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**Effects of different ageing methods on colour, yield, oxidation and sensory qualities of Australian
beef loins consumed in Australia and Japan**

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Abstract

This study investigated the effect of three ageing methods (dry, wet and stepwise wet-then-dry) and ageing time on pH, colour, yield, lipid and protein oxidation and eating quality of beef loins using Meat Standards Australia (MSA) sensory protocol with 900 and 540 consumers in Australia and Japan, respectively. Australian beef loins (*Longissimus thoracis et lumborum*) at four days post mortem were subjected to wet ageing (boneless; for 7, 21, 35 or 56 days), dry ageing (bone-in; for 35 or 56 days) or a wet-then-dry ageing method (bone-in; 21 days wet ageing followed by 35 days dry ageing). The pH was higher in dry aged than wet aged beef loins ($P < 0.001$). Instrumental measurement of surface colour of trimmed dry and wet aged steaks showed significant differences in a^* , b^* and hue angle. Weight loss was higher in dry aged primals ($P < 0.001$), however, total water content was similar among the two ageing methods ($P = 0.934$). Retail yield did not differ between 35 and 56 days dry aged primals. Lipid (TBARS) and protein (total carbonyl content) oxidation between the dry and wet aged samples differed depending on the ageing time. When comparing the wet-then-dry and 56 days dry aged samples, only pH and retail yield differed. Australian and Japanese consumers rated dry aged steaks significantly higher ($P < 0.001$) than the wet aged counterparts for tenderness, juiciness, flavour, overall liking and weighted palatability scores. The wet-then-dry steaks were also rated higher than the 56 days wet aged steaks for flavour, overall liking and palatability within the Japanese sensory panels. The Japanese consumers also consistently rated all MSA sensory attributes lower ($P < 0.001$) than the Australian consumers. The results from this study show dry ageing provides a value adding opportunity for the meat industry in both domestic and export markets.

Keywords: Dry ageing; wet ageing; *Longissimus*; stepwise; Meat Standards Australia

1. Introduction

Ageing of beef is a long-established preservation method to improve tenderness, flavour and overall acceptance (Nair et al., 2019, Khan et al., 2016). This was traditionally achieved by 'hanging' the carcass, quarter or primal cut of meat in a cool place. With the advent of vacuum packaging, selected primal cuts could be aged under more controlled conditions, resulting in improvements in yield, processing, transport and shelf life. This method of meat preservation is often referred to as 'wet' ageing. Dry ageing is the ageing of a primal (usually unpackaged) in air under strictly controlled temperature, air velocity and relative humidity. Dry aged beef is marketed as a premium product and served in a small number of upscale restaurants and retailers around the world. Due to regulatory limitations, Australian beef destined to be dry aged in Japan are first vacuum packed, then take up to three weeks to reach Japan by sea where they are then subjected to dry ageing. Little is known in the literature about the effect of dry ageing of a pre-wet aged beef on instrumental and sensory measurements.

Instrumental colour measurement showed dry aged steaks from beef loins to be darker and more colour stable with increasing ageing time compared to wet aged steaks (Dikeman et al., 2013, Kim et al., 2016, Li et al., 2013). While oxidation of lipids and proteins is known to influence colour and sensory attributes of aged beef, little is known about the differential effect of dry and wet ageing on oxidation. The study of DeGeer et al. (2009), comparing lipid oxidation in beef loins dry aged with and without dry ageing bags, suggested lipid oxidation may contribute to development of the dry aged flavour.

Results comparing the sensory quality of dry and wet aged beef from previous studies are inconsistent. US studies reported no effects of dry ageing on sensory quality (Dikeman et al., 2013, Laster et al., 2008, Smith et al., 2008, Lepper-Blilie et al., 2016, Sitz et al., 2006), while others in Europe, Japan and New Zealand have shown beneficial effects of dry ageing on consumer sensory assessments of quality (Iida et al., 2016, Li et al., 2014, Li et al., 2013, Stenstrom et al., 2014, Kim et

al., 2016). Amongst studies that showed differential eating quality between dry and wet ageing, flavour was consistently the dominating attribute (Iida et al., 2016, Kim et al., 2016). Iida et al. (2016) showed that the umami (a highly desirable flavour) intensity was highest in highly marbled beef after dry ageing. It is noted lipid oxidation during meat ageing may also increase in highly marbled beef, thus contributing to a decrease in sensory flavour liking in prolonged ageing (Iida et al., 2016).

Tenderness may also contribute to a higher consumer score for dry aged beef. Kim et al. (2016) reported beef strip loins wet aged for 3 weeks at 1 °C produced higher shear force (tougher meat) than dry ageing for at 1 °C for the same period; however, this difference was not detected by the untrained consumer panel. Differences in sensory results reported in previous studies were likely due to differences in dry ageing conditions (Kim et al., 2016) and primal quality and composition, e.g. muscle, intramuscular fat and carcass quality (Smith et al., 2014, Iida et al., 2016, Lepper-Blilie et al., 2016). Also, the use of different sensory protocols may have contributed to differences reported.

