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Research Article

Quality and Acceptability of Fresh and Long-Term Frozen In-Bag Dry-Aged Lean Bull Beef

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In-bag dry-aged lean beef was produced using a stepwise ageing process. Lean bull beef striploins were dry-aged at 2°C, 75% RH under three different air velocities: 0.5, 1.5, and 2.5 m·s^{-1} for 7 days followed by wet-ageing for 14 days. The quality and acceptability of the dry-aged beef were compared with equivalent beef dry-aged for 21 days at 0.5 m·s^{-1} which served as a control. Two portions of the dry-aged beef (7/21 days) were randomly selected and held frozen at −18°C for 12 months. Shear force, drip, and cook loss decreased significantly ($p < 0.05$) with dry-ageing time. Increased air velocities accelerated dehydration process with no negative impact on the meat quality, microbiological safety, and consumer acceptability compared to the control ($p > 0.05$). Frozen storage for 12 months had little or no effect on the quality and acceptability of the dry-aged lean beef ($p > 0.05$). Dry-aged lean beef of equivalent quality and palatability, with a lower level of surface microorganisms and higher yield compared to the control, could be produced using the stepwise ageing process.

1. Introduction

Postmortem ageing of fresh beef for retail and foodservice is essential in meeting the high demands and expectations of discerning consumers seeking exceptional eating experience. Ageing improves tenderness [1, 2] and flavour [3]. Ageing of beef up to 14 days has been reported to increase the fatty and distinct aged flavours (beefy, brothy, sweet, and browned caramel), and these contribute positively towards the consumers' liking of premium beef cuts from the loin and rib [4]. The most widely used ageing practice is wet-ageing, which refers to ageing of a piece of meat in a moisture and air impermeable vacuum package bag under chilled storage conditions. Traditional dry-ageing involves ageing of the primal and subprimal cuts without the use of any packaging or ageing bags to produce meat considered superior to wet-aged meat by purveyors due to the intense beefy and roasted flavour from the process [5]. Dry-aged beef is typically described as having a buttery, rich, nutty, and/or earthy flavour profile [6].

Dry-ageing requires critical control of processing parameters including temperature, air velocity, and relative humidity, to prevent excessive weight loss and growth of microorganisms. A new method called "in-bag dry-ageing" has emerged over the last decade to address the concerns associated with the traditional dry-ageing process [7, 8]. The dry-ageing bags allow loss of moisture from the meat in a similar way to that of the traditional dry-ageing method. The dry-ageing bags act as an oxygen barrier to reduce oxygen exchange with the surrounding air, thereby limiting oxidation and its associated consequences, which include oxidative deterioration which produces rancid or off-flavour [9]. The bags also act as protective barriers to prevent contamination from the surroundings and reduce the proliferation of spoilage microorganisms during the ageing process [10], which in turn reduces the need of excessive trimming. Excessive trimming cannot be avoided with the traditional dry-ageing method and often causes a loss to the meat purveyors. A novel dry-ageing strategy called "smart ageing" proposed by Kim et al. [11] has shown improvement

in meat quality and value through modification of specific postmortem ageing conditions. Stepwise ageing is one of the smart-ageing strategies which combine different ageing methods to produce dry-aged beef of equivalent quality compared with those using dry-ageing only [12].

Most dry-aged beef is produced from well-marbled premium beef cuts from prime steers or heifers with high intramuscular fat (IMF) and consumed locally rather than exported and fresh rather than thawed. Lean bull beef, on the contrary, is characterised as low-value beef with fat content of 1-2%, reduced juiciness, and tough texture. As a result, it is usually processed to sausages, patties, and other further processed meat products and hardly used for premium products such as the dry-aged products. Recently, dry-aged *Longissimus thoracis et lumborum* from young bull (IMF around 2%) was produced and rated to be preferred over the wet-aged counterparts from the consumers [13], suggesting the potential to produce dry-aged beef products from lean cuts. The use of lean beef may offer further advantages over the prime fatty beef for storage stability and reduced off-flavours associated with high fat content and the interaction of the latter with storage time and temperature.

Long-term storage of dry-aged beef may need to be considered if the meat industry was to produce dry-aged meat for commercial export as it currently does for chilled and frozen wet-aged meat. Freezing of meat at −18°C during storage and distribution is a common practice in the meat industry, particularly for the export market. A processing strategy called "aged and then frozen" was of great interest over the last decade. This strategy refers to applying a certain period of wet-ageing (2–4 wks) prior to the frozen storage. It has been proven to improve the colour stability, tenderness, and water-holding capacity without negative impact on the meat quality [14–18]. Kim et al. [12] reported on the improvements of water-holding capacity and shear force tenderness with no impact on colour and sensory quality of stepwise aged beef (dry-aged 10 d + wet-aged 7 d) followed by frozen storage for 6 months. However, whether dry-aged lean beef can be frozen without deterioration in quality or not and how long the frozen storage can be continued without deterioration in quality remain unknown. Answers to these questions are required if dry-aged meat was to be commercially traded globally and particularly to growing markets in countries like China where frozen rather than chilled meat imports is the norm.

This study aimed to investigate the effects and interactions of air velocities, ageing time, and long-term frozen storage on the meat quality and acceptability of in-bag dry-aged striploins from lean bull beef using the stepwise ageing regime. The current study was carried out to test the hypothesis that the combination of in-bag dry-ageing at higher air velocities for shorter ageing time followed by wet-ageing in vacuum barrier bags would produce dry-aged meat of equivalent quality, long-term frozen stability, and acceptability to in-bag dry-ageing meat produced using longer dry-ageing time and with no wet-ageing involved.

2. Materials and Methods

2.1. Sample Collection and Dry-Ageing Procedure. A total of 15 pairs ($n = 30$) of beef striploins (*longissimus lumborum*) from bull (≈2-year-old, boneless) beef carcasses were obtained on the slaughter day from a local meat processing plant. All the loins were held at 12°C for 12 hrs until they entered rigor and then randomly assigned to four treatments (Figure 1): T1: in-bag dry-ageing for 21 d (control, $n = 6$) at an air velocity of 0.5 m·s^{-1}; T2 – T4: in-bag dry-ageing for 7 d + wet-aged for 14 d at an air velocity of 0.5 m·s^{-1} (T2), 1.5 m·s^{-1} (T3), and 2.5 m·s^{-1} (T4) ($n = 8$ for each stepwise ageing treatment). All the loins were vacuum packaged in dry-ageing bags (TUBLIN® 10 and 50 μm thick, polyamide mix with a water vapour transmission rate of 2.5 kg/50 μ/m^2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark) and laid out on wire racks inside a dry-ageing chamber set at 2 ± 0.5°C and relative humidity of 75 ± 5%. Samples were weighed during the ageing process (0 d, 3 d, 5 d, 7 d, 13 d, 16 d, 18 d, and 21 d), and the weights were used to calculate the % weight loss: % weight loss = [(initial weight of the sample before ageing – weight at a given time point)/initial weight before ageing] × 100.

A thin layer of dried and discoloured surface (including subcutaneous fat) was trimmed off from the striploins aged for 7 d and 21 d and then fabricated into 2 cm thick steaks. Minimum three steaks were taken from each loin of each treatment (T1–T4) and at different ageing time points (0, 7, and 21 d) for further analysis of fresh beef. No subsample was taken at 7 d of ageing time for the control (T1). Another three fresh steaks (minimum) were obtained from each loin of each treatment and ageing time point, as described above, vacuum packed immediately after ageing, and stored frozen at −18°C for 12 months to determine the effect of long-term frozen storage on the quality of dry-aged lean beef.

