



Effect of Postmortem Aging and Fast-Freezing on Meat Quality of Various Lamb Cuts Under Prolonged Frozen Storage and Repeated Freezing/Thawing Conditions

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Abstract: This study aimed to investigate the effects of aging and fast-freezing on meat quality attributes of various lamb cuts under conditions of prolonged frozen storage (Experiment 1) and repeated freezing/thawing cycles (Experiment 2). The paired lamb muscles including *longissimus dorsi*, *gluteus medius*, *quadriceps femoris*, *semimembranosus*, and *biceps femoris* from 15 lamb carcasses were collected at 1 d postmortem. In Experiment 1, the muscles were vacuum-packaged and randomly assigned to one of 3 treatments: (1) non-frozen control (aged only for 5 wk at -1.5° C); (2) aging for 4 wk, fast-frozen in a -18° C glycol immersion chamber, and frozen storage in -18° C for 1 week; or (3) aging for 4 wk, fast-frozen, and frozen storage for 24 wk. In general, regardless of muscle cuts, samples that were aged, fast-frozen, stored for 1 wk, and thawed exhibited similar water-holding capacity, shear force, and color attributes as those of the aged-only (never frozen) lamb muscles (P > 0.05). Furthermore, extending the frozen storage duration up to 24 wk did not result in any adverse effects on color, shear force, purge loss, or microbiological attributes of the aged/fast-frozen/thawed lamb muscles (P > 0.05). In Experiment 2, the repeated freezing and thawing of aged and fast-frozen lamb loins (n = 8) had no adverse impacts on color, tenderness, or microbiological attributes (P > 0.05), although slight increases in purge and cook losses were observed compared to non-repeated slow-frozen loins. The findings of the present study suggest that the combined treatment of aging and fast-freezing can minimize changes in meat quality during the freezing and thawing process, even with prolonged frozen storage of up to 24 wk.

Keywords: aging, fast-freezing, lamb, thawing, tenderness, water-holding capacityMeat and Muscle Biology 8(1): 17604, 1–10 (2024)doi:10.22175/mmb.17604Submitted 19 December 2023Accepted 26 January 2024

Introduction

Freezing is widely recognized as an effective and convenient method for preserving meat, employed in both households and the meat industry. While freezing significantly extends the shelf life of meat by inhibiting the growth of microorganisms, it can also lead to the deterioration of meat quality, such as increases in purge/thaw loss, discoloration, and/or texture defects (Kim et al., 2018). These quality deteriorations can be attributed to physicochemical and structural damage caused by ice crystals to muscle cell membranes and muscle tissues (Kiani and Sun, 2011).

The quality attributes of frozen meat are primarily influenced by the formation pattern of ice crystals, known as ice crystallization. In particular, the rate of freezing determines the location, distribution, size, and number of ice crystals in muscle tissues, which in turn impact the meat quality attributes (Leygonie et al., 2012). In general, slow-freezing rates lead to the formation of extracellular large ice crystals, which can substantially damage muscle cells and structures upon thawing. This results in excessive water loss and protein denaturation in frozen/thawed meat (Chabela and Oyague, 2004). In contrast, fast-freezing, which forms fine and uniform intracellular ice crystals, has been recognized as a method to minimize the negative impacts of freezing on meat quality attributes. Several studies reported decrease in purge loss (Kim et al., 2018), no adverse impacts on shear force and texture (Kim et al., 2015; Kim et al., 2023), and an increase in protein solubility (Tuell et al., 2020) of meat undergone fast-freezing.

In addition to freezing rate, another crucial postharvest factor with a significant impact on the quality attributes of frozen/thawed meat is the extent of postmortem aging prior to freezing. During the aging process, natural endogenous enzymes hydrolyze the myofibrillar structural proteins, which consequently allow muscle cells to swell and entrap more moisture within the intracellular space. The swollen muscle structure with a fragmented protein matrix due to aging results in a reduction in the extent of mechanical damage caused by the formation of ice crystals (Kim et al., 2018; Zhang et al., 2023). Significant improvements in instrumental tenderness (measured by shear force values) and water-holding capacity of aged/frozen/ thawed meat by decreasing either purge or drip loss have been observed (Farouk et al., 2009a; Farouk et al., 2009b; Kim et al., 2011a; Wiklund et al., 2009). In this context, a study conducted by Kim et al. (2015) revealed the positive impacts of integrating postmortem aging and fast-freezing on the quality attributes of beef loins. The research demonstrated a significant decrease in purge/thaw loss and drip loss of beef loins subjected to aging followed by fast-freezing.

Taken together, it can be proposed that applying postmortem aging along with fast-freezing could be an effective value-adding process to improve meat quality and value of frozen/thawed meat. However, the efficacy of the combined application of aging and fast-freezing on various lamb muscle cuts has never been thoroughly investigated. Furthermore, there is limited information on how aging, when combined with fast-freezing, may impact the quality attributes of lamb muscle cuts during prolonged frozen storage. This storage period could lead to the formation of large ice crystals due to the growth and fusion of ice crystals (Wang et al., 2020). Additionally, given that the practice of repeated freezing/thawing is prevalent in retail conditions and/or consumers' households, it would be valuable to evaluate the effects of multiple cycles of freezing and thawing on the meat quality attributes of lamb loins. These loins, having undergone aging and fast-freezing initially, would

provide insights into the cumulative impacts of those combined treatments on meat quality attributes. Therefore, in two separate experiments, the objectives of this study were to determine the effect of aging and fast-freezing on meat quality attributes of various lamb muscle cuts under extended frozen storage (up to 24 wk) and repeated freezing/thawing (2 cycles) conditions.