The Meat Standard Australia (MSA) sensory testing protocol was developed specifically for the testing of whole meats (Watson et al., 2008). Sensory experiments using the MSA protocol involve a substantial number of untrained consumers and strict standards for meat preparation, cooking and serving, complex randomisation of carcasses, carcass side, cut, position and consumer groups. Large consumer panels reflecting the wide variation in consumer preference are regularly conducted as part of meat quality assurance schemes in many countries (Bonny et al., 2017).

This study, using current industry meat ageing practices, examined the effect of dry ageing and dry ageing of pre-wet (wet-then-dry) aged Australian beef loins and compare with conventional wet aged products using instrumental (pH, colour and yield) and chemical (lipid and protein oxidation) measurements. Sensory evaluation of the beef products was conducted using the MSA sensory protocol with untrained consumer panels in both Australia and Japan.

2. Materials and methods

2.1. Animal and carcass selection

Bos Taurus pasture raised cattle were purchased from three commercial beef properties in Tasmania, Australia. The cattle were mixed breeds of predominantly Angus, Hereford and Murray Grey, less than 24 months old and were hormone- and antibiotic-free. Following slaughter on the same day, carcasses (n=24) from 16 steers and 8 heifers with normal ultimate pH (≤ 5.7) were selected at 24-hour post mortem. *Achilles* tendon hung carcasses were graded using MSA grading standards with the average hot carcass weight of 157 ± 29 kg, ultimate pH of 5.55 ± 0.07 , ultimate temperature of 7 ± 1 °C, colour score of 3.8 ± 0.8 , ossification score of 154 ± 23 , rib fat score of 7 ± 2 and marbling score of 305 ± 71 . Bone-in striploins and OP ribs were removed from both sides of the 24 carcasses to obtain full length *Longissimus thoracis et lumborum*. A total of 48 striploins and 48 OP ribs were transported under refrigerated conditions to Top Cut Foods (Gold Coast, QLD) for processing and ageing. The use of commercial beef breeds, supply chain and beef cuts in this study was to provide results that are relevant to existing meat ageing practices in industry.

2.2. Meat cutting and ageing

The ageing method by ageing time treatment combination allocated within the two *longissimus thoracis et lumborum* from one carcass were; wet aged for 7, 21, 35 or 56 days; dry aged for 35 or 56 days; and wet-then-dry (21 day-wet ageing followed by 35 day-dry ageing). The 7- and 21- day wet aged samples were aged in steak size whereas all other ageing treatments were conducted with primals. The experimental design is illustrated in Fig. 1. The treatments were allocated to the OP ribs and strip loins within each carcass, allowing for randomisation of position within the cuts and sides. All products were weighed prior to ageing. Boned products were aged (wet) in vacuum pouches for 7, 21, 35 or 56 days at 4 °C. Primals allocated to dry ageing were kept bone-in and transferred to a dry ageing room and stored for 35 or 56 days. For the wet-then-dry treatment, bone-in primals (n=24) were wet aged in vacuum pouches with bone guards. At 21 days post mortem, the primals were removed from packaging materials, dried with paper towels and transferred to the dry ageing room and aged for a further 35 days. After 35 or 56 days, all dry aged primals were boned and

trimmed by commercial boners and 2.5 cm thick steaks were excised from the centre of all primals. Steaks were immediately frozen and stored at -20 °C. Duplicates of the 56 days wet ageing and 56 days dry ageing samples from each carcass and the wet-then-dry dry ageing samples were despatched to Japan for sensory analysis.

2.3. Dry ageing room specifications

The dry ageing room used at Top Cut Foods (Gold Coast, QLD) was a multi batch chiller loaded with movable racks. Whole racks containing meat products were moved to different positions within the chiller depending on the stage of dry ageing and length of time of ageing. The level of relative humidity (RH) in the chamber is critical to encourage drying. The RH in the dry ageing chamber at Top Cut Foods ranged from 53.5% to 100.0% with an average RH of 89.4% over the experimental period. The temperature of the chamber varied from 1.3 °C to 4.1 °C an average recorded temperature of 2.1 °C. In a central position air speed varied between 0.75-1.2 m/s. There were two UV light units fitted to the ceiling of the chiller.

2.4. Sampling for objective, chemical and sensory analysis

2.4.1. Wet ageing

For day 7 and 21 aged samples, steaks were removed from vacuum packing material, patted dry with paper towels and weighed. Steaks for sensory assessment were frozen immediately while steaks for chemical analysis were used to measure pH and colour before being stored at -20 °C. Whole primals wet aged for 35 and 56 days were cut into 2.5 cm thick steaks using a cutting jig to ensure accurate thickness.