2.2. Surface Microbial Growth and Water Activity (A_w). Microorganisms from the untrimmed surface of fresh (unfrozen) beef samples were enumerated before (0 d) and after the ageing process (21 d) for all four treatments using standard methods in the Compendium of Methods of Microbiological Examination of Foods [19] for *Escherichia coli* (*E. coli*, Chapter 8.91), aerobic bacteria (Chapter 7.62), lactic acid bacteria (Chapter 19.522), Enterobacteriaceae (Chapter 8.63), and moulds and yeast (Chapter 20.51).

A_w of the fresh beef samples (one steak per loin) collected on 0 d and 21 d of ageing from all four treatments was measured in duplicate at 20°C using a water activity meter (Aqua lab CX-2, Decagon Devices, Inc., Washington, US). The water activity meter was calibrated using a saturated potassium sulphate solution and water at 20°C.

2.3. pH and Proximate Content. The pH of fresh and frozen (thawed at 4°C overnight) in-bag dry-aged beef samples (T1–T4 at all ageing time points, one steak per loin) obtained from *longissimus lumborum* was measured by inserting a calibrated pH probe (Hanna 99,163 pH meter with a FC232D combined temperature and pH insertion probe,

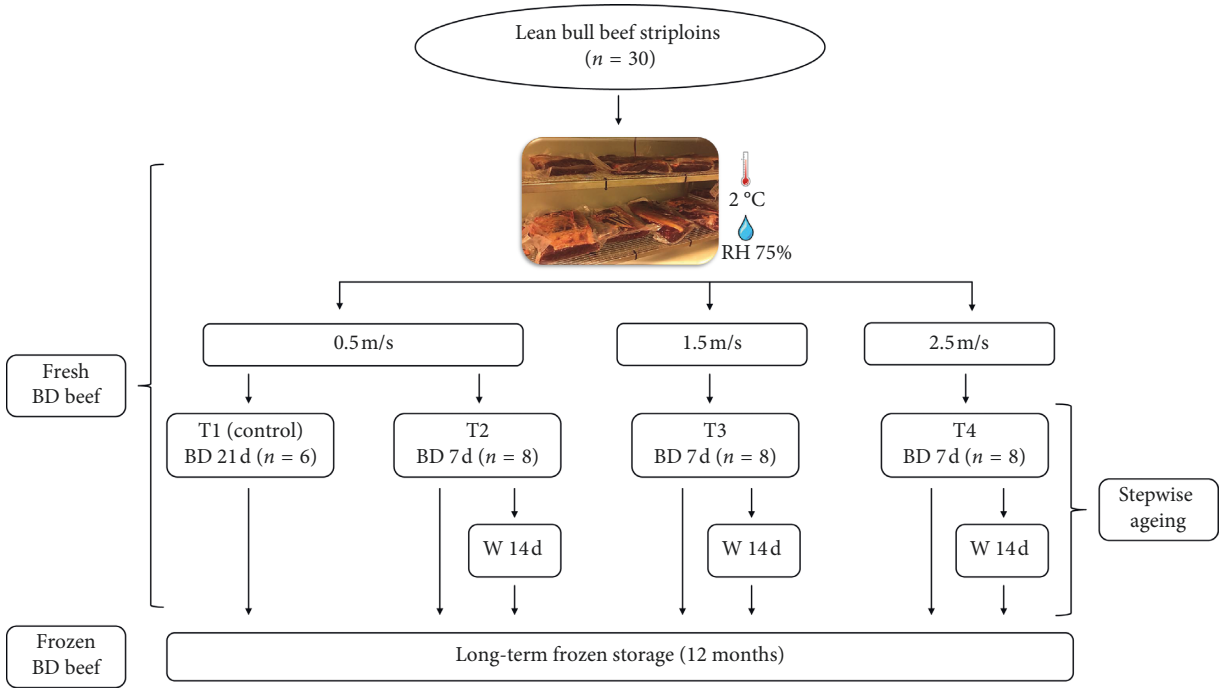

Figure 1: Schematic illustration of the ageing process and treatment combinations in the current study. BD: in-bag dry-ageing; W: wet-ageing; T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d.

Rhode Island, USA) directly into the beef samples. Triplicate measurements were taken and averaged.

Beef steaks from pH measurement were minced individually after trimmed off intramuscular fat and subsamples were collected for proximate analysis. Moisture content was measured using the oven-drying method described in AOAC 950.46 [20]. Crude fat content was measured using the Soxhlet extraction method of AOAC 960.39 [20]. The extraction of total muscle protein was as described in Lomiwes et al. [21] using an extraction buffer consisting of 50 mM Tris-HCl (pH = 5.8), 10% glycerol, 2% SDS, and 2% β-mercaptoethanol. Protein content of total muscle extracts was determined using a RC-DC protein assay kit (Bio-Rad® Laboratories, Hercules, CA, USA) based on Lowry assay [22]. Muscle protein concentrations were determined from the standard curve prepared with bovine serum albumin of concentrations from 0 to 2.0 mg·mL^{-1}.

2.4. Instrumental Colour. The steaks (one steak per loin) from fresh (0, 7, and 21 d) and frozen (7 and 21 d, thawed at 4°C overnight) in-bag dry-aged beef loins (T1–T4) were placed on a polypropylene foam tray lined with moisture absorbent pads and then overwrapped with the polyvinyl chloride (PVC) film and allowed to bloom for 30~60 min under simulated retail display light at 4°C. Surface colour was measured using a Minolta Chroma Meter (CR-400; Konica Minolta Photo Imaging Inc., Mahwah, NJ, USA) that had been calibrated using a standard white tile. CIE (L* (lightness), a* (redness), and b* (yellowness)) values were measured (Illuminant D65, 8 mm diameter aperture, 10 standard observers) through the PVC film at three random locations on each steak.

2.5. Water-Holding Capacity. The water-holding capacity was evaluated in the form of % drip loss for the fresh beef (7 and 21 d of in-bag dry-aged), % thaw + drip loss for the frozen beef (7 and 21 d of in-bag dry-aged), and % cook loss for both the fresh and frozen samples (one steak per loin).

2.5.1. Drip Loss. The bag drip method by Honikel [23] with some minor changes proposed by Kim et al. [24] was used on the collected samples from 7 d to 21 d of ageing time for both the fresh and frozen in-bag dry-aged beef samples. The % drip loss was calculated as follows: % drip loss = [(initial weight − weight after hanging for 48 hrs)/initial weight] × 100. The drip loss of frozen storage samples was measured from the frozen state. Therefore, the drip loss was expressed as the total loss from thawing and suspension in drip bags which was calculated as % thaw + drip loss = [(initial weight at frozen − weight after suspension for 48 hrs)/initial weight at frozen] × 100.

2.5.2. Cook Loss. Fresh beef portions (one portion per loin, 6 cm thickness) with weight of approximately 400 g from 7 d to 21 d of ageing were cooked in a boiling water bath (99°C) to the internal temperature of 70°C. Frozen steaks (one steak per loin, thaw at 4°C overnight) of 2 cm thickness from 7 d to 21 d of ageing time were cooked sous vide at 70°C for 1 hr.