Materials and Methods

Raw materials and sampling procedure

Experiment 1-Effect of aging then fast-freezing on quality attributes of various lamb cuts during extended frozen storage. Fifteen lambs (about 4 mo old; average hot-carcass weight 18 kg) were selected at a local meat processing plant. The ultimate pH of all lamb carcasses, measured by inserting a portable pH meter (Testo 205 pH meter, Lenzkirch, Germany; calibrated by standard solutions pH 4.01 and 7.00) directly into the loin (longissimus) muscle between the 11th and 12th rib of each carcass, was found to be less than 5.8 at 24 h postmortem. Five different muscle cuts including the loin (longissimus dorsi [LD]), rump (gluteus medius [GM]), knuckle (quadriceps femoris [QF]), inside round (semimembranosus [SM]), and outside round (biceps femoris [BF]), were immediately excised from both sides of each carcass, vacuum packaged, and then transported to AgResearch (Ruakura, New Zealand) via a refrigerated truck under a chilling condition (approximately 3-5°C). Each lamb muscle from each side of carcasses was randomly assigned to 3 different treatments based on the balanced incomplete block design [(2 sides \times 15 carcasses)/3 treatments, n = 10]. Three aging/freezing treatments were prepared as follows: (1) aging at -1.5° C for 5 wk (A5, aged only-never frozen control); (2) aging at -1.5° C for 4 wk, then rapidly freezing in a -18° C glycol immersion chamber and frozen storage in a -18° C air blast freezer for 1 wk (A4F1, short period of frozen storage); and (3) aging at -1.5° C for 4 wk, rapidly freezing in a -18°C glycol immersion chamber and storage in a -18°C air blast freezer for 24 wk (A4F24, prolonged frozen storage). At the end of each assigned frozen storage treatment, the frozen muscles were thawed at 3°C overnight and processed to evaluate meat quality assessments such as pH, water-holding capacity (purge loss, drip loss, and cooking loss), shear force, initial color characteristics (lightness, redness, and chroma), and microbial quality attributes.

Experiment 2-Effect of postmortem aging and fast-freezing with repeated freezing/thawing on quality characteristics of lamb loins. Paired loins (LD) were separated at 1 d postmortem from 8 lamb carcasses (about 4 mo old; average hot-carcass weight $18 \text{ kg}; \leq pH 5.8 \text{ at } 24 \text{ h postmortem}$, vacuum packaged, and randomly assigned to either aging/slow-freezing/ thawing (A-SFT) or aging/fast-freezing/thawing/slowfreezing/thawing (A-FT-SFT) [(2 sides \times 8 carcasses)/ 2 treatments, n = 8]. (In detail, A-SFT: aging at -1.5° C for 4 wk, slow-freezing and frozen storage in a $-18 \pm 2^{\circ}$ C consumer type chest freezer for 2 wk and then thawing at 4° C; and A-FT-SFT: aging at -1.5° C for 4 wk, fast-freezing in a -18° C immersion glycol tank at stored for 1 wk, thawing at 4°C, freezing again in a $-18 \pm 2^{\circ}$ C consumer type chest freezer for 2 wk and then thawed at 4°C.) The A-FT-SFT treatment was designed with the consideration for simulating repeated freezing/thawing in home (slow freezing by chest freezer) after purchasing aged/fast-frozen/thawed meat.

Fast-freezing and thawing process

The samples assigned to fast-freezing were placed in a glycol immersion tank $(-18^{\circ}C)$, while the samples assigned to slow-freezing (in Experiment 2) were located in an air-freezer $(-18^{\circ}C)$. The freezing temperature was monitored using an Agilent HP 75000 Series C Data Acquisition & Control unit set at 1-min intervals for both fast and slow conditions.

The immersion tank was a 250-liter insulated tank fitted with a heat exchanger. The tank heat exchanger was connected to a pump, which, in turn, was linked to an in-house designed refrigeration unit. The unit comprised a Kirby Polar Pack condenser unit equipped with a titanium plate heat exchanger. The glycol solution was circulated via the cooling unit and also employed as the working fluid in the immersion tank.

For the immersion trials, the tank was operated at -17 to -18° C before the sample placement. The lamb muscle samples were fitted with T-Type thermocouples in sets of 4 mounted on thin rods—3 placed within 5 mm of each other to ensure a center temperature and a 4th inserted just under the sample surface to monitor the surface temperature. All thermocouples were calibrated at 0°C in an ice reference before use, and the difference from zero was added or removed before data analysis. The changes in temperature of the muscle samples are shown in Figure 1.

рΗ

The pH of the lamb muscles was determined by inserting a calibrated pH probe (Testo 205 pH meter, Lenzkirch, Germany) directly into the meat.

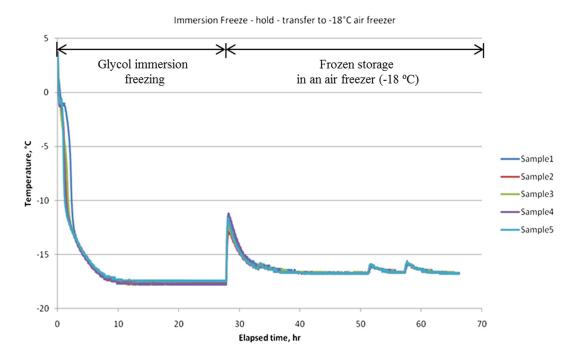


Figure 1. Temperature decline trends of the lamb muscles through a glycol immersion fast-freezing and temperature monitoring during storage in $a - 18^{\circ}C$ air freezer.

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Purge loss, drip loss, and cooking loss

To determine water-holding capacity of the sample during aging/freezing/thawing procedure, various water loss measurements which included purge loss, drip loss, and cooking loss were conducted as described by Setyabrata and Kim (2019). For purge loss measurement, the sample weight prior to vacuum-packaging was measured as the initial sample weight. Then, after each assigned storage time, the samples were thawed in a 4°C cooler (about 24 h), removed from the vacuum-packaged bags, lightly blotted with paper towels on the surface of the sample, and reweighed to estimate purge loss as the weight difference between initial and final weights.

To determine drip loss following each assigned storage period, a cubic size sample of meat (approximately 50 g) with any visible fat and connective tissue removed was weighed and then individually placed in a plastic mesh and suspended by a hook within a closed Ziploc container. After 48 h at 4°C, the sample was blotted dry and then reweighed. Drip loss was determined as the percentage of weight lost relative to the original sample weight.