2.4.2. Dry ageing

Whole primals retrieved from the dry ageing room were weighed followed by boning, trimming and reweighing. Steaks measuring 2.5 cm thick were obtained from each primal, vacuum packed and frozen at -20 °C. Steaks for chemical analysis were used to measure pH and colour before freezing.

2.5. pH and colour measurements

After cutting at each ageing time point, steaks measuring 2.5 cm thick were used to measure pH and colour. The pH of the interior of the steaks was measured by insertion of a spear-head pH probe attached to WP-80 pH-mV-temperature meter (TPS Pty Ltd., Brisbane, QLD). Temperature compensation was allowed using a TPS temperature probe. After 30 min of blooming, instrumental colour measurement on the cut surface of the meat was conducted using a Hunterlab Miniscan EZ (Hunter Assoc. Labs Inc., Virginia, USA) calibrated against white and black reference tiles. Duplicate surface colour measurements were taken with D65 illuminant and 10° observer angle. CIE L* (lightness), a* (redness) and b* (yellowness) values were obtained from the average values of two readings on the surface of loin samples. Hue angle ($\tan^{-1}(b^*/a^*)$) and chroma ($((a^{*2}+b^{*2})^{1/2})$) were obtained. Surface oxymyoglobin and metmyoglobin (%) were calculated using reflectance values at wavelength 630nm and 580nm as described by Khliji et al. (2010).

2.6. Weight loss during ageing

Loss of water during ageing was calculated by weighing meat steaks or whole primal before and after ageing. Total water loss was determined from the equation below:

$$\text{Weight loss (\%)} = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100$$

2.7. Yield at point of sale (for dry ageing only)

Yield at point of sale was defined as the total amount of loss from water and trimming after the dry ageing process using the equation below:

$$\text{Total yield (\%)} = \frac{(\text{weight before ageing} - \text{weight after ageing and trimming})}{\text{weight before ageing}} \times 100$$

Primals used for wet ageing were boned-out prior to ageing and no trimming was required after ageing, thus yield for wet aged products was not calculated.

2.8. Total water content

Total water content of meat was determined by the oven method. Meat samples (4g) were minced and dried in a convection oven set at 105 °C for 24h. The samples were cooled to room temperature in a desiccator and re-weighed. Total water content was calculated as:

$$\text{Total water content (\%)} = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100$$

2.9. Myofibrillar fragmentation index

Samples from 12 randomly selected carcasses were used for myofibrillar fragmentation index (MFI), lipid oxidation and total carbonyl content assays.

MFI measurement was conducted in duplicates for all treatments as per the method of Culler et al. (1978). Frozen raw muscle tissue (4 g) was weighed and homogenised using a Polytron PT 10-35GT homogeniser (Kinematica AG, Switzerland) in 7 mL of ice-cold extraction buffer (50 mM Tris/HCl; 10 mM EDTA, pH 8.3) at 13,000 rpm for 10 s. The tubes were centrifuged at 1500 × g for 10 min at 2 °C and the supernatant was discarded. The pellet was re-suspended in 25 mL of extraction buffer. This process was repeated twice. After the third wash, the pellet was re-suspended in 5 mL of extraction buffer. The suspension was filtered through a tea strainer to remove fat and connective tissue, followed by the addition of another 5 mL of extraction buffer to wash the strainer. Protein concentration was determined using Biruet assay with bovine serum albumin as standard. The myofibrillar suspension was diluted to a final protein concentration of 0.5 mg/mL with extraction buffer and the absorbance at 540 nm was determined in triplicate. MFI was calculated as $A_{540} \times 200$.

2.10. Thiobarbituric acid reactive substances (TBARS) assay

Lipid oxidation was quantified for the 35 and 56 day aged samples using an established TBARS assay with modifications (Sorensen and Jorgensen, 1996). Beef samples (4 g) were homogenised in 7.5 mL of 10% TCA solution containing 0.1% EDTA and 0.1% PG using Polytron PT 10-35GT (19000 rpm) for 60 s, then centrifuged at 2 °C and 2000 g for 8 min in a Rotina 380R Hettich Centrifuge. The supernatant was filtered through n° 1 Whatman filter paper. Following addition of thiobarbituric acid solution, the mixture was incubated at 95 °C for 60 min. The absorbance at 532 nm was measured with correction for nonspecific turbidity at 600 nm. Results were expressed as mg MDA/kg of meat using a calibration curve of 1,1,3,3-tetraethoxy-propane (TEP).

2.11. Carbonyl content assay

Total carbonyl group was quantified for the 35 and 56 day aged samples (n = 12 for each treatment) as per the method of (Lund et al., 2007). The samples (3 g) were homogenised using a Polytron PT 10-35GT (19000 rpm) for 30 s. Absorbance at 280 nm and 370 nm of the samples at room temperature was measured and the carbonyl content (nmol) per mg protein was calculated. The blank value was subtracted from the corresponding sample value. Means from duplicate measurements for each sample were used for statistical analysis.