Immediately after cooking, the cooked samples were transferred into an ice bath for 30 min to prevent further cooking, blotted dry, and weighed. The % cook loss was calculated as follows: % cook loss = [(initial weight − cooked weight)/initial weight] × 100.

2.6. Instrumental Texture. Cooked steaks from Section 2.5.2 were further analysed for instrumental texture. Tenderness of cooked fresh beef was measured with a MIRINZ tenderometer [25]. A 10 mm × 10 mm cross section was cut from each cooked steak to measure the force required to shear through the sample at a right angle to the fibre axis. The results were expressed as shear force (N) from the average shear force values of ten replicates for each of the cooked sample. A tender meat is defined as the meat of the shear force value ≤88 N (9 kgF) measured by the MIRINZ tenderometer [25].

The texture profile of long-term frozen in-bag dry-aged lean beef was analysed using texture profile analysis (TPA) according the procedure described by Zhang et al. [26]. In brief, a cube (1 cm^3) was measured using Stable Micro Systems TA.HD Plus texture analyser (Surry, UK) with a 50 mm cylinder probe at 50% strain setting using a test speed of 5.0 $m \cdot s^{-1}$. Maximum load force of 50 kg was used with trigger force of 5 g at the auto mode. A minimum of ten replicates from each steak were measured and averaged.

2.7. Consumer Sensory Testing

2.7.1. Cooking and Sample Preparation. Fresh in-bag dry-aged steaks (21 d, minimum three steaks per loin) were cooked in a conventional oven at 170°C until the core temperature reached 70°C. Frozen in-bag dry-aged steaks (21 d, minimum three steaks per loin) were thawed at 4°C chiller overnight and precooked sous vide at 70°C for 1 h and reheated on a grill set at 230°C for 90 s on each side. Once cooked, each steak was cut across the grain into a 1.3 × 1.3 × 2.0 cm piece and randomly assigned to a plastic cup. All the cups were prelabelled with unique codes made of panelist numbers (1, 2, 3, etc.) × sample ID (A, B, C, and D). Each panellist was asked to taste one sample each time in the order from A to D. Each sample ID (A–D) of the same panellist corresponded to one of the ageing treatments (T1–T4). The panellists may taste the same sample from the same steak more than once due to the randomised design model. Water and water crackers were provided as palate cleansers. Consumers were asked to take a bite of cracker, rinsed their mouths, and rest for 30 s between the samples. Consumers have been informed that swallowing was allowed but not compulsory.

2.7.2. Sensory Evaluation of Fresh and Long-Term Frozen Beef

(1) Fresh In-Bag Dry-Aged Beef. The aim of the first sensory session was to determine the effect of stepwise ageing and ageing chamber air velocity on the palatability of fresh in-bag dry-aged lean bull beef. A total of 44 untrained panellists (20 to 65 years old) who are frequent meat consumers and familiar with sensory evaluation of various meat products have participated in the study. Each consumer was provided with a computer to accomplish the webpage-based questionnaire. Consumer panels were asked to evaluate the acceptance and liking of the steak samples in terms of aroma, tenderness, juiciness, flavour, and overall liking on a scale of 1–100, where 1 represented "extremely dislike," and 100 represented "extremely like." Consumers were also asked to rate the degree of off-flavours where 1 represented "not detected" and 100 represented "detected very strongly." After tasting every two samples (A and B or C and D), panellists were also asked to rank for a preferred sample.

(2) Frozen In-Bag Dry-Aged Beef. The aim of the second sensory session was to determine the acceptability of long-term frozen stored (for 12 months) in-bag dry-aged lean bull beef. A total of 72 panellists (20 to 65 years old), consisting of 40% female and 60% male, participated in this study. Each panellist evaluated four samples from four different ageing treatments in a random order. Panellists were asked to rate their acceptance on a 9-point hedonic scale using a printed questionnaire where 1 represented "extremely dislike" and 9 represented "extremely like." Detection of the off-flavour was evaluated on a 5-point hedonic scale where 1 represented "not detected" and 5 represented "detected very strongly."

2.8. Statistical Analysis. A randomised trial was designed with 30 striploins from 15 beef carcasses ($n = 30$) which were unevenly assigned to four different treatment combinations (1 control dry-ageing; T1: $n = 6$ and 3 stepwise ageing regimes; T2–T4: $n = 8$ for each). Linear mixed effect regression analyses were performed on the data using R (version 3.4.1), with the "lme4" package to determine the difference between four treatment combinations across the ageing time. The ageing treatments (T1–T4) and ageing time (0, 7, and 21 d) were included as fixed effects, where the sample ID (loin number) was included as a random effect to account for the uneven number of samples between the control and other treatments. One-way analysis of variance (ANOVA) was performed to determine the effect of air velocity, stepwise ageing, and ageing time on the fresh and frozen in-bag dry-aged beef. The effect of air velocity was determined by the comparison between T2, T3, and T4 at 7 d of ageing time. The effect of stepwise ageing was determined by the comparison between T1 and T2 at 21 d of ageing time. The interaction of stepwise ageing and air velocity was determined by the comparison between all four treatments at the 21 d of ageing time. The effect of ageing time on each treatment was analysed separately by the comparing across the ageing time from 0 to 21 d. The effect of frozen storage on the proximate content (0, 7, and 21 d), pH (7 and 21 d), and instrumental colour (7 and 21 d) of in-bag dry-aged beef was analysed by comparing fresh and frozen beef samples aged for the same ageing time individually. Post hoc comparison of means was performed using Fisher's least significant differences (LSD) and Tukey's (HSD) test at the 5% significance level.

3. Results and Discussion

3.1. Effect of Dry-Ageing Chamber Air Velocity on the Physicochemical Properties of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.1.1. Fresh In-Bag Dry-Aged Beef. Weight loss of lean beef during in-bag dry-ageing increased ($p < 0.05$) with the increase in air velocity over the first 7 d of ageing time, as shown in Figure 2. The highest weight loss was associated with the highest air velocity of 2.5 m·s^{-1} (T4, 11.19%) compared with those of medium (T3, 10.57%) and low velocity (T2, 9.37%). Significantly higher weight loss with increased velocity from 0.2 to 0.5 m·s^{-1} after 21 d of ageing time has been reported for dry-aged prime steer beef loins [24].

A significantly lower ($p < 0.05$) moisture content was found in beef that was in-bag dry-aged at medium velocity of 1.5 m·s^{-1} (T3, Table 1). However, the % moisture differences were less than 1% between different air velocities. Therefore, the effect of ageing chamber air velocity on the moisture content of in-bag dry-aged beef was minimum. This could be explained by the moisture loss during dry-ageing being mainly on the surface and the outer section of the beef at higher velocities forming "crust-like" dry skin faster compared to lower velocities, which might have reduced moisture loss. Hence, the higher loss at medium velocity was observed as compared to the lower or higher velocities. The significant difference ($p < 0.05$) detected in the crude fat content was likely to be caused by the variations of moisture content.

Meat colour is widely used by consumers to determine the freshness of the meat products [27]. Colour is closely associated with pH values of the meat. As shown in Tables 2 and 3, increase of air velocity had no impact on the pH and instrumental colour of in-bag dry-aged beef except for the lightness (L*). L* decreased significantly ($p < 0.05$) with the increase of velocity to 2.5 m·s^{-1}. Similar findings have been reported with the increase in air velocity from 0.2 to 0.5 m·s^{-1} in dry-aged beef [24]. The reduced lightness could be attributed to the moisture loss on the surface of the meat, resulting in more light absorption and darker colour [28].