Cooking loss was measured in accordance with Kim et al. (2015) after the storage period subsequent to the cooking procedure. The samples underwent cooking in a water bath at 99°C until reaching an internal temperature of 75°C, monitored by a Digi-Sense scanning temperature logger (Eutech Instruments Pte Ltd., Singapore) with a thermocouple placed in the center of each sample. Post-cooking, the samples were transferred to an ice-water slurry for at least 10 min, and the weight of the cooked sample was measured. Cooking loss was then calculated as the difference between weights before and after cooking, expressed as a percentage of the pre-cooked weigh.

Shear force

Following the assessment of cooking loss, the meat samples were utilized for shear force analysis. Shear force was measured by determining the force needed to shear through a 10 mm \times 10 mm cross-section sample at right angles to the fiber axis, employing the MIRINZ tenderometer as described by Chrystall and Devine (1991) and MacFarlane and Marer (1966). Ten replicates were measured for each pre-cooked sample, and the results were presented as shear force (kgF).

Color measurement

The aged meat removed from the vacuum package was conducted for an hour bloom to return deoxymyoglobin to oxymyoglobin. Meat surface color after aging/freezing/thawing treatments was measured using a Minolta Color Meter (CR-300; Konica Minolta Photo Imaging Inc., Mahwah, NJ, USA) calibrated using a standard white tile (Y = 93.5, x = 0.3132, and y = 0.3198). CIE L* (lightness), a* (redness), and b* (yellowness) values were measured (Illuminant D65, 1 cm diameter aperture, 10° standard observer) through overwrapping with polyvinylchloride (PVC) film at 3 different locations per each sample. CIE L*a*b* values were used to calculate hue angle [(b*/a*)^{tan-1}] and chroma [(a*²+b*²)^{1/2}] (King et al., 2023).

Aerobic plate count

Aerobic plate count (APC) of the lamb muscles after assigned aged/frozen storage was conducted in accordance with the procedure outlined in the AOAC (2020) proficiency testing program 990.12.

Data analysis

The data were analyzed using the ANOVA with REML directive of GenStat (12th Edition, 2010; GenStat for Windows, version 12.2.0.3717, VSN International, Oxford). Animal served as the random effect, while treatments and the type of lamb muscles were considered fixed effects. Experiment 1 consisted of a total of 10 replicates, while Experiment 2 comprised 8 replicates. Least-squares means for all relevant traits were differentiated using an F test (P < 0.05) and least significant differences.

Results and Discussion

Experiment 1—Effect of aging then fastfreezing on quality attributes of various lamb cuts during extended frozen storage

Temperature decline. The fast-freezing of lamb muscles was accomplished by immersing the vacuumpackaged muscle samples in a glycol immersion tank, reaching -15° C in less than 4 h. Notably, within just 1 h, the internal temperature of the samples traversed the critical zone, ranging from -1 to -8° C, wherein the size and characteristics of ice crystals are consistently determined (Kiani and Sun, 2011). It has been wellestablished that the formation of fine ice crystals through fast-freezing ensures a uniform distribution both inside and outside the muscle cells. Consequently, this could minimize the degradation of product quality during freezing and thawing by inflicting less damage to the muscle tissues (Zhang et al., 2023).

pH value and water-holding capacity (purge, drip, and cooking loss). The impact of aging then fast-freezing on the pH values of various lamb muscles during extended frozen storage conditions is presented in Table 1. Neither freezing nor the duration of frozen storage had an influence on the pH value (P > 0.05). A significant difference in pH values, however, was observed between the muscle cuts, where SM and GM had lower values compared to LD, BF, and QF (P < 0.05). These findings align with a study conducted by Tschirhart-Hoelscher et al. (2006), who observed lower pH values for SM and GM muscles between a set of 18 lamb muscles. The pH variation among muscles has been extensively explored in previous research and can be attributed to differences in muscle fiber composition and the associated energy metabolism (Tarrant and Sherington, 1980; Tschirhart-Hoelscher et al., 2006). These muscle-specific pH variations can have broader implications for meat quality attributes such as waterholding capacity, tenderness, and color characteristics (Picard and Gagaoua, 2020).

No significant difference in purge loss was found between the aged-only (A5; never frozen control) samples and aged/fast-frozen thawed (A4F1) and aged/ fast-frozen/long-term stored/thawed (A4F24) samples (P > 0.05; Table 1). A significant muscle effect was observed, with SM exhibiting the highest purge loss, followed by BF, LD, GM, and QF, which had the least purge loss (P < 0.001).

The thawing process of frozen meat is wellestablished as an integral step that can result in a significant loss of water from the meat (Xia et al., 2009). This loss, also referred to as thawing loss, is a result of damage to cell membranes and muscle tissues caused by ice crystal formation, as well as protein denaturation due to increased ionic strength (Leygonie et al., 2012). Furthermore, prolonged frozen storage, which allows for the growth and fusion of ice crystals, results in increased loss of water-soluble components such as sarcoplasmic proteins, vitamins, and minerals, in addition to water itself (Wang et al., 2020). The results of the present study, however, showed that the combined treatment of aging and fast-freezing did not adversely affect purge/thaw loss of lamb cuts throughout the freezing, frozen storage, and thawing processes when compared to aged-only (never frozen) control. While purge/thaw loss stands out as a prominent quality defect associated with frozen/thawed meat, several studies have highlighted the positive impacts of postmortem aging before freezing in mitigating the initial purge loss of thawed meat from various species, including beef, lamb, and pork (Wiklund et al., 2009; Kim et al., 2018; Setyabrata et al., 2019). This phenomenon can be explained by the "sponge effect." Farouk et al. (2012) reported that increasing the aging time before freezing reduced thawing loss and expressible water due to an improvement in water-holding capacity by aging. Alterations in muscle structure, induced by the hydrolysis of endogenous proteolytic enzymes during aging, can result in the development of a spongelike complex. This intricate structure serves to enhance the retention of water molecules, thereby contributing to a reduction in purge loss upon the thawing of meat (Farouk et al., 2012).