2.12. Consumer testing

Sensory assessments were conducted per established MSA protocols (Watson et al., 2008) using samples from all 24 carcasses. Frozen steaks were thawed at 4 °C the day before testing and grilled for 5 minutes using a clamshell grill (Silex, Marrickville, Australia) set at 220 °C. The samples were rested at room temperature for 3 min before serving. The “link” sample followed MSA protocol aimed at familiarising consumers with the procedure and starting with a midrange sample to avoid potential risk of biasing subsequent samples from serving an initial high or low quality in first position and to minimize sample carry-over effects. Meat from each muscle allocated to a treatment was evaluated by ten consumers and each consumer tasted seven samples including the “link” sample. A total of 900 Australian (45 tasting sessions) and 540 Japanese (27 tasting sessions)

untrained consumers participated. Conduction of the consumer panels in both Australia and Japan was overseen by the same team. The consumers evaluated tenderness, flavour, juiciness and overall liking on a 100 mm line scale. A composite overall quality score (MQ4) on a 1 to 100 scale was calculated from these four sensory attributes. The MQ4 was calculated both as a mean of 10 consumers for each sample per validated procedures. The MQ4 score (also known as meat quality score or palatability score) is therefore a numerical value to quantify consumer's acceptability of a beef product. Clipped means of all sensory attributes and the MQ4 score were used in subsequent statistical analyses.

2.13. Statistical analysis

All instrumental parameters were analysed by the method of restricted maximum likelihood (REML). For all instrumental measurements, ageing method (dry ageing, wet ageing and wet-then-dry ageing), ageing time nested within ageing method (35 and 56 days for dry ageing; 7, 21, 35 and 56 days for wet ageing; and 56 days for wet-then-dry ageing), and animal sex (M or F) were fitted as fixed effects. Carcass source, number, side, cut and cut position (all nested within i.e. carcass source/number/side/cut/cut position) were fitted as random effects. For the Australian sensory measurements, the fixed effects were: Ageing method (dry ageing, wet ageing and wet-then-dry ageing); ageing time nested within ageing method (35 and 56 days for dry ageing; 7, 21, 35 and 56 days for wet ageing; and 56 days for wet-then-dry ageing); country (Australia and Japan); and animal sex (M or F). Taster group, carcass source, number, side, cut and cut position (all nested within) were fitted as random effects. The same random effects were used for the MSA sensory scores from the Japanese consumer panels while ageing method was fitted as fixed effect. Comparison of MSA sensory scores between countries (Australia vs. Japan) was conducted only with the data of the 56 days dry-aged and 56 days wet-aged treatments tested in the two countries with ageing method and country fitted as fixed effects and the same random effects as the two consumer sensory data analyses above. Data was transformed where necessary. Metmyoglobin, oxymyoglobin,

retail yield and total water content required angular transformation. All statistical analyses were performed using GENSTAT (18th Edition, VSN International Ltd, Hemel Hempstead, UK).

3. Results and discussion

3.1. Dry ageing room specifications

Due to the rack system of the multi batch chiller at Top Cut Foods, meat products at different heights were exposed to different temperatures, airspeeds and UV at any given time. The level of RH in the chamber was critical to encourage drying and minimise microbial growth. The combination of temperature <0.5 °C, Relative Humidity <80%, an air speed varying 0.5-2.0 m/s was observed to deliver primals with ideal dehydration and minimal spoilage, resulting in highest yield. Variable fan speed evaporators are recommended to control air speed at distinct stages of treatment for optimal results.

3.2. pH

Dry aged samples had a higher pH compared to the wet aged samples (Table 1). There was also a significant interaction between ageing method and ageing time ($P < 0.001$). The pH of wet aged beef was relatively stable up to 21 days of ageing and significantly declined up to 56 days of ageing. The pH of dry aged beef at 35 days was higher than the equivalent wet aged samples. The pH of the dry and wet-then-dry aged samples was significantly higher than the wet aged samples at 56 days. The pH of meat affects Maillard reaction during cooking which is responsible for flavour of cooked meat (Madruga and Mottram, 1995). It is postulated that the higher pH of dry aged products at both 35 and 56 days contributed to the higher flavour scores of dry aged beef compared to the wet aged counterparts presented in Tables 3 and 4. A correlation between pH and flavour scores of aged beef products has been observed in several studies. A higher pH accompanied by a higher sensory flavour scores in dry aged beef was reported (Li et al., 2014, Li et al., 2013, Ahnstrom et al., 2006) while similar pHs coinciding with no difference in flavour scores were observed in other studies (DeGeer et al., 2009, Dikeman et al., 2013). It is noted that the study of Kim et al. (2016) reported a difference in flavour

scores of dry and wet aged beef loins which did not differ in pH. In the current study, pH as a covariate did not significantly influence the MSA flavour score in either Australian ($P = 0.521$) or Japanese ($P = 0.633$) consumer panels (data not shown). Thus, pH likely contributes but is not solely the driver behind higher MSA flavour scores of dry aged beef presented in Tables 3 and 4. Sex (males or females) did not have a significant effect ($P > 0.05$) on pH or any other measurables in this study (data not shown).