Water-holding capacity (Table 4) and shear force tenderness (Table 5) of fresh in-bag dry-aged samples were not influenced by the increase of air velocity ($p > 0.05$) which agreed with the findings reported by Kim et al. [24] on dry-aged beef.

3.1.2. Frozen In-Bag Dry-Aged Beef. There was no interaction ($p > 0.05$) between air velocity and frozen storage on the quality and physicochemical properties of in-bag dry-aged lean bull beef. Air velocity had no effect on the proximate content, pH, water-holding capacity, instrumental colour, and instrumental texture of in-bag dry-aged beef (7 d of ageing, T2–T4) stored frozen at −18°C for 12 months. This suggests that the use of higher air velocity in the dry-ageing of lean bull beef would not negatively influence the meat quality of in-bag dry-aged lean beef frozen for 12 months.

3.2. Effect of Stepwise Ageing on the Physicochemical Properties and Acceptability of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.2.1. Effect of Stepwise Ageing on the Physicochemical Properties. Stepwise ageing involving the in-bag dry-ageing of lean bull beef for 7 d followed by 14 d of wet-ageing (T2) significantly ($p < 0.0001$) reduced % weight loss of the dry-aged meat compared to the control (T1, in-bag dry-aged straight for 21 d). In this study, stepwise ageing had no effect on the pH, proximate content, and instrumental colour of fresh in-bag dry-aged beef ($p > 0.05$, Table 1) as was previously reported [29–32]. The water-holding capacity (measured as drip and cook losses) of fresh in-bag dry-aged lean beef (21 d, T1 and T2) were not affected by the stepwise ageing regimes, as shown in Table 4. Conflicting findings reported by Kim et al. were [12] that stepwise ageing (dry-aged 10 d + wet-aged 7 d) significantly decreased the drip loss but did not differ in cook loss as compared to dry-aged only. They attributed the higher drip loss from the dry-aged-only beef loins to the possible higher protein oxidation in the samples which resulted in the decrease of water-holding capacity. The lack of difference in cook loss between ageing regimes observed in the current study was in line with the outcomes of most of the previous studies on dry-aged beef [13, 24, 33, 34].

The instrumental tenderness (shear force) of dry-aged lean beef produced using stepwise ageing did not differ with its control counterpart produced by dry-ageing of lean beef for 21 d straight (Table 5). This result contradicts the findings reported Kim et al. [12] that stepwise ageing produced significantly lower ($p < 0.05$) shear force compared with traditional dry-ageing. The difference between our finding and that of [12] could be the type of beef used in the studies particularly the difference in the fat content of our lean bull beef and the beef (USDA low choice) used in [12].

There was no effect of stepwise ageing on the measured quality parameters of frozen in-bag dry-aged lean beef (21 d, T1 and T2). Therefore, the use of stepwise ageing produced in-bag dry-aged lean beef of equivalent quality to those of in-bag dry-ageing only without an adverse effect, even after a long-term frozen storage.

3.2.2. Interaction between Air Velocity and Stepwise Ageing. A significantly higher ($p < 0.05$) average % weight loss (20.48%) was found in the control (in-bag dry-ageing only, T1) at low velocity of 0.5 m·s^{-1} compared to the other treatments (T2 = 9.80%, T3 = 10.92%, and T4 = 11.54%), as shown in Figure 2. There was no significant ($p > 0.05$) weight loss arising from the extended wet-ageing process for T2, T3, and T4 regardless of the velocity as expected. The pH, b*, and chroma of stepwise ageing were similar to those of dry-ageing-only beef samples (Tables 2 and 3). Although the values slightly ($p < 0.05$) decreased at the high velocity (T4), the difference was negligible and should not impact meat quality. The other colour attributes, proximate content and water-holding capacity of fresh in-bag dry-aged beef (21 d, T1–T4), did not differ between the treatment combinations

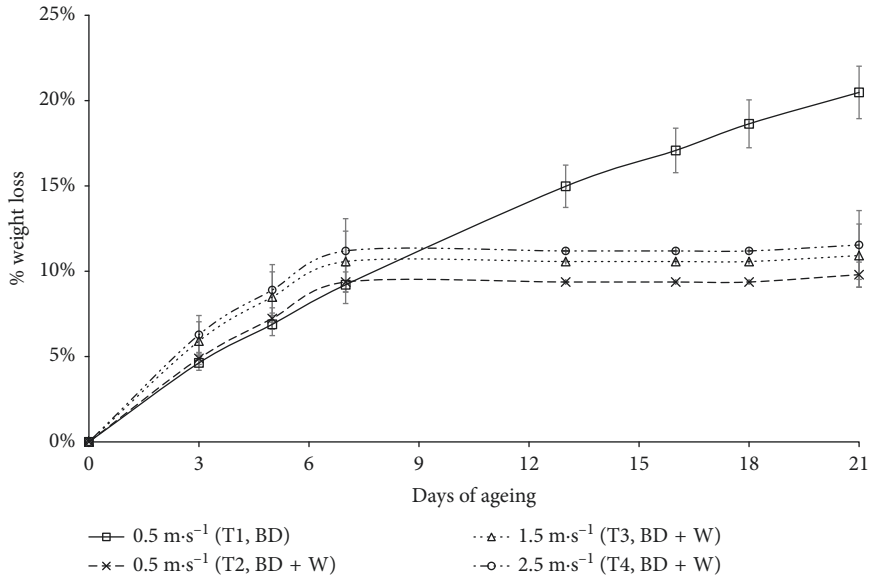

Figure 2: Average % weight loss of lean beef striploins of four different ageing treatments across different ageing times (days). BD: in-bag dry-ageing; W: wet-ageing. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d.

Table 1: Effect of ageing treatments, ageing time, and frozen storage on the proximate content of in-bag dry-aged lean bull beef.

Attributes/ storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (unaged)	p storage (7 d ageing)	p storage (21 d ageing)
% moisture												
Fresh	0 d	76.22x	76.29x	75.84x	75.81x	0.43	0.029	0.532	0.450	0.561	0.727	0.810
	7 d		75.83ay	74.77by	75.84ay							
	21 d	74.93y	75.19z	74.67z	74.33z							
	p ageing time	0.001	***	***	***							
Frozen	0 d	76.27	76.37	76.55	75.69	0.82	0.002	0.824	0.494			
	7 d		76.22a	74.83b	75.69a							
	21 d	74.93	75.09	74.57	74.08							
	p ageing time	0.241	0.145	0.068	0.075							
% crude fat												
Fresh	0 d	0.69	0.64	1.10	1.17	0.26	0.018	0.796	0.163	0.201	0.370	0.832
	7 d		0.63a	1.21b	0.62a							
	21 d	0.66	0.69	1.05	1.25							
	p ageing time	0.799	0.654	0.298	0.146							
Frozen	0 d	1.09	0.99	0.88	2.00	0.33	0.056	0.394	0.222			
	7 d		0.73a	1.28b	0.85a							
	21 d	0.68	0.78	1.13	1.08							
	p ageing time	0.232	0.541	0.600	0.116							
% muscle protein												
Fresh	0 d	22.31x	22.17x	22.26x	22.26x	0.29	0.948	0.709	0.236	***	***	***
	7 d		22.65y	23.21y	22.77y							
	21 d	23.48y	23.33z	23.70z	23.77z							
	p ageing time	0.003	<0.001	***	***							
Frozen	0 d	19.01x	18.25x	18.65	20.34	1.04	0.198	0.874	0.432			
	7 d		19.78xy	21.37	20.12							
	21 d	21.35y	21.23y	21.11	22.11							
	p ageing time	0.043	0.044	0.125	0.058							