Also, the benefit of applying fast-freezing, which yields fine and uniform intracellular ice crystals within

	Treatments (T) ¹				Lamb cuts (C) ³						Significance of P value ⁴		
Traits	A5	A4F1	A4F24	SED^2	QF	LD	SM	GM	BF	SED	Т	С	T×C
pH value	5.91	5.90	5.89	0.019	5.97 ^a	5.95ª	5.81 ^b	5.80 ^b	5.96 ^a	0.042	NS	***	NS
Water-holding capacity													
Purge loss (%)	3.66	3.34	3.57	0.187	1.96 ^d	2.81°	5.44 ^a	2.63°	4.79 ^b	0.279	NS	***	NS
Drip loss (%)	1.20 ^b	1.70 ^a	1.76 ^a	0.075	1.32 ^b	1.72 ^{ab}	1.37 ^b	1.48 ^{ab}	1.87 ^a	0.086	***	***	NS
Cooking loss (%)	27.69 ^b	27.54 ^b	29.54 ^a	0.817	28.83 ^{ab}	27.49 ^b	29.77ª	28.01 ^{ab}	27.17 ^b	0.920	*	*	NS

Table 1. pH value and water-holding capacity (purge loss, drip loss, and cooking loss) of aged/fast-frozen/thawed various lamb cuts (n = 10)

^{a-c}Means with different letters within each effect (treatment or lamb cut) are significantly different (P < 0.05).

¹Treatments (T): A5: aged-only control for 5 wk; A4F1: aging for 4 wk, fast-freezing and frozen storage for 1 wk; A4F24: aging for 4 wk, fast-freezing and frozen storage for 24 wk.

²SED: standard errors of difference.

³Lamb cuts: QF, *Musculus quandriceps femoris*; LD, *Longissimus dorsi*; SM, *Semimembranosus*; GM, *Gluteus medius*; BF, *Biceps femoris*. ⁴Significance of *P* value: NS, no significance (P > 0.05); *P < 0.05; ***P < 0.001. muscle fibers, has been well-established as an effective method for mitigating the detrimental effects of freezing and frozen storage, particularly decreasing purge/ thaw loss (Choi et al., 2018). Balan et al. (2019) reported a substantial decrease in purge loss of aged lamb *longissimus lumborum* when subjected to fastfreezing compared to slow-frozen counterparts.

Conversely, a higher drip loss was observed in the aged/frozen/thawed cuts (A4F1 and A4F24 treatments) compared to the aged-only control across all 4 lamb cuts (P < 0.001). While a significant difference was found, the difference between the treatments was less than 0.5%, indicating that it would be unlikely to have a practical impact on meat quality attributes.

Similar to the findings for drip loss, cooking loss was influenced by the freezing and frozen storage treatment (P < 0.05) and the type of lamb cuts (P < 0.05). The A4F24 treatment demonstrated a higher cooking loss than both A5 and A4F1 (P < 0.05) regardless of the cuts. This observation indicates that prolonged frozen storage duration resulted in increased cooking loss compared to the aged-only and aged/frozen short-term treatments (P < 0.05). Studies have concluded that during long-term frozen storage, the storage temperature may hold more significance than the initial freezing rate (Farouk et al., 2004; Choi et al., 2018). Considering the lack of significant differences in cooking loss between A5 and A4F1, it can be suggested that, for meat

subjected to prolonged frozen storage, the storage duration (and possibly storage temperature) may be more critical than the initial freezing rate. For the lamb cut effect, the SM exhibited a significantly higher cooking loss compared to LD and BF. Tschirhart-Hoelscher et al. (2006) previously reported that LD had lower expressible moisture compared to SM, suggesting a potential association between lower expressible moisture and higher muscle pH, which aligns with our findings.

Shear force. The impact of aging then fastfreezing on the shear force values of various lamb cuts under prolonged frozen storage duration is depicted in Table 2. A significant interaction between freezing treatments and different lamb muscle types on instrumental tenderness was found (P < 0.05). No difference in shear force values between the treatments was observed in LD and BF (P > 0.05). However, for SM and GM, a slightly lower shear force value was found in A4F1 compared to A5 and A4F24 (P < 0.05), while no difference was found between A5 and A4F24 (P > 0.05). Interestingly, for the QF muscle, an increase in shear force attributed to prolonged frozen storage (A4F24) was determined (P < 0.05), while A5 and A4F1 showed no difference (P > 0.05). These observations could indicate muscle-specific responses to aging/fast-freezing/frozen duration in the instrumental tenderness values. It has to be noted, however, that the shear force values of meat samples were

Traits	Treatments ¹ (aging/freezing periods)	QF	BF	SED ³	P value			
Shear force (kgF)	A5	3.19 ^{c,y}	3.13 ^c	5.18 ^{a,xy}	3.94 ^{b,x}	3.56 ^b	0.07	0.04
	A4F1	3.33 ^{b,y}	3.00 ^b	4.75 ^{a,y}	3.31 ^{b,y}	3.37 ^b		
	A4F24	4.14 ^{ab,x}	3.24 ^b	5.29 ^{a,x}	3.92 ^{ab,x}	3.41 ^b		
L*	A5	42.3 ^a	38.1 ^{b,x}	37.3 ^{b,x}	42.7 ^a	36.6 ^{b,y}	0.89	0.006
	A4F1	43.9 ^a	35.2 ^{d,y}	35.2 ^{d,y}	41.1 ^b	38.7 ^{c,xy}		
	A4F24	42.6 ^a	35.9 ^y	36.1 ^{c,xy}	41.3 ^{ab}	39.8 ^{b,x}		
a*	A5	22.1 ^{a,x}	20.1 ^b	23.2 ^{a,x}	22.7 ^{a,x}	23.2 ^{a,x}	0.73	0.004
	A4F1	18.7 ^{b,y}	19. 4 ^{ab}	19.9 ^{ab,y}	20.5 ^{a,y}	20.5 ^{a,y}		
	A4F24	22.9 ^{a,x}	19.7 ^b	21.8 ^{a,x}	21.6 ^{a,xy}	23.0 ^{a,x}		
Chroma	A5	23.3 ^{a,x}	21.5 ^b	25.0 ^{a,x}	24.3 ^{a,x}	25.0 ^{a,x}	0.88	0.005
	A4F1	19.3 ^{b,y}	20.4 ^{ab}	21.0 ^{ab,y}	21.6 ^{a,y}	21.9 ^{a,y}		
	A4F24	24.5 ^{a,x}	21.0 ^b	23.3 ^{a,x}	23.1 ^{a,xy}	24.6 ^{a,x}		

Table 2. Shear force and color characteristics of aged/fast-frozen/thawed various lamb cuts (n = 10)

^{a-c}Means within a row with different letters are significantly different (P < 0.05).