3.3. Surface colour

Significant differences between dry and wet aged samples were observed for a^* , b^* and Hue angle. Ageing time affected the instrumental colour measurements of the wet and dry aged samples differently (Table 1). For the dry aged samples, ageing time significantly affected redness (a^*) and yellowness (b^*) but not lightness (L^*). For the wet aged samples, ageing time resulted in an increase in lightness and yellowness but did not affect redness. When comparing wet and dry aged samples at day 35, similar results were obtained for all L^* , a^* and b^* . However, steaks in the dry and wet-then-dry ageing treatment had lower L^* and b^* values and greater a^* value than the wet aged counterparts at day 56. Lower L^* and b^* and greater a^* values were also observed in wet aged compared to dry aged beef in previous studies (Kim et al., 2016, Dikeman et al., 2013).

Although reflectance measurement closely matches what the eye and brain can see, differences in muscle structure, surface moisture, fat content and pigment concentrations change how light is reflected. It can be corrected by using ratios of reflectance at different wavelengths or by using differences between reflectance at different wavelengths. Reflectance ratio of 630/580 nm ($R_{630/580}$) is useful indication of redness development or decline in meat (Strange et al., 1974). Hue angle, which has a strong correlation with visual colour, represents the meat colour change from red to yellow and a larger hue angle value generally indicates a shift to lower redness and higher yellowness (Brewer et al., 2001). Hue angle after 30 min blooming was lower in dry and wet-then-dry aged steaks compared to the wet aged steaks, suggesting that dry and wet-then-dry ageing

treatments resulted in a slower discolouration. There was no significant difference in chroma (indication of colour intensity) was observed between the wet and dry ageing treatments. The lower L^* and higher a^* , b^* , hue angle and $R_{630/580}$ of dry aged steaks compared to wet aged samples at 56 days was consistent the more intensely redness in visual appearance (Fig. 2).

Surface oxymyoglobin decreased with ageing time in both dry and wet aged samples. However, dry and wet-then-dry aged samples at 56 days had a higher surface oxymyoglobin content than the wet aged at 56 days. Inversely, the wet aged samples at 56 days had a higher surface metmyoglobin than their dry aged counterparts and metmyoglobin increased with ageing time in both dry and wet ageing. Deoxymyoglobin was negligible in all samples (data not shown), mostly likely due to the 30 min blooming. Together these results point to a lower oxidation of myoglobin in the dry and wet-then-dry samples compared to the wet aged samples.

3.4. Weight loss, total water content and retail yield

Weight loss during ageing (Table 2) was higher in the dry aged than the wet aged samples. Weight loss also significantly ($P < 0.001$) increased with ageing time in dry ageing and up to 35 days in wet ageing, in agreement with previous studies (Kim et al., 2016, Dikeman et al., 2013). Contrary to findings in previous studies, total water content of wet and dry aged beef samples in this study (Table 2) was similar ($P = 0.934$). It is noted that samples for total water content in the current study was obtained from a middle steak of each primal. Li et al. (2014) measured total water content of two different layers (outside and inner) of dry and wet aged beef steaks after trimming. The difference in total water content between the two ageing methods was only in the outside layer and there was a clear difference between the two layers of dry aged steaks.

Compared to the conventional wet ageing, more trimming is required for dry and wet-then-dry aged meat due to substantial dehydration of the surfaces. There was no significant difference in retail yield between meat dry aged for either 35 or 56 days. In this study, to closely match commercial processing, wet ageing was conducted with boneless meat which did not result in boning or

trimming. Thus, retail yield of wet aged products was not calculated to avoid misleading data interpretation. Dry ageing lead to a significant loss in retail yield (approximately 43%) due to surface drying and the need to trim off darkened meat. A previous study (Kim et al., 2016) comparing wet and dry aged bone-in beef primals found that wet and dry aged beef had final yields of 55% and 46%, suggesting that bone weight contributes more to retail yield than weight loss due to ageing. Similar results were found for dry ageing of bone-in primals in other studies (Smith et al., 2008). The study of Smith et al. (2008) also found the difference in realisable profit margin between dry and wet ageing depended on the grade of meat (US Choice or US Select) prior to ageing, saleable components (porterhouse, T-bone steak, top loin steak, lean trimmings or stew beef) and ageing time (7, 14, 21 or 35 days). Thus, together with total weight loss and retail yield, other meat processing aspects will need to be considered in deciding cost recovery, retail price and profit margin.