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 2: Effect of ageing treatments, ageing time, and frozen storage on pH of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (7 d ageing)	p storage (21 d ageing)
pH											
Fresh	0 d	5.34x	5.34x	5.36x	5.32x	0.04	0.082	0.232	0.035	0.226	0.654
	7 d		5.74y	5.60y	5.69y						
	21 d	5.66ay	5.62abz	5.64ay	5.58bz						
	p ageing time	***	***	***	***						
Frozen	7 d		5.66	5.65	5.62	0.03	0.420	0.644	0.525		
	21 d	5.63	5.62	5.61	5.6						
	p ageing time		0.068	0.299	0.588						

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 3: Effect of ageing treatments, ageing time, and frozen storage on the instrumental colour of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (7 d ageing)	p storage (21 d ageing)
L *											
Fresh	0 d	33.51x	33.34x	33.05x	32.65x	0.62	0.022	0.588	0.411	***	***
	7 d		40.78ay	41.28ay	38.54by						
	21 d	39.80y	39.46z	39.46z	38.85y						
	p ageing time	<0.001	***	***	***						
Frozen	7 d		35.99	36.52	35.44	0.97	0.492	0.712	0.926		
	21 d	36.22	35.86	36.08	36.48						
	p ageing time		0.897	0.678	0.232						
a *											
Fresh	0 d	18.05	18.21x	17.44x	17.47x	0.90	0.797	0.927	0.084	0.004	***
	7 d		15.25y	15.29y	14.62y						
	21 d	18.45a	18.55ax	17.06abx	16.38bz						
	p ageing time	0.232	<0.001	0.004	0.001						
Frozen	7 d		13.37	11.79	13.82	0.81	0.075	0.312	0.742		
	21 d	13.77	13.02	13.34	13.04						
	p ageing time		0.686	0.303	0.157						
b *											
Fresh	0 d	11.84x	11.74x	11.44	11.31x	0.49	0.059	0.53	0.018	***	***
	7 d		10.81y	11.39	10.23y						
	21 d	12.93ay	12.69az	11.98ab	11.44bx						
	p ageing time	0.005	0.001	0.298	0.058						
Frozen	7 d		6.56	5.53	6.87	0.58	0.116	0.53	0.471		
	21 d	7.43	6.93	7.17	6.55						
	p ageing time		0.547	0.154	0.355						
Chroma											
Fresh	0 d	21.59x	21.68x	20.87x	20.82x	0.99	0.585	0.347	0.047	***	***
	7 d		18.70y	19.08y	17.84y						
	21 d	22.54ay	22.48ax	20.86abx	19.98bx						
	p ageing time	0.034	<0.001	0.048	0.005						
Frozen	7 d		14.9	13.03	15.44	0.94	0.078	0.994	0.653		
	21 d	15.66	14.00	15.15	14.6						
	p ageing time		0.888	0.236	0.169						
Hue											
Fresh	0 d	33.36x	32.85x	33.34x	32.93x	0.75	0.267	0.885	0.775	***	***
	7 d		35.42y	36.79y	35.01y						
	21 d	35.25y	34.42z	35.14y	34.95y						
	p ageing time	0.01	<0.001	0.001	***						
Frozen	7 d		26.04	24.89	26.38	1.22	0.401	0.442	0.544		
	21 d	28.34	27.92	28.2	26.67						
	p ageing time		0.061	0.158	0.755						

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 4: Effect of ageing treatments, ageing time, and frozen storage on water-holding capacity of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments				SED	p AV	p SA	p SA × AV
		T1	T2	T3	T4				
% drip loss									
	7 d		3.86x	3.35x	3.26x	0.29	0.226	0.152	0.257
Fresh	21 d	1.35	1.56y	1.58y	1.92y				
	p ageing time		<0.001	0.001	0.001				
% thaw + drip loss									
	7 d		12.38x	10.36x	11.75x	1.41	0.335	0.322	0.443
Frozen	21 d	5.79	7.49y	5.86y	7.33y				
	p ageing time		0.009	0.013	0.003				
% cook loss									
	7 d		30.22	29.46	32.18x	1.69	0.519	0.979	0.204
Fresh	21 d	27.77	27.74	25.98	28.69y				
	p ageing time		0.240	0.120	0.020				
	7 d		31.81	31.81	33.04x	1.04	0.174	0.498	0.644
Frozen	21 d	29.60	30.29	30.59	29.40y				
	p ageing time		0.103	0.371	0.003				

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$).

Table 5: Effect of ageing treatments, ageing time, and frozen storage on instrumental texture of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments				SED	p AV	p SA	p SA × AV
		T1	T2	T3	T4				
Shear force (N)									
	0 d	132.00x	116.70x	131.81x	114.25x	12.45	0.071	0.932	0.919
Fresh	7 d		89.34xy	69.24y	83.95y				
	21 d	72.67y	71.98y	68.26y	70.61y				
	p ageing time	0.001	0.011	***	0.007				
Texture profile analysis									
Frozen									
	7 d		2.49	2.81	2.72	0.37	0.348	0.601	0.920
Hardness (kg)	21 d	3.02	2.88	3.11	3.10				
	p ageing time		0.404	0.362	0.193				
	7 d		0.53	0.51	0.54	0.02	0.678	0.634	0.969
Springiness	21 d	0.53	0.53	0.53	0.52				
	p ageing time		0.778	0.051	0.582				
	7 d		0.55	0.53	0.55	0.41	0.313	0.609	0.410
Cohesiveness	21 d	0.55	0.54	0.54	0.54				
	p ageing time		0.380	0.388	0.537				
	7 d		0.72	0.75	0.81	0.12	0.394	0.547	0.944
Chewiness (kg)	21 d	0.89	0.83	0.89	0.89				
	p ageing time		0.440	0.159	0.509				
	7 d		0.23	0.22	0.22	0.01	0.522	0.909	0.276
Resilience	21 d	0.23	0.22	0.22	0.22				
	p ageing time		0.181	0.473	0.773				

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$).

of air velocity and stepwise ageing ($p > 0.05$). As discussed above, air velocity and stepwise ageing process mainly influenced the water fraction on the surface of the samples.

A significantly ($p < 0.05$) higher amount of yeast was detected in the control (in-bag dry-ageing only) compared to those from stepwise ageing although the growth of surface microorganisms in all samples was low (Table 6). The results are supported by the outcomes of other studies that reported higher yeast counts in the beef samples of in-bag dry-ageing as compared to the wet-aged counterparts [8, 10, 29, 34]. The lactic acid bacteria, Enterobacteriaceae, *E coli*, and moulds did not differ between the treatment combinations of air velocity and stepwise ageing because the proliferation of these microorganisms was low and below the detection limit (Table 6). The use of different ageing regimes (dry/wet-ageing) had no impact on growth of Enterobacteriaceae and

Table 6: Effect of ageing treatments and ageing time on A_w and surface microbial growth of fresh-and-never frozen in-bag dry-aged lean bull beef.