^{x,y}Means within a column with different letters are significantly different (P < 0.05).

¹Treatments: A5: aged-only control for 5 wk at -1.5° C; A4F1: aged/fast-frozen/thawed muscles—after aging for 4 wk at -1.5° C, fast frozen (glycol immersion tank at -18° C), stored for 1 wk at -18° C, and thawed at 4°C overnight; A4F24: aged/fast-frozen/thawed muscles—aged for 4 wk at -1.5° C, fast frozen (glycol immersion tank at -18° C), stored for 24 wk (6 mo) at -18° C, and thawed at 4°C overnight.

²Lamb cuts: QF, Musculus quandriceps femoris; LD, Longissimus dorsi; SM, Semimembranosus; GM, Gluteus medius; BF, Biceps femoris.

³SED: standard errors of difference.

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approximately 4 kgF or below, with the exception of SM, which measured around 5 kgF, likely attributed to background toughness. These findings suggest the beneficial impact of extended aging on meat tenderization. Additionally, fast-freezing of aged meat resulted in similar (or numerically improved) instrumental tenderness values of frozen/thawed lamb cuts. Previous studies reported freeze/thaw-induced meat tenderization, where a decrease in shear force values of meat samples was observed likely due to additional muscle fiber fragmentation facilitated through the formation of intra-/inter-cellular ice crystals in muscle (Chabela and Oyague, 2004; Vieira et al., 2009). This partially aligns with our findings. However, the underlying cause of the increased shear force in the QF muscle with prolonged storage (when no similar observation was determined in other muscles) remains unclear.

Color characteristics. A significant interaction between lamb cut types and freezing treatments was found in the color characteristics of lamb muscles (Table 2). While muscle-specific responses were observed in most of color attributes, in general, freezing/thawing resulted in a decrease in L* values (lightness) of the lamb cuts. Specifically, the lightness values of the LD, SM, and BF appeared to be most affected by the freezing/thawing compared to the aged-only treatment (A5) (P < 0.05), while QF and GM did not show substantial changes in lightness values by the treatments (P > 0.05). A decrease in lightness of frozen/thawed lamb loins was previously reported by Kim et al. (2011a), where they postulated a decrease in myoglobin oxygenation (blooming) ability of aged/frozen/thawed meat compared to never-frozen (aged-only) counterparts. Vieira et al. (2009) reported that beef muscle aged for 10 d exhibited slightly lower lightness than that aged for 3 d. Furthermore, they observed a continuous decrease in lightness over 90 d of frozen storage.

The a^* values (redness) and chroma values (color intensity) of the lamb cuts were influenced by freezing and frozen storage treatments (P < 0.01; Table 2).

Although slight decreases in redness and color intensity of lamb cuts due to freezing/thawing were observed, the surface redness and color intensity of the lamb muscles remained at acceptable levels (the lowest a^* values > 14.8; Khliji et al., 2010). Notably, the redness of LD remained unaffected by the freezing/thawing treatment (P > 0.05). This observation could be attributed to aging prior to freezing, as Kim et al. (2011a) also reported that lamb loins subjected to aging (2 or 3 wk) followed by freezing maintained higher redness values compared to loins treated with frozen/thawed only (no aging). Oxygen utilizing enzymes in mitochondria in muscle cells may regain their respiratory activities upon thawing, resulting in less oxygen for myoglobin for oxygenation. However, allowing sufficient aging periods prior to freezing of meat may decrease oxygen consumption rate of respiratory enzymes in mitochondria, leading to relatively less impact on oxygenation ability of myoglobin (Kim et al., 2011a).

Aerobic plate count. There was no significant difference in APC attributable to fast-freezing and frozen storage. However, there were variations in APC among lamb cuts, with LD having the highest counts among other muscles (P < 0.05). The differences in APC are likely associated not only with the slaughtering and deboning processes but also with the location of the muscles in the animal (Voloski et al., 2016; Van Ba et al., 2019). It is worth noting, however, that although LD showed the highest APC, it remained below the threshold of 4.3 log10 CFU/cm², which is considered the upper limit for microbiological quality attributes for plants (e.g., abattoirs and meat cutting establishments) as a measure of hygiene (Todd, 2004).

Experiment 2—Effect of aging and fastfreezing with repeated freezing/thawing on quality characteristics of lamb loins

The impact of multiple cycles of freezing and thawing on the meat quality attributes of lamb loins,

Table 3. Aerobic plate count $(\log_{10}CFU/cm^2)$ of aged/fast-frozen/thawed various lamb cuts (n = 10)

	Treatments (T) ¹			L	amb cuts (C) ³			Signi	ficance of I	^p value ⁴
A5	A4F1	A4F24	SED^2	QF	LD	SM	GM	BF	SED	Т	С	T×C
2.15	1.82	1.98	0.349	1.99 ^b	3.02 ^a	1.42 ^c	1.45 ^c	2.03 ^b	0.255	NS	***	NS

^{a-c}Means with different letters within each effect (treatment or lamb cut) are significantly different (P < 0.05).

¹Treatments (T): A5: aged-only control for 5 wk; A4F1: aging for 4 wk, fast-freezing and frozen storage for 1 wk; A4F24: aging for 4 wk, fast-freezing and frozen storage for 2 wk.

²SED: standard errors of difference.