3.5. Myofibrillar fragmentation index (MFI)

MFI is highly correlated with beef tenderness determined by both shear force and sensory evaluation (Olson and Parrish, 1977, Culler et al., 1978). The MFI value of the beef samples (Table 2) were significantly affected by ageing method ($P < 0.001$) and interaction between ageing method and ageing time ($P < 0.001$). MFI values of the dry aged samples were significantly higher than the wet aged samples at both 35 and 56 days. There was no significant difference between the MFI values of the 56 days dry aged and wet-then-dry samples. Studies on beef strip loins (Kim et al., 2016) and beef sirloins (Laster et al., 2008) reported a lower shear force of dry aged compared to the equivalent wet aged products. However, both studies did not find a difference in sensory tenderness between the two ageing treatments. Studies comparing dry and wet aged beef loins (Dikeman et al., 2013, Sitz et al., 2006, Li et al., 2014, Smith et al., 2008) and sirloin (Li et al., 2013) found no difference in shear force or sensory tenderness results between the two treatments. MFI was not measured in these studies.

To mimic commercial processing, primals allocated to wet ageing in this study were boned prior to ageing whereas bone-in primals were used for dry ageing. Difference in MFI of wet and dry samples observed in the current study is not currently understood, however, wet aged bone-in beef loins were found to have lower shear force than wet aged boneless loins (Jeremiah and Gibson, 2003).

3.6. Oxidation

A significant interaction between ageing method and ageing time ($P < 0.001$) was observed for lipid oxidation. Dry and wet-then-dry ageing induced more lipid oxidation in than wet ageing at day 56 compared to wet ageing while there was no difference between dry and wet ageing at 35 days (Fig. 3). Correlation between lipid oxidation and production of free radicals, colour and shelf life of meat is well established (Faustman et al., 2010). An increase in lipid oxidation also leads to a more rancid flavour of meat. The molecular connection of lipid oxidation and rancidity has not been fully established. The study of Campo et al. (2006) linked TBARS values with sensory qualities of beef and showed that rancid overpowered beef flavour at the TBARS value of 2 mg MDA/kg meat. The level of MDA of all samples in this study was below the 2 mg/kg meat recommended by Campo et al. (2006), suggesting an acceptable lipid oxidation level for flavour acceptability. It is noted that MDA is only a part of the total odour complex as volatile odour compounds produced by mechanisms other than lipid oxidation also contribute to the rancid flavour of meat (Casaburi et al., 2015). Interestingly, DeGeer et al. (2009) showed increase in lipid oxidation in dry aged beef loins was associated with higher 'overall aged beef' flavour score, indicating involvement of lipid oxidation in dry aged flavour development.

There was no significant difference ($P = 0.834$) in total carbonyl content between dry and wet aged beef, however, there was a significant interaction between ageing method and ageing time ($P = 0.032$). A significant increase in total carbonyl content was observed only for the wet aged samples. The higher total carbonyl content in wet aged samples compared to those in dry and wet-then-dry aged treatments is consistent with a higher percentile of metmyoglobin (Table 1). Protein

carbonylation is promoted by reactive oxygen species. Primary protein carbonylation such as oxidation of side chains of L, R, P, and T amino acids produces DNPH detectable protein product. DNPH derivatizable protein adducts can also be formed via the addition of aldehydes such as those generated from lipid peroxidation (Suzuki et al., 2010). Aldehydes and other carbonyls are known to react readily with Mallard intermediates which generate additional aroma compounds, thus modifying the overall flavour of cooked meat (Mottram, 1998).

3.7. Consumer sensory evaluation

The 56 days wet-then-dry aged samples were only tasted by consumers in Japan together with the 56 days wet aged and 56 days dry aged samples, whereas Australian consumers tasted the 7, 21, 35 and 56 wet aged and 35 and 56 days dry aged samples.

For the Australian sensory results (Table 3), dry aged samples had higher MSA consumer scores than the wet aged samples for all four eating attributes tenderness, juiciness, flavour and overall liking ($P < 0.001$ for all four traits). Sensory tenderness results were consistent with those of MFI. Sensory juiciness results showed a clear difference between dry and wet aged samples at both 35 and 56 days, despite a higher weight loss during ageing of dry aged primals and a similar total water content (Table 2). Dikeman et al. (2013) comparing dry and wet aged loins reported a higher raw moisture content but a higher cooking loss of wet aged steaks. A lower cooking loss of dry aged beef loins may have contributed to a higher sensory juiciness score in the current study.

Insignificant differences were observed for the sensory attributes of the two dry aged samples at 35 vs. 56 days. However, ageing time appeared to influence sensory attributes of wet aged samples with those aged for 21 days receiving highest juiciness, flavour and overall liking scores. Data for wet ageing in this study, however, suggest that ageing of beef loins beyond 21 days provided no additional benefit to tenderness and was even detrimental to juiciness, flavour and overall liking.

