. W. (1991). Using calcium chloride injection to improve tenderness of beef from mature cows. *Journal of Animal Science,* 69**,** 4469-4476.

Muela, E., Sañudo, C., Campo, M. M., Medel, I. & Beltrán, J. A. (2010). Effect of freezing method and frozen storage duration on instrumental quality of lamb throughout display. *Meat Science,* 84**,** 662-669.

Muela, E., Sañudo, C., Campo, M. M., Medel, I. & Beltrán, J. A. (2012). Effect of freezing method and frozen storage duration on lamb sensory quality. *Meat Science,* 90**,** 209-215.

Muela, E., Monge, P., Sañudo, C., Campo, M. M. & Beltrán, J. A. (2015). Meat quality of lamb frozen stored up to 21 months: Instrumental analyses on thawed meat during display. *Meat Science,* 102**,** 35-40.

Muela, E., Monge, P., Sañudo, C., Campo, M. M. & Beltrán, J. A. (2016). Sensory quality of lamb following long-term frozen storage. *Meat Science*, 114, 32-37.

Newton, K. G. & Gill, C. O. (1981). The microbiology of DFD fresh meats: A review. *Meat Science,* 5**,** 223-232.

Ngapo, T. M., Barbare, I. H., Reynolds, J. & Mawson, R. F. (1999). Freezing and thawing rate effects on drip loss from samples of pork. *Meat Science*, 53, 149-158.

Nollet, L. M. L. (2012). Packaging and freezing of beef as related to sensory properties. In: Nollet, L. M. L. (Ed.). *Handbook of meat, poultry and seafood quality* (pp. 246-253), John Wiley & Sons, Inc.

Ouali (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Meat Science*, 74, 251-265.

Pannier, L., Gardner, G. E., Pearce, K. L., McDonough, M., Ball, A. J., Jacob, R. H. & Pethick, D. W. (2014a). Associations of sire estimated breeding values and objective meat quality measurements with sensory scores in Australian lamb. *Meat Science*, 96, 1076-1087.

Pethick, D. W., Rowe, J. B. & Tudor, G. (1995). Glycogen metabolism and meat quality. *Recent Advances in Animal Nutrition in Australia*, 97-103.

Petrović, L., Grujić, R., Petrović, M. (1993). Definition of the optimal freezing rate - 2. Investigation of the physico-chemical properties of beef *M. longissimus dorsi* frozen at different freezing rates, *Meat Science*, 33, 319-331.

Ponnampalam, E. N., Trout, G. R., Sinclair, A. J., Egan, A. R. & Leury, B. J. (2001). Comparison of the color stability and lipid oxidative stability of fresh and vacuum packaged lamb muscle containing elevated omega-3 and omega-6 fatty acid levels from dietary manipulation. *Meat Science,* 58**,** 151-161.

Ponnampalam, E. N., Sinclair, A. J., Egan, A. R., Ferrier, G. R. & Leury, B. J. (2002). Dietary manipulation of muscle long-chain omega-3 and omega-6 fatty acids and sensory properties of lamb meat. *Meat Science*, 60, 125-132.

Ponnampalam, E. N., Norng, S., Burnett, V. F., Dunshea, F. R., Jacobs, J. L. & Hopkins, D. L. (2014a). The synergism of biochemical

components controlling lipid oxidation in lamb muscle. *Lipids*, 49, 757-766.

Ponnampalam, E. N., Butler, K. L., Pearce, K. M., Mortimer, S. I., Pethick, D. W., Ball, A. J. & Hopkins, D. L. (2014b). Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights. *Meat Science*, 96, 1095-1103.

Ponnampalam, E. N., Burnett, V. F., Norng, S., Hopkins, D. L., Plozza, T. & Jacobs, J. L. (2016). Muscle antioxidant (vitamin E) and major fatty acid groups, lipid oxidation and retail colour of meat from lambs fed a rougage based diet with flaxseed or algae. *Meat Science*, 111, 154-160.

Ponnampalam, E. N., Plozza, T., Kerr, M. G., Linden, N., Mitchell, M., Bekhit, A. E.-D. A., Jacobs, J. L. & Hopkins, D. L. (2017). Interaction of diet and long ageing period on lipid oxidation and colour stability of lamb meat. *Meat Science*, 129, 43-49.