³Lamb cuts: QF, *Musculus quandriceps femoris*; LD, *Longissimus dorsi*; SM, *Semimembranosus*; GM, *Gluteus medius*; BF, *Biceps femoris*. ⁴Significance of *P* value: NS, no significance (*P* > 0.05); ****P* < 0.001. which were initially subjected to aging/fast-freezing/ thawing, is presented in Table 3. The experiment aimed to replicate common practices in retail conditions and/ or the handling of purchased meat by consumers in a household setting.

pH and water-holding capacity. The repeated freezing and thawing of the previously aged/fast-frozen/thawed loin (A-FT-SFT) did not affect the pH when compared to the aged/slow-frozen/thawed loin (A-SFT) (P > 0.05). However, the loins assigned to the multiple freezing/thawing cycle treatment (A-FT-SFT) resulted in higher purge and cooking losses than the one time freezing/thawing (A-SFT) treatment (P < 0.05). This observation could indicate that repeated freezing and thawing leads to increased water loss throughout the freezing/thawing and cooking processes. However, there was no significant difference in drip loss between the two treatments. Several studies reported that an increase in the number of freezing/thawing cycles can have a negative impact on water-holding capacity (Ali et al., 2015; Tippala et al., 2021; Liu et al., 2022). However, Tuell et al. (2020) emphasized the importance of the initial freezing rate for the repeated freeze-thaw processes, suggesting that an initial fast-freezing could mitigate some of the negative effects associated with repeated freeze/ thaw-cycling pork muscles and final frozen/thawed patty products. In the current study, with regard to the experimental design, the absence of a slow-freezing counterpart for the initial freezing precludes a direct comparison. Nevertheless, it is reasonable to assume that lamb loins might exhibit increased purge/thaw loss if subjected to slow-freezing as a counterpart to fastfreezing for the initial freezing, followed by repeated freezing/thawing. Further investigation is warranted to validate this assumption.

Shear force. The repeated freezing and thawing did not have an impact on the shear force values of the lamb loins (P > 0.05). Notably, the average shear force value for the aged/frozen/thawed loins was 2.9 kgF, which is considered very tender (Bickerstaffe et al., 2001). This observation reaffirms the positive effect of aging prior to freezing on meat tenderness, indicating that aging before freezing can preserve the improved tenderness achieved through aging (Wiklund et al., 2009; Kim et al., 2011b; Kim et al., 2012). This benefit persists even when the meat undergoes repeated freezing and thawing processes, a common practice among consumers at home.

Color. Instrumental color measurements revealed that there were no significant differences in L^* (lightness), b^* (yellowness), and hue angle (discoloration)

between the two freezing and thawing cycles. Interestingly, the A-FT-SFT treatment exhibited higher *a** values (redness) and color intensity (chroma value) compared to the A-SFT treatment (P < 0.05), indicating that repeated freezing and thawing of the previously aged/fast-frozen/thawed loins did not result in surface color deterioration. Previous research has reported that repeated freezing/thawing cycles can lead to a decrease in a^* values (Qi et al., 2012). This is often attributed to the loss of myoglobin along with meat exudate and the formation of metmyoglobin during repeated freezing and thawing. Conversely, aging before freezing, as suggested by Alvarenga et al. (2019), may potentially increase a^* values compared to muscles that undergo aging alone. Although the authors did not discuss the causes in detail, this change is likely related to alterations in the oxygenation of myoglobin (Aroeira et al., 2017). The repeated freezing and thawing cycles may suppress oxygen utilizing enzymes in mitochondria, allowing myoglobin to react with more residual oxygen to form oxymyoglobin (Kim et al., 2011a).

Aerobic plate count. The repeated freezing and thawing of the previously aged/fast-frozen/thawed loins did not have an impact on the APC (P > 0.05; Table 4), indicating that repeated freezing/thawing

Table 4. Effect of repeated freezing/thawing on meat quality attributes of aged/fast-frozen/thawed lamb loins (*m. longissimus dorsi*, n = 8)

Attributes	A-SFT ¹	A-FT-SFT	SED ²	Significance of P value ³
pH	5.83	5.86	0.04	NS
Water-holding capacity				
Purge loss (%)	4.3 ^b	5.4 ^a	0.81	*
Drip loss (%)	1.7	1.8	0.39	NS
Cooking loss (%)	25.2 ^b	28.1 ^a	2.00	*
Instrumental tenderness				
Shear force (kgF)	2.8	3.0	0.21	NS
Color characteristics				
L^*	36.9	35.5	1.87	NS
a*	18.5 ^b	20.1 ^a	1.35	*
b^*	6.6	7.1	0.93	NS
Hue angle	19.5	19.2	1.23	NS
Chroma	19.6 ^b	21.4 ^a	1.57	*
Aerobic plate count (log ₁₀ CFU/cm ²)	3.00	3.80	1.08	NS

^{a,b}Means with different letters are significantly different (P < 0.05).

¹A-SFT: aging for 4 wk, slow-freezing, frozen storage for 2 wk and thawing treatment; A-FT-SFT: aging for 4 wk, fast-freezing, frozen storage for 1 wk, thawing, slow-freezing again, frozen storage for 1 wk and thawing treatment.

²SED: standard errors of difference.

³Significance of *P* value: NS, no significance (P > 0.05); **P* < 0.05.

itself would not affect microbiological quality characteristics.

Conclusions

The results from the present study demonstrate that, in general, aged/fast-frozen/thawed lamb cuts stored for a short duration maintain similar water-holding capacity, instrumental tenderness, and color characteristics as aged-only lamb cuts (never frozen). Also, regardless of muscle cuts, the aged/fast-frozen/thawed meat samples had similar purge (thaw) loss compared to agedonly control counterparts. This confirms the positive impacts of combining aging and fast-freezing on the quality characteristics of frozen/thawed meat. Prolonged frozen storage of muscle cuts treated with fastfrozen could lead to muscle-specific responses in certain quality attributes, such as drip loss, cooking loss, and shear force. However, these differences would be practically less meaningful given the overall extent of variations. Additionally, the repeated freezing and thawing of previously aged/fast-frozen/thawed loins did not have a detrimental impact on meat quality attributes, although slight increases in purge and cook losses were observed. The findings of the current study suggest that the combined treatment of aging and fast-freezing could be an effective value-adding process, mitigating defects frequently associated with frozen/thawed lamb muscle cuts, particularly an increase in purge/thaw loss resulting from freezing and thawing, even during prolonged frozen storage of up to 24 wk. Considering the musclespecific responses in meat quality attributes to the aging/ fast-freezing/thawing treatment for long-term storage, further research to identify optimal regimes for aging/ freezing/thawing and subsequent storage times based on muscle types can provide valuable insights for maximizing the meat quality of frozen/thawed meat.