Meat quality score (MQ4) (Table 3) was calculated as weighted results of the four sensory traits tenderness, juiciness, flavour and overall liking (Watson et al., 2008). A beef description system based on the MQ4 score with some adjustments to the weightings and cut-off values has been shown useful in comparatively describing the eating quality of beef across several countries (Polkinghorne et al., 2014, Thompson et al., 2008). The MQ4 score of dry aged samples was higher ($P < 0.001$) than that of wet aged beef. However, 21 days ageing appeared to be the optimal ageing time for wet ageing, consistent with the juiciness, flavour and overall liking results.

The results in this study is contrary to those of previous studies which compared wet and dry aged beef from US Choice and US Select grades and showed no difference in any sensory attributes (Smith et al., 2014, Laster et al., 2008, Smith et al., 2008). Dry ageing of some muscles such as *Spinalis thoracis* and *Gluteobiceps* even produced products with a lower overall liking score than their wet aged counterparts (Smith et al., 2014), suggesting only high value muscles such as *Longissimus* should be used for dry ageing. However, the difference between consumer sensory results in the current study and in that of Smith et al. (2008, 2014) demonstrates the need for similar experiments using different muscles of Australian beef.

Similar to the Australian consumer data, the Japanese consumer panels preferred the 56 days dry aged samples to the wet aged samples for all sensory attributes. To investigate opportunities for dry ageing of Australian beef for the Japanese market, the 21 days wet aged followed by 35 days dry aged (wet-then-dry) treatment was included for the Japanese consumers. While there were insignificant differences in tenderness and juiciness, the wet-then-dry samples received higher scores for flavour, overall liking and MQ4 compared to the wet aged products. Thus, sensory evaluation with the Japanese consumer panels indicate that the wet-then-dry ageing treatment provides an opportunity to create premium products with exported Australian beef in overseas markets.

Interestingly, all sensory attributes of dry and wet aged beef at 56 days were consistently rated lower ($P < 0.001$ for all) in the Japanese consumers compared with the Australian consumers (Table 4). The study of Polkinghorne et al. (2014) comparing sensory evaluation of beef between Australian and Japanese consumers found that grilled steaks were downgraded by Japanese consumers regardless of muscle types, consistent with results found in the current study. Polkinghorne et al. (2014) also found that juiciness as a sensory trait was more important for Japanese consumers than Australian consumers. Further investigation on both contributing factors to the enhanced sensory quality of dry aged beef, and difference in consumer preference for beef products in different countries is needed.

4. Conclusion

Compared with conventional wet ageing, dry ageing of Australian beef loins produced products with more intense redness, less myoglobin oxidation and preferred sensory qualities. The significant difference in instrumental colour measurement and sensory eating qualities between wet and dry aged beef demonstrates the potential of dry ageing to value-add to the red meat industry. Retail yield was lower in dry aged beef which may lead to an increase in production cost for processors. Limiting dry ageing time to 35 days may reduce production cost while maintaining optimal eating quality. Stepwise (wet-then-dry) dry ageing beef was preferred to wet aged beef for flavour, overall liking and palatability by the Japanese consumers, thus presenting new export opportunities and processing flexibilities for the red meat industry. However, a more thorough understanding of difference in consumer preference between countries, such as doneness level and cooking method, is needed to maximise the value of dry aged Australian beef in overseas markets.

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Table 1. Effects of ageing method and ageing time on pH and instrumental measurements of surface colour.

	Ageing method*				Ageing method by Ageing time									
	Dry ^A	Wet ^B	s.e.d	P-value	Dry 35	Dry 56	Wet 7	Wet 21	Wet 35	Wet 56	Wet-then-dry 56 ^C	s.e.d	P-value	r.d.f.
pH	5.59	5.42	0.02	<0.001	5.62	5.54	5.43	5.52	5.44	5.28	5.59	0.02	<0.001	146
L*	34.74	35.23	0.43	0.234	34.44	34.88	33.48	35.37	35.82	36.32	35.14	0.71	0.001	160
a*	23.98	23.34	0.29	0.035	24.57	23.36	22.92	21.78	25.17	22.89	22.97	0.57	<0.001	160
b*	21.07	21.68	0.23	0.011	21.82	20.55	21.43	19.93	22.53	22.18	20.11	0.48	<0.001	160
Hue angle	41.5	43.09	0.24	<0.001	41.64	41.39	43.33	42.67	42.1	44.38	41.5	0.43	<0.001	160
Chroma	31.91	31.86	0.34	0.885	32.87	31.04	31.27	29.41	33.78	31.97	30.9	0.69	<0.001	160
R _{630/580} ^D	7.93	7.77	0.41	0.486	8.57	7.37	8.7	7.11	8.56	6.5	7.06	0.36	<0.001	160
Oxy-myoglobin (%)	73.44	74.73	0.75	0.096	75.46	71.67	80.24	75.89	76.29	67.65	71.04	1.08	<0.001	160
Met-myoglobin (%)	16.56	15.27	0.75	0.096	14.54	18.33	9.76	14.11	13.71	22.35	18.96	1.08	<0.001	160

*The wet-then-dry ageing method had only one ageing time (56 days) and is presented under the 'Ageing method by Ageing time' section for ease of comparison. ^ADry = dry aged 35 or 56 days; ^BWet = wet aged 7, 21, 35 or 56 days; ^CWet-then-dry = wet aged for 21 days then dry aged for 35 days; ^DR_{630/580} = the ratio of light reflectance at 630 nm and 580 nm; s.e.d. = standard errors of differences between the predicted means; r.d.f. = residual degrees of freedom.