Attributes	Ageing time	Treatments				SED	p SA × AV
		T1	T2	T3	T4		
A_w	0 d	0.992x	0.993x	0.994x	0.992x	0.001	0.325
	21 d	0.987y	0.987y	0.988y	0.986y		
	p ageing time	0.005	***	0.003	***		
Microbial load (log cfu/g)							
APC	0 d	4.21x	2.62	3.09	2.74x	0.61	0.281
	21 d	2.50y	3.29	2.56	2.00y		
	p ageing time	0.038	0.459	0.494	***		
LAB	0 d	1.15	ND	1.50	ND	0.07	—
	21 d	ND	ND	ND	ND		
	p ageing time	—	—	—	—		
Mould	0 d	1.39	ND	ND	ND	0.05	—
	21 d	ND	1.00	ND	ND		
	p ageing time	—	—	—	—		
Yeast	0 d	2.21x	ND	1.48	1.39	0.40	0.003
	21 d	4.06ay	1.57b	2.12b	2.41b		
	p ageing time	0.007	—	0.237	0.076		
Enterobacteriaceae	0 d	3.16	1.84	1.38	1.71	0.71	—
	21 d	ND	2.69	ND	1.35		
	p ageing time	—	—	—	—		
E. coli (MPN/g)	0 d/21 d	ND	ND	ND	ND	—	—

ND: not detected; BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

moulds [8, 10] and *E. coli* [9]. Some studies have suggested that wet-ageing may contribute to a higher amount of lactic acid bacteria and lower aerobic bacteria count [8–10, 34, 35] postageing compared with (in-bag) dry-ageing due to the anaerobic ageing condition favouring the proliferation of lactic acid bacteria and suppressing that of aerobic bacteria. The aerobic bacteria counts tended to decrease ($p < 0.05$) with the air velocity in stepwise aged beef samples (T2–T4, Table 6). Current findings of lactic acid bacteria and aerobic bacteria could be explained by the faster dehydration on the meat surface at higher air velocities playing a major role in creating an adverse environment for the growth of both bacteria.

The quality parameters of a long-term frozen-stored in-bag dry-aged lean beef (21 d, T1–T4) were not affected by the treatment combinations of air velocity and stepwise ageing. The differences in colour (a*, b*, and chroma) observed in the fresh in-bag dry-aged beef due to ageing treatments disappeared after long-term frozen storage (Table 3). This could be due to the biochemical changes of muscle cells and myoglobin during the frozen storage.

3.2.3. Sensory Quality of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

(1) Acceptability of Fresh In-Bag Dry-Aged Beef. The consumer panel could not detect any difference ($p > 0.05$) between the samples produced using the four ageing treatment combinations in terms of aroma, texture, tenderness, juiciness, flavour, and overall liking (Table 7). The lack of differences in the consumer acceptability of the tenderness and texture of the in-bag dry-aged samples (21 d) from the four ageing treatments agreed with the instrumental shear force measurement of tenderness (Table 5). Kim et al. [12] also found no difference in the sensory acceptability of dry-aged beef loins when comparing the stepwise ageing regimes with those traditional dry-aged only.

Debate on the consumer preference and acceptability of dry-aged beef over the equivalent wet-aged beef is ongoing. Some of the studies found no significant difference in the consumer acceptability of the tenderness and juiciness of dry-aged as compared to the wet-aged beef [13, 24, 33, 36, 37]; others reported higher preference of (in-bag) dry-aged beef compared to the wet-aged in terms of tenderness and juiciness [1, 8, 10, 13, 38]; while others suggested the tenderness of wet-aged beef was more acceptable/preferred than the dry-aged counterparts [4, 32, 39]. The differences arising from ageing time, ageing conditions, and muscle types across the studies of dry-ageing may contribute to the inconsistent findings in consumer acceptability.

For the overall liking and the flavour liking, findings from previous studies were also controversial. Thus, the conclusion over the most effective ageing method to maximise palatability cannot be easily drawn. In the current study, consumers gave similar ratings of overall liking to all four samples; however, different findings were observed when they were asked to express their preference between samples. As shown in Table 7, about 94% of the panellists were able to discriminate the difference between four treatment combinations. The control (in-bag dry-aged straight for 21 d) was the most preferred by the consumers

Table 7: Effect of ageing treatment combinations and frozen storage on sensory acceptability of in-bag dry-aged lean beef for 21 days.

Attributes	Storage type	Treatments				SED	p SA × AV
		T1	T2	T3	T4		
	Fresh						
Aroma		56.53	55.58	55.78	56.60	2.81	0.977
Tenderness		53.62	48.73	45.84	55.01	4.88	0.224
Juiciness		54.39	53.64	48.35	47.80	4.12	0.244
Flavour		44.85	44.76	41.87	42.95	4.35	0.881
Off-flavour		20.68	16.73	19.16	17.79	3.66	0.727
Overall liking		59.77	58.20	52.88	57.17	3.33	0.218
Preference ranking (%)		28.41	22.73	22.73	20.45		
	Frozen						
Aroma		5.75	5.81	5.81	6.00	0.19	0.548
Tenderness		5.74	5.46	5.75	6.02	0.30	0.397
Juiciness		5.86a	4.55b	5.24c	5.91a	0.26	***
Flavour		6.12	5.76	5.96	6.33	0.23	0.126
Off-flavour		1.71	1.89	1.85	1.78	0.12	0.401
Overall liking		6.03a	5.37b	5.89a	6.25a	0.24	0.006

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

(28.41%) in the current study compared to other ageing treatments which were equally preferred (20–22%).

(2) Acceptability of Frozen Dry-Aged Beef. For the first time, we report the sensory quality of dry-aged beef that were frozen for 12 months. Despite the negative impression over the frozen storage of beef, the sensory quality of the in-bag dry-aged beef (21 d) of all the ageing treatments, including aroma, tenderness, flavour, and overall liking, was rated to be higher than that of the fresh (never frozen) in-bag dry-aged counterparts (Table 7). No significant difference ($p > 0.05$) was found in tenderness, between the four ageing treatments, which is in agreement with the instrumental texture profile analysis of the corresponding frozen in-bag dry-aged samples. Panellists failed to differentiate the control in-bag dry-aged beef (T1) and stepwise aged samples except for those aged at low ageing chamber air velocity (T2), and this was the least preferred by the consumers at a significant level ($p = 0.006$). This may have been caused by significantly ($p < 0.05$) lower rating of juiciness, which is further supported by higher % thaw + drip loss and % cook loss compared to the other ageing treatments (Table 4).

Rancidity, noted as the off-flavour, is another important indicator for the consumers to determine the freshness of cooked meat. Low mean values of the off-flavour were found in the cooked steaks of all the treatments, suggesting the difficulty in recognising the rancid flavour from the frozen in-bag dry-aged (21 d) lean beef samples. The rancidity note generated from the deteriorated meat is mainly caused by the oxidation and hydrolysis of the fat in meat. The meat samples containing higher fat (such as prime cuts and wagyu meat) were more susceptible to oxidation and consequently give off the rancid flavour. It is worth noting that the beef samples used in this study were lean bull beef, which only contained approximately 1% of IMF (Table 1). Therefore, the low level of rancidity in the beef samples after long-term frozen storage was expected. Kim et al. [12] recently reported no effect of short-term (1 month) freezing on the sensory quality of beef loins (USDA low choice) dry-aged by the stepwise ageing process. However, how the sensory quality would change if the storage time was extended (e.g., ≥6–12 months) and how the long-term frozen storage would affect the sensory quality of dry-aged beef with different IMF contents have not been explored. Answers to these questions would be of great significance for the export of frozen dry-aged beef.