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Literature Cited

Ali, S., W. Zhang, N. Rajput, M. A. Khan, C. B. Li, and G. H. Zhou. 2015. Effect of multiple freeze–thaw cycles on the quality of chicken breast meat. Food Chem. 173:808–814. https://doi. org/10.1016/j.foodchem.2014.09.095

- Alvarenga, T. I., D. L. Hopkins, E. M. Ramos, A. K. Almeida, and G. Geesink. 2019. Ageing-freezing/thaw process affects blooming time and myoglobin forms of lamb meat during retail display. Meat Sci. 153:19–25. https://doi.org/10.1016/ j.meatsci.2019.02.016
- AOAC. 2000. Official methods of analysis. 17th ed. AOAC International, Arlington, VA.
- Aroeira, C. N., R. de Almeida Torres Filho, P. R. Fontes, A. D. L. S. Ramos, L. A. de Miranda Gomide, M. M. Ladeira, and E. M. Ramos. 2017. Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle. Meat Sci. 125:16–21. https://doi.org/10.1016/j. meatsci.2016.11.010
- Balan, P., Y. H. B. Kim, A. D. Stuart, R. Kemp, M. Staincliffe, C. Craigie, and M. M. Farouk. 2019. Effect of fast freezing then thaw-aging on meat quality attributes of lamb M. longissimus lumborum. Anim. Sci. J. 90:1060–1069. https://doi.org/10. 1111/asj.13216
- Bickerstaffe, R., A. E. D. Bekhit, L. J. Robertson, N. Roberts, and G. H. Geesink. 2001. Impact of introducing specifications on the tenderness of retail meat. Meat Sci. 59:303–315. https:// doi.org/10.1016/S0309-1740(01)00083-3
- Chabela, M. L. P., and J. M. Oyague. 2004. Frozen meat: Quality and shelf life. In: Y. H. Hui, P. Cornillon, I. G. Legaretta, M. H. Lim, K. D. Murrell, and W.-K. Nip, editors, Handbook of frozen foods. Vol. 133. Marcel Dekker.
- Choi, M. J., T. Abduzukhurov, D. H. Park, E. J. Kim, and G. P. Hong. 2018. Effects of deep freezing temperature for longterm storage on quality characteristics and freshness of lamb meat. Korean J. Food Sci. An. 38:959. https://doi.org/10. 5851/kosfa.2018.e28
- Chrystall, N. B., and C. E. Devine. 1991. Quality assurance for tenderness. Meat Industries Research Institute of New Zealand Publication No. 872.
- Farouk, M. M., N. Md. Mustafa, G. Wu, and G. Krsinic. 2012. The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with aging. Meat Sci. 90:670–677. https://doi.org/10.1016/j.meatsci. 2011.10.012
- Farouk, M. M., K. J. Wieliczko, and I. Merts. 2004. Ultra-fast freezing and low storage temperature are not necessary to maintain the functional properties of manufacturing beef. Meat Sci. 66:171–179. https://doi.org/10.1016/S0309-1740 (03)00081-0
- Farouk, M., E. Wiklund, A. Stuart, and P. Dobbie. 2009a. Aging prior to freezing improves the color of frozen-thawed beef and venison. In: 55th ICoMST, Copenhagen, Denmark. pp. 786–790.
- Farouk, M., E. Wiklund, A. Stuart, and P. Dobbie. 2009b. Aging prior to freezing improves waterholding capacity in beef and venison. In: 55th ICoMST, Copenhagen, Denmark. pp. 781–785.
- GenStat. 2010. GenStat for Windows. 12th edition, Version 12.2.0.3717. VSN International, Oxford.
- Khliji, S., R. Van de Ven, T. A. Lamb, M. Lanza, and D. L. Hopkins. 2010. Relationship between consumer ranking of lamb color and objective measures of color. Meat Sci. 85:224–229. https://doi.org/10.1016/j.meatsci.2010.01.002