Popova, T. & Marinova, P. (2013). Lipid oxidation in *M. longissimus dorsi* and *M. semimembranosus* in lambs reared indoors and on pasture. *Iranian Journal of Applied Animal Science*, 3, 533-537.

Popova, T. (2014). Fatty acid composition during storage in *Longissimus dorsi* and *Semimembranosus* in lambs, reared indoors and on pasture. *Emirates Journal of Food and Agriculture*, 26, 302-308.

R Core Team (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from www.R-project.org.

R Core Team (2016). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. URL www.R-project.org.

Rahelić, S., Puač, S. & Gawwad, A. H. (1985). Structure of beef *Longissimus dorsi* muscle frozen at various temperatures: Part 1—histological changes in muscle frozen at −10, −22, −33, −78, −115 and −196°C. *Meat Science,* 14**,** 63-72.

Reis, M. M., Reis, M. G., Mills, J., Ross, C. & Brightwell, G. (2016). Characterization of volatile metabolites associated with confinement odour during the shelf-life of vacuum packed lamb meat under different storage conditions. *Meat Science*, 113, 80-91.

Risvik, E. (1994). Sensory properties and preferences. *Meat Science,* 36**,** 67-77.

Rosenvold, K. & Wiklund, E. (2011). Retail colour display life of chilled lamb as affected by processing conditions and storage temperature. *Meat Science*, 88, 354-360.

Rousset-Akrim, S., Young, O. A. & Berdagué, J. L. (1997). Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science*, 45, 169-181.

Rowe, L. J., Maddock, K., Lonergan, S. M. & Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. *Journal of Animal Science,* 82**,** 3254-3266.

Samouris, G., Kasapiou, E., Ioannidou, M. & Eleftheriadou, A. (2011). Changes in lipid oxidation levels and fatty acid composition in the

meat of milk-fed lambs during refrigerated and frozen storage – Nutritional indices. *Jounral of Food Safety and Food Quality*, 62, 217-222.

Savell, J. & Cross, H. (1988). The role of fat in the palatability of beef, pork, and lamb. In: Call, D. L. *et al.* (Eds.). *Designing foods: Animal product options in the marketplace* (pp. 345-355)**,** Washington, DC: National Academy Press.

Shanks, B. C., Wulf, D. M. & Maddock, R. J. (2002). Technical note: The effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. *Journal of Animal Science,* 80**,** 2122-2125.

Shorthose, W. R. & Harris, P. V. (1991). Effects of growth and composition on meat quality. In: Pearson, A. M. & Dutson, T. R. (Eds.), *Growth regulation in farm animals*, Vol. 17 (pp. 515-555), London: Elsevier Applied Science.

Sigma-Aldrich (2015). *Peroxidase acitivty assay kit, technical bulletin MAK092*. Sigma Aldrich Co, LLC.

Sigma-Aldrich (2016). *Glycogen assay kit, technical bulletin MAK016*. Sigma-Aldrich Co. LLC.

Small, A. H., Jenson, I., Kiermeier, A. & Sumner, J. (2012). Vacuum-packed beef primals with extremely long shelf life have unusual microbiological counts. *Journal of Food Protection,* 75**,** 1524-1527.

Smith, M. A., Bush, R. D., van de Ven, R. J. & Hopkins, D. L. (2017). The effect of grain supplementation on alpaca (*Vicunga pacos*) production and meat quality. *Small Ruminant Research*, 147, 25-31.

Smulders, F. J. M., Marsh, B. B., Swartz, D. R., Russell, R. L. & Hoenecke, M. E. (1990). Beef tenderness and sarcomere length. *Meat Science,* 28**,** 349-363.

Spooncer, W. F. (1998). Vacuum-packed meat. *Meat quality, packaging new products workshop (Chapter 4).* Richmond, NSW: CSIRO. Available at: http://www.meatupdate.csiro.au/data/Meat_quality_and_packaging_04-88.pdf

Starkey, C. P., Geesink, G. H., Oddy, V. H. & Hopkins, D. L. (2015). Explaining the variation in lamb *longissimus* shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Science,* 105**,** 32-37.