3.3. Effect of Dry-Ageing Time on the Physicochemical Properties of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.3.1. Fresh In-Bag Dry-Aged Beef. The length of dry-ageing time had a significant ($p < 0.05$) impact on the % weight loss observed in the present study as expected (Figure 2). There was a significant ($p < 0.05$) decrease in moisture content with the increased ageing time (Table 1). This finding is consistent with the outcomes of some previous studies [29, 40] and contradicted others [9]. The variation in the outcomes reported in the literature may have arisen from the different sampling methods used in the studies though often these were not clearly stated in the studies. The slight increase in the protein content ($p < 0.05$) with ageing time observed in the current study (Table 1) was more likely to be due to the decrease in moisture content ($p < 0.05$) in the in-bag dry-aged samples (T1–T4) over the ageing time.

Overall, the pH of in-bag dry-aged striploins increased significantly ($p < 0.05$) after 21 d of ageing (Table 2) as compared to unaged counterparts which was in agreement with other studies on dry-aged beef [24, 29, 39]. Within the first 7 d of in-bag dry-ageing, the pH increased from an average of 5.34 to 5.74 (T2), 5.60 (T3), and 5.69 (T4). Although a slight but significant decrease ($p < 0.05$) of pH values during the extended 14 d of wet-ageing was detected

in T2 and T4 samples, the actual difference was minimal (0.1 units). The increase of pH values after dry-ageing could be associated with the generation of nitrogenous compounds caused by proteolysis as suggested by Aksu et al. [41].

After in-bag dry-ageing for 21 d (T1, Table 3), all the colour parameters (L*, b*, chroma, and hue) measured in this study increased significantly ($p < 0.05$) except for a* which did not change. However, a* has been reported in other studies to increase with ageing time on dry-aged beef using ageing bags [8] and traditional (no bag) dry-ageing [34]. The stepwise ageing process affected the colour properties differently from the control across the ageing time. The first 7 d of the in-bag dry-ageing process significantly ($p < 0.05$) increased the L* and hue angle, decreased a* and chroma compared with unaged (0 d) counterparts (T2–T4, Table 3). The extended wet-ageing from 7 d to 21 d of ageing slightly decreased L* (T2 and T3) and hue (T2) but significantly increased ($p < 0.05$) a* and chroma (T2–T4) to similar levels as unaged counterparts. The inconsistent changes of a* and chroma over the stepwise ageing process is unclear. It could be associated with the difference of metmyoglobin reducing ability and stability of myoglobin at different ageing times. The increase of L* has been linked with the myofibrillar protein denaturation which consequently gave rise to a tighter and more opaque structure [42]. The lack of change in L*, a*, and b* values over the dry-ageing process was also reported in previous studies [8]. The stepwise ageing has been reported to have no negative effect on the instrumental colour quality [12]. However, to the best of our knowledge, the effect of stepwise ageing time on the instrumental colour of in-bag dry-aged beef has not been explored in previous studies.

Drip loss significantly decreased ($p < 0.05$) in in-bag dry-aged beef at 21 d compared to 7 d regardless of the ageing treatment combinations (Table 4). This could be due to the significant amount of moisture lost by evaporation after 21 d of ageing thereby reducing the amount of moisture that could be lost as drip compared to 7 d, as discussed above. The reduced drip loss at 21 d could also be attributed to the higher muscle protein breakdown with 21 d of ageing compared to 7 d resulting in the "sponge effect" proposed by Farouk et al. [43], which physically entrap the water and improve the water-holding capacity by lowering the water loss by gravity. The water-holding capacity of lean beef increased with ageing time, particularly in terms of the decrease in drip loss. The cook loss, on the contrary, could be another indicator of the water-holding capacity under extreme conditions, i.e., heating. The cook loss of in-bag dry-aged lean beef decreased with the ageing time from 7 d to 21 d in general, but the significant decrease was only seen in the beef aged at highest air velocity (T4). Other studies also did not find any difference in cook loss of dry-aged beef from 14 to 35 d [9, 29, 44].

Shear force values decreased significantly ($p < 0.05$) with dry-ageing time at the first 7 d from an average of approximately 120 N (untender) to 80 N (tender) and then further decreased slightly ($p > 0.05$) during the extended 14 d of the wet-ageing period to a similar level (approx. 70 N), regardless of the ageing treatments (Table 5). The majority of studies on dry-aged beef reported no difference of shear force between wet-ageing, traditional dry-ageing, and in-bag dry-ageing of beef [2, 4, 9, 10, 36], which further support the findings of the current study. Therefore, it was the ageing time rather than the ageing methods that played the key role in tenderisation of beef. A significant decrease of shear force occurring within the first 14 d of ageing time has been observed by Gudjónsdóttir et al. [34]. Extension of ageing time beyond 4 weeks showed a minor effect on the improvement of tenderness [29, 36, 39, 44]. Therefore, the most rapid improvement of in-bag dry-aged beef tenderness occurs within the first 7 to 14 d of ageing time. This could be explained by the activity of endogenous enzymes (mainly μ-calpain) which plays a significant role in the tenderisation through proteolysis [45]. The activity is decreased significantly after 7 d of ageing time [46]. A more recent study by Velotto et al. [37] observed slightly faster decline of μ-calpain activity in dry-aged beef compared to the wet-aged over 15 d of ageing time. Further tenderisation during extended ageing time was mainly affected by more stable lysosomal proteases (mainly cathepsin B and B + L), though the rate of tenderisation decreased. Measurement of shear force at 0 d and/or 7 d of ageing time is absent in many of the studies to date, which may have contributed to overlooking of the fact that the longer ageing time is not necessary for the improvement of tenderness. Though tenderisation is well known to improve the eating quality of meat, it should be critically controlled to avoid overtenderisation and loss of texture and mouth appeal.

A_w is one of the most important parameters to indicate the shelf life of a food product. A_w of beef samples from all four treatments decreased significantly ($p < 0.05$) from an average value of 0.993 to a similar level of 0.987 after 21 d of ageing time, as shown in Table 6. In general, a low level of growth of surface microorganisms was found before and after ageing. The significant decrease ($p < 0.05$) of the aerobic bacteria counts in the in-bag dry-aged beef of T1 and T4 may be associated with the decline of A_w because of the surface being dried. The proliferation of aerobic bacteria with the increase of dry-ageing time has been reported [1, 7, 9]. However, contradictory findings were reported in Ahnström et al. [29] that no difference of aerobic bacteria counts on dry-aged beef regardless of ageing methods and ageing time. Yeast in the present study increased with ageing time ($p < 0.05$) in the control in-bag dry-aged beef (T1) which is in line with other studies on (in-bag) dry-aged beef reported by Degeer et al. [9]. The increase of yeast could be attributed to yeast species being able to grow on the dry meat surface with low moisture content compared to other microorganisms.