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- Kiani, H., and D. W. Sun. 2011. Water crystallization and its importance to freezing of foods: A review. Trends Food Sci. Tech. 22:407–426. https://doi.org/10.1016/j.tifs.2011.04.011
- Kim, Y. H. B., M. Frandsen, and K. Rosenvold. 2011a. Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Sci. 88:332–337. https://doi.org/10.1016/j. meatsci.2010.12.020
- Kim, H. W., J. H. Kim, J. K. Seo, D. Setyabrata, and Y. H. B. Kim. 2018. Effects of aging/freezing sequence and freezing rate on meat quality and oxidative stability of pork loins. Meat Sci. 139:162–170. https://doi.org/10.1016/j.meatsci.2018.01.024
- Kim, Y. H. B., C. Liesse, R. Kemp, and P. Balan. 2015. Evaluation of combined effects of ageing period and freezing rate on quality attributes of beef loins. Meat Sci. 110:40–45. https://doi. org/10.1016/j.meatsci.2015.06.015
- Kim, J., J. K. Seo, H. W. Kim, and Y. H. B. Kim. 2023. Combined impacts of initial freezing rate and thawing/cooking conditions on physicochemical and textural properties of pork patties. Meat Muscle Biol. 7:16190, 1–11. https://doi.org/10. 22175/mmb.16190
- Kim, Y. H. B., A. Stuart, C. Black, P. Dobbie, and K. Rosenvold. 2011b. Effect of ageing prior to freezing and impact of slaughter processing conditions on meat quality attributes of lamb loins. Ruakura Research Centre: AgResearch (AGFH 01493).
- Kim, Y. H. B., A. Stuart, C. Black, and K. Rosenvold. 2012. Effect of lamb age and retail packaging types on the quality of longterm chilled lamb loins. Meat Sci. 90:962–966. https://doi. org/10.1016/j.meatsci.2011.11.040
- King, D. A., M. C. Hunt, S. Barbut, J. R. Claus, D. P. Cornforth, P. Joseph, Y. H. B. Kim, G. Lindahl, R. A. Mancini, M. N. Nair, K. J. Merok, A. Milkowski, A. Mohan, F. Pohlman, R. Ramanathan, C. R. Raines, M. Seyfert, O. Sørheim, S. P. Suman, and M. Weber. 2023. American Meat Science Association guidelines for meat color measurement. Meat Muscle Biol. 6:12473, 1–81. https://doi.org/10.22175/mmb.12473
- Leygonie, C., T. J. Britz, and L. C. Hoffman. 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Sci. 91:93–98. https://doi.org/10.1016/j.meatsci.2012.01.013
- Liu, C., L. Kong, P. Yu, R. Wen, X. Yu, X. Xu, and X. Peng. 2022. Whey protein hydrolysates improved the oxidative stability and water-holding capacity of pork patties by reducing protein aggregation during repeated freeze–thaw cycles. Foods 11:2133. https://doi.org/10.3390/foods11142133
- MacFarlane, P. G., and J. M. Marer. 1966. An apparatus for determining the tenderness of meat. Food Technol. 838:134–135.
- Picard, B., and M. Gagaoua. 2020. Muscle fiber properties in cattle and their relationships with meat qualities: An overview. J. Agr. Food Chem. 68:6021–6039. https://doi.org/10.1021/ acs.jafc.0c02086
- Qi, J., C. Li, Y. Chen, F. Gao, X. Xu, and G. Zhou. 2012. Changes in meat quality of ovine longissimus dorsi muscle in response to repeated freeze and thaw. Meat Sci. 92:619–626. https:// doi.org/10.1016/j.meatsci.2012.06.009
- Setyabrata, D., and Y. H. B. Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physicochemical properties of beef muscles. Meat Sci. 151:64–74. https://doi.org/10.1016/j.meatsci.2019.01.007

- Setyabrata, D., J. R. Tuell, B. Kim, and Y. H. B. Kim. 2019. The effect of aging/freezing sequence and freezing rate on quality attributes of beef loins (M. longissimus lumborum). Meat Muscle Biol. 3:488–499. https://doi.org/10.22175/mmb.11234
- Tarrant, P. V., and J. Sherington. 1980. An investigation of ultimate pH in the muscles of commercial beef carcasses. Meat Sci. 4:287–297. https://doi.org/10.1016/0309-1740(80)90028-5
- Tippala, T., N. Koomkrong, and A. Kayan. 2021. Influence of freeze-thawed cycles on pork quality. Animal Bioscience 34:1375. https://doi.org/10.5713/ajas.20.0416
- Todd, E. C. 2004. Microbiological safety standards and public health goals to reduce foodborne disease. Meat Sci. 66:33– 43. https://doi.org/10.1016/S0309-1740(03)00023-8
- Tschirhart-Hoelscher, T. E., B. E. Baird, D. A. King, D. R. McKenna, and J. W. Savell. 2006. Physical, chemical, and histological characteristics of 18 lamb muscles. Meat Sci. 73:48–54. https://doi.org/10.1016/j.meatsci.2005.10.015
- Tuell, J. R., J. K. Seo, and Y. H. B. Kim. 2020. Combined impacts of initial freezing rate of pork leg muscles (M. biceps femoris and M. semitendinosus) and subsequent freezing on quality characteristics of pork patties. Meat Sci. 170:108248. https://doi.org/10.1016/j.meatsci.2020.108248
- Van Ba, H., H. W. Seo, P. N. Seong, S. M. Kang, S. H. Cho, Y. S. Kim, B. Y. Park, S. S. Moon, S. J. Kang, Y. M. Choi, and J. H. Kim. 2019. The fates of microbial populations on pig carcasses during slaughtering process, on retail cuts after slaughter, and intervention efficiency of lactic acid spraying. Int. J. Food Microbiol. 294:10–17. https://doi.org/10.1016/j. ijfoodmicro.2019.01.015
- Vieira, C., M. T. Diaz, B. Martínez, and M. D. García-Cachàn. 2009. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of aging. Meat Sci. 83:398–404. https://doi.org/10.1016/j.meatsci.2009.06.013
- Voloski, F. L. S., L. Tonello, T. Ramires, G. G. Reta, C. Dewes, M. Iglesias, R. G. Mondadori, E. A. Gandra, W. P. da Silva, and E. H. Duval. 2016. Influence of cutting and deboning operations on the microbiological quality and shelf life of buffalo meat. Meat Sci. 116:207–212. https://doi.org/10.1016/j. meatsci.2016.02.020
- Wang, Y., H. Liang, R. Xu, B. Lu, X. Song, and B. Liu. 2020. Effects of temperature fluctuations on the meat quality and muscle microstructure of frozen beef. Int. J. Refrig. 116:1– 8. https://doi.org/10.1016/j.ijrefrig.2019.12.025
- Wiklund, E., M. M. Farouk, A. Stuart, S. Cartwright, N. Penney, and K. Rosenvold. 2009. Quality of chilled-never-frozen versus chilled-frozen-thawed lamb. In: *Proceedings of the New Zealand Society of Animal Production*, Christchurch, New Zealand. New Zealand Society of Animal Production. Vol. 69: pp. 108–111.
- Xia, X., B. Kong, Q. Liu, and J. Liu. 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci. 83:239– 245. https://doi.org/10.1016/j.meatsci.2009.05.003
- Zhang, R., C. E. Realini, Y. H. B. Kim, and M. M. Farouk. 2023. Challenges and processing strategies to produce high quality frozen meat. Meat Sci. 205:109311. https://doi.org/10.1016/j. meatsci.2023.109311