Sumner, J. (2016). *The impact of transport to Australia's distant markets*. Sydney, Australia: Australian Meat Processor Corporation. Retrieved from: http://www.ampc.com.au/2016/05/The-impact-of-transport-to-Australias-distant-markets.

Sumner, J. & Jenson, I. (2011). The effect of storage temperature on shelf life of vacuum-packed lamb shoulders. *Food Australia,* 63, 249-251.

Tapp, W. N. III, Yancey, J. W. S. & Apple, J. K. (2011). How is the instrumental color of meat measured? *Meat Science,* 89**,** 1-5.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. & Dugan Jr, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society,* 37**,** 44-48.

Thompson, J. M., Hopkins, D. L., D'Souza, D. N., Walker, P. J., Baud, S. R. & Pethick, D. W. (2005). The impact of processing on sensory and objective measurements of sheep meat eating quality. *Australian Journal of Experimental Agriculture*, 45, 561-573.

UK P & I Club (2006). *Carefully to carry: Meat and meat products in containers*. London, UK: Thomas Miller P & I Ltd, available at: https://www.ukpandi.com/fileadmin/uploads/uk-pi/LP%20Documents/Carefully_to_Carry/Meat%20and%20meat%20products%20in%20containers.pdf.

USDA (2013). Freezing and food safety. United States Department of Agriculture: Food Safety and Inspection Service, available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/freezing-and-food-safety/CT_Index.

Verbyla, A. P., Cullis, B. R., Kenward, M. G. & Welham, S. J. (1999). The analysis of designed experiments and longitudinal data by using smoothing splines. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, 48, 269-311.

Vieira, C., Diaz, M. T., Martínez, B. & García-Cachán, M. D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science,* 83**,** 398-404.

Warner, R. D., Greenwood, P. L., Pethick, D. W. & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. *Meat Science*, 86, 171-183.

Watson, R., Gee, A., Polkinghorne, R. & Porter, M. (2008). Consumer assessment of eating quality – development of protocols for Meat Standards Australia (MSA) testing. *Australian Journal of Experimental Agriculture,* 48**,** 1360-1367.

Wiklund, E., Farouk, M. M., Stuart, A., Cartwright, S., Penney, N. & Rosenvold, K. (2009a). Quality of chilled-never-frozen versus chilled-frozen-thawed lamb. *Proceedings of the New Zealand Society of Animal Production*, Vol. 69.

Wiklund, E., Farouk, M. M., Stuart, A. & Dobbie, P. (2009b). Consumer evaluation of chilled-never-frozen versus chilled-frozen-thawed beef and venison. *Proceedings: 55th ICoMST, Copenhagen, Denmark* (pp. 1206-1209).

Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics,* 64**,** S113-S119.

Winger, R. J. (1984). Storage life and eating-related quality of New-Zealand frozen lamb: A compendium of irrepressible longevity In: Zeuthen, P. (Ed.), *Thermal processing and quality of foods* (pp. 541-543). London: Elsevier.

Winger, R. J. & Hagyard, C. J. (1994). Juiciness — its importance and some contributing factors. In: Pearson, A. M. & Dutson, T. R. (Eds.), *Advances in Meat Research* (pp. 94-124), Glasgow: Chapman & Hall.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I. & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society,* 58**,** 363-370.

Wood, J. D., Richardson, R. I., Nute, G.R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R. & Enser, M. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66, 21-32.

Xia, X., Kong, B., Liu, Q. & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze–thaw cycles. *Meat Science,* 83**,** 239-245.

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. In: Decker, E. A., Faustman, C. & Lopez-Bote, C. J. (Eds.), *Antioxidants in muscle foods: nutritional strategies to improve quality* (pp. 85-111). New York: Wiley.

Young, O. A., Berdagué, J. L., Viallon, C., Rousset-Akrim, S. & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science,* 45**,** 183-200.

ZeptoMetrix (2011). *OXItek TBARS assay kit: Thiobarbituric acid reactive substances, technical bulletin (Revision 2).* ZeptoMetrix Corporation. Retrieved from: http://www.zeptometrix.com/docs/PI0801192.pdf?1456512819.

Zhou, G. H., Xu, X. L. & Liu, Y. (2010). Preservation technologies for fresh meat – a review. *Meat Science*, 86, 119-128.