Table 2. Effects of ageing method and ageing time on weight loss, total water content, retail yield and myofibrillar fragmentation index.

	Ageing method*				Ageing method by Ageing time									
	Dry ^A	Wet ^B	s.e.d	P-value	Dry 35	Dry 56	Wet 7	Wet 21	Wet 35	Wet 56	Wet-then-dry 56 ^C	s.e.d	P-value	r.d.f.
Weight loss (%)	14.43	5.24	0.67	<0.001	11.16	17.55	2.97	4.98	6.62	6.03	16.54	0.59	<0.001	95
Total water content (%)	58.4	58.38	0.22	0.934	58.86	57.91	58.59	58.75	58.33	57.84	58.46	0.34	0.004	130
Retail yield ^D (%)	39.67		1.342	<0.001	40.97	38.36					66.06	1.52	0.091	69
MFI ^E	52.57	35.69	2.88	<0.001	45.94	59.2	25.97	31.92	39.41	45.44	58.37	2.48	<0.001	70

*The wet-then-dry ageing method had only one ageing time (56 days) and is presented under the 'Ageing method by Ageing time' section for ease of comparison. ^ADry = dry aged 35 or 56 days; ^BWet = wet aged 7, 21, 35 or 56 days; ^CWet-then-dry = wet aged for 21 days then dry aged for 35 days; ^DRetail yield = the sum of weight loss and trim loss (including bone) calculated for the dry aged primals only; ^EMFI = myofibrillar fragmentation index; s.e.d. = standard errors of differences between the predicted means; r.d.f. = residual degrees of freedom.

Table 3. Effects of ageing method and ageing time on MSA consumer sensory scores with Australian consumers. The scores were the 'clipped' scores (see method section 2.12).

	Ageing method (Australia only)				Ageing method by Ageing time (Australia only)								
	Dry ^A	Wet ^B	s.e.d	P-value	Dry 35	Dry 56	Wet 7	Wet 21	Wet 35	Wet 56	s.e.d	P-value	r.d.f.
Tenderness liking	77.92	70.74	1.89	<0.001	76.03	79.88	68.41	71.53	72.43	70.52	2.35	0.172	92
Juiciness liking	75.09	68.76	1.04	<0.001	75.05	75.14	70.47	72.83	67.39	64.26	2.17	0.002	94
Flavour liking	73.25	67.75	1.29	<0.001	74.25	72.18	68.11	70.06	68.49	64.18	2.25	0.141	95
Overall liking	75.29	69.36	1.38	<0.001	75.8	74.74	69.75	70.95	69.62	66.87	2.29	0.49	97
MQ4	74.78	68.74	1.299	<0.001	74.62	74.9	68.17	70.69	69.5	66.4	2.08	0.337	94
Satisfaction	3.85	3.61	0.07	0.002	3.81	3.88	3.62	3.65	3.6	3.57	0.11	0.922	93

^ADry = dry aged 35 or 56 days; ^BWet = wet aged 7, 21, 35 or 56 days; s.e.d. = standard errors of differences between the predicted means; r.d.f. = residual degrees of freedom.

Table 4. Comparisons of the effects of ageing method and country on MSA consumer sensory scores with Japanese consumers. The scores were the 'clipped' scores (see method section 2.12).

	Ageing method (Japan only)						Country ^D				
	Dry 56 ^A	Wet 56 ^B	Wet-then-dry 56 ^C	s.e.d	P-value	r.d.f.	Australia	Japan	s.e.d	P-value	r.d.f.
Tenderness liking	69.65	63.22	65.32	2.04	0.014	30	75.19	67.12	1.33	<0.001	42
Juiciness liking	57.7	49.16	52.61	2.59	0.011	31	69.79	53.82	1.58	<0.001	34
Flavour liking	60	46.46	54.26	1.93	<0.001	34	68.5	53.43	1.68	<0.001	43
Overall liking	64.48	51.46	56.41	1.94	<0.001	31	71.07	58.03	1.71	<0.001	42
MQ4	63.91	53.41	57.31	1.54	<0.001	32	70.93	58.96	1.31	<0.001	39
Satisfaction	2.48	2.18	2.27	0.07	<0.001	32	3.75	2.34	0.07	<0.001	44

^ADry = dry aged 56 days; ^BWet = wet aged 56 days; ^CWet-then-dry = wet aged for 21 days then dry aged for 35 days; ^DCountry = location (Australian vs. Japan) in which consumer sