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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 oxidation and fatty acid profiles

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ABSTRACT

Lipid oxidation and fatty acid (FA) profiles were evaluated for beef *M. longissimus lumborum* (LL) stored under different chilled-then-frozen storage combinations (up to 5 and 52 weeks, respectively) and two frozen holding temperatures (−12 °C and −18 °C). 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.

1. Introduction

Beef is a rich source of dietary fat that includes many essential and beneficial fatty acids (FA) important to a balanced diet (Williams, 2007; Wyness et al., 2011). Production systems and genetic tools have been applied to optimise beef FA composition (Howes, Bekhit, Burritt, & Campbell, 2015) and in doing so, capitalise of consumer preference for healthy and nutritious food options (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017). The level of polyunsaturated FA (PUFA) in beef 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).

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, Holman, Friend, & Hopkins, 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, McPhail, Kearney, Clarke, & Warner, 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 et al., 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.

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Table 1
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, C13:0, 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, C18:3 t, 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, C18:3 t, C18:3ω3, C18:3ω6,C20:2ω6, C20:3ω3, C20:3ω6, C20:4ω3, ***C20:4ω6***, C20:5ω3, C22:2ω6, C22:5ω3, C22:6ω3
Omega-6 s (n-6)	***C18:2ω6***, C18:2ω6t, C18:3ω6,C20:2ω6, C20:3ω6, ***C20:4ω6***, C22:2ω6
Omega-3 s (n-3)	***C18:3ω3***, C20:3ω3, C20:4ω3, ***C20:5ω3***, C22:5ω3, C22:6ω3
Health claimable fatty acids (EPA + DHA)	C20:5ω3, C22:6ω3

Holman, Coombs, Morris, Kerr, and Hopkins (2017) have already discussed the potential benefits to industry from better understanding long term chilled-then-frozen storage effects on beef quality – including improvements to market access; economic management of exporting shipping speeds, production gluts and distribution logistics; and optimising sub-zero frozen holding temperatures. These would be enhanced by assuring beef that was a source or good source of omega-3s when first processed will remain as such upon delivery to sometimes geographic and chronologically distant markets. As a consequence, we aimed to explore chilled-then-frozen storage period combination effects on beef's capacity for lipid oxidation and its influence on FA profiles, when two different holding temperatures were applied.

2. Materials and methods

2.1. Experimental design and sampling

This study was undertaken parallel and complementary to Holman, Coombs, et al. (2017) and as such, the duplicities between experimental design and statistical analyses are acknowledged. In brief, a total of 48 beef *M. longissimus lumborum* (LL) were randomly selected from the boning room of a commercial Australian abattoir. These were each divided into four equal portions ($n = 192$) and assigned to each of four chilled storage periods (0, 2, 3 and 5 weeks) × six subsequent frozen storage periods (0, 4, 8, 12, 24 and 52 weeks) × two frozen storage holding temperatures (−12 °C and −18 °C, observed mean ± standard deviations equal to −11.5 ± 0.7 °C and −18.0 ± 0.4 °C respectively) combinations. Portion assignments were balanced within LL, as near as possible, over frozen storage period and the remaining combinations fitted with randomly allotted LL portions.

On-site at the abattoir, these LL portions were vacuum-packaged, processed and stored for the duration of their assigned chilled storage period under refrigeration (observed mean ± standard deviations equal to 0.1 ± 0.4 °C) before, and only when allocated for frozen storage (frozen storage period > 0), being frozen using an industrial plate freezer. Frozen LL portions were transported to the Cowra Meat Laboratory (Centre for Red Meat and Sheep Development, New South Wales, AUS) where they were held as per their frozen storage periods within one of two replicate freezers set at each frozen storage holding temperature (total: four freezers). Once storage treatment combinations were complete, the corresponding LL portions were sectioned with care taken to maintain aseptic conditions and status (i.e. frozen LLs were sectioned still frozen and not allowed to thaw). Subsections not immediately tested were kept at −80 °C until analysis.

As per the complementary study; in an effort to improve readability, chilled storage period and following frozen storage period 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.

2.2. Fatty acid profile analysis

A portion from each sample was freeze-dried at −50 °C (ScanVac CoolSafe™, LaboGene Ltd., Lynge, DEN) and then ground in a sample mill (model 1095, Knifetech™, FOSS Pacific Ltd., New South Wales, AUS) to produce a homogenous sample. A protocol adapted from Ponnampalam et al. (2014) was then used to determine FA profiles in a commercial laboratory, wherein approximately 5.0 mg freeze-dried sample 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 μL 10 N potassium hydroxide (KOH; Sigma-Aldrich Ltd., New South Wales, AUS) and 5.3 mL of methanol and incubated for 90 min at 55 °C with 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 again repeated. 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 autosampler vials to be analysed by gas chromatography (GC).

CG column settings were: 60 m × 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 (Table 1) and the ratio of omega-3 to omega-6 (Rn3n6).

2.3. Thiobarbituric acid reactive substance analysis

Sample TBARS quantification method was adapted from Hopkins et al. (2014) and used 500.0 μL Radioimmunoprecipitation assay (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 analysed 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. Technical duplicates were averaged and expressed as mg malondialdehyde (MDA) per kg LL.

2.4. Peroxidase activity analysis

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