There was no difference in lactic acid bacteria, Enterobacteriaceae, and moulds across ageing time in the current study. *E. coli* was not detected in all the samples. Inconsistent results have been reported that Enterobacteriaceae [8], *E. coli* [9] and moulds [34] increased with ageing time. The decreased [9, 29] or unchanged [1] counts of lactic acid bacteria were also observed on dry-aged beef across the ageing. The proliferation of microorganisms has been observed in the traditional dry-ageing process which could cause the

spoilage of meat, and some toxin-producing pathogens may lead to serious food poisoning and even death [47]. On the contrary, microorganisms could facilitate the deterioration of meat quality and generation of off-flavours such as cheesy and dairy [48] and discolouration [49]. Therefore, the strict control of the processing hygiene and monitoring of the level of microorganism contamination are extremely important in terms of meat quality and food safety assurance. Current dry-ageing treatment combinations were able to produce microbiologically safe dry-aged lean beef products after 21 d of ageing time. This enables the meat industry to produce dry-aged products that satisfy the food safety standard for both local and export markets.

3.3.2. Frozen In-Bag Dry-Aged Beef. After long-term frozen storage, the proximate content of in-bag dry-aged beef from all four ageing treatments did not differ across the ageing time except for the muscle protein content which increased with ageing (Table 1). Significant ($p < 0.05$) increase of muscle protein content was seen in T1 and T2 which was likely to be attributed to the decrease of moisture content with ageing time. The water-holding capacity of frozen in-bag dry-aged samples increased significantly ($p < 0.05$) with ageing time in terms of % thaw + drip loss which was also evident in the fresh counterparts (Table 4). In general, the cook loss of frozen in-bag dry-aged samples decreased with ageing though the significant ($p < 0.05$) decrease was only found in the samples aged at highest air velocity (T4). Therefore, the water-holding capacity improved by ageing time was stable over the long-term frozen storage which was in agreement with Farouk et al. [50].

The pH, colour, and texture profile of in-bag dry-aged lean beef was not affected by ageing time ($p > 0.05$) after long-term frozen storage. The differences of pH, instrumental colour, and texture detected in fresh in-bag dry-aged samples between 7 d and 21 d of ageing were not observed after long-term storage. The changes in texture could be explained by two theories suggested in Dransfield [51]: (1) proteolysis during long-term frozen storage although the rate was slow because of calpain activity being suppressed; (2) a more rapid proteolysis occurred when thawing prior to freezing due to the reactivation of calcium-depend proteases. Another possible explanation could be the interaction between ageing time and frozen storage as suggested by Vieira et al. [52] that the beef aged for 3 d showed significant decrease on shear force during longer frozen storage time as compared to those aged for 7 d which did not change. Therefore, understanding the texture profile of in-bag dry-aged lean beef which has been long-term frozen stored is more important than the shear force tenderness because the frozen storage could further tenderise the meat as discussed above. The changes of other texture properties are not able to determine by single shear force measurement.

3.4. Effect of Frozen Storage on Lean Dry-Aged Beef for Different Ageing Times. The pH of in-bag dry-aged beef was not affected by long-term frozen storage ($p > 0.05$). This supports the outcomes of the 6 months frozen storage of stepwise dry-aged beef reported by Kim et al. [12]. Proximate content of in-bag dry-aged beef at all three time points (0, 7, and 21 d) was not affected by the frozen storage except for the muscle protein content which decreased significantly ($p < 0.0001$) after frozen storage. A consistent moisture and fat content over the frozen storage of up to 52 weeks was also reported by Holman et al. [53] on beef, with wet-ageing for up to 5 weeks prior to freezing. The muscle protein content in this study was measured from the extracted muscle protein solution. The decrease of muscle protein content was more likely to be attributed to the decrease of protein solubility in the extraction buffer due to protein denaturation after long-term frozen storage which has also been reported by Farouk et al. [50].

Frozen storage had the major effect on the instrumental colour of in-bag dry-aged beef (Table 3). All colour attributes have significantly ($p < 0.05$) decreased for in-bag dry-aged samples (7 and 21 d) after the frozen storage. Thawed in-bag dry-aged beef in the current study became darker but still within the consumer acceptable range (approx. 35–40 of L^*) [54]. a^* declined to around 13.0 which was slightly below the threshold of 14.5, according to their study. However, a lesser brown colour (lower hue) as compared to the fresh counterparts was detected. Decrease of hue in the current study was attributed to the significant decrease in b^*. The change in colour observed in this study due to long-term frozen storage could be partially explained by the damage of muscle cells which altered the optical properties of the meat [55]. This may support the loss of lightness and redness of meat colour. The decreases in b^* [52] and hue [50] of beef over the frozen storage have also been reported previously. The decrease in b^* may have been caused by the migration of the oxygenated layer to a deeper position due to the reduced oxygen consumption rate over long-term frozen storage, and this may have resulted in a delay of the oxidation of myoglobin to metmyoglobin. Another possible reason may be that, due to the low fat content of the lean bull beef used in this study, a lower level of lipid oxidation could contribute lesser to the generation of metmyoglobin and yellowness of beef [56]. A significant decrease of L^* and a^* and increase of b^* and hue have also been reported by Kim et al. [12] on dry-ageing/stepwise aged-then-frozen beef loins.

The water-holding capacity of frozen in-bag dry-aged beef decreased due to the extrafluid loss upon thawing (Table 4). Cook loss also tended to increase after frozen storage which may be associated with the decrease of juiciness [57]. However, there was no clear decline in the juiciness rating of frozen in-bag dry-aged samples in this study (Table 7), which was also observed on beef [52] and lamb of other studies [58].

4. Conclusion

The increase of dry-ageing chamber air velocity accelerated the weight loss of in-bag dry-aged lean bull beef but had no other negative effects on meat quality, microbiological safety, and consumer palatability. Ageing time, on the contrary, played a more important role in improving the quality of the dry-aged products. Combining in-bag dry-

ageing with traditional wet-ageing as a stepwise ageing strategy was able to produce dry-aged lean beef of equivalent quality compared to those of dry-ageing only for the same period of ageing time but with lower weight loss/higher yield. In-bag dry-aged lean bull beef products could be long-term frozen stored for up to 12 months and still be acceptable to consumers.

5. Implications of the Study

The following are some of the implications particularly the stepwise ageing process used in the present study:

(i) The process can be applied by the meat industry to shorten the turnover time of the dry-ageing chamber because the wet-ageing component can be accomplished during chill chain distribution without any loss in quality.

(ii) The process produced microbiologically safe dry-aged products with improved ease of handling and potentially free of trimming and increased yield. This enables the meat industry to produce dry-aged products easier, safer, and cheaper for both local and export markets.

(iii) Long-term frozen storage of in-bag dry-aged lean beef produced using the process had no effect on the quality of the thawed product except for the minor discolouration. Thus, postthawing display may not be recommended for long-term frozen in-bag dry-aged lean beef. Exporting the product frozen in vacuum packages or supplying the product pre-cooked in sous vide for local and international restaurants and markets is suggested to retain the value of the in-bag dry-aged products.

(iv) In-bag dry-aged lean beef from the process have potential as a value-added product for the low marbled fresh and frozen beef market locally and globally. Future work regarding the oxidative changes of lipids and proteins, the changes of flavour precursors from the ageing treatments and frozen storage, and their impact on the shelf life and functionality of the products need to be explored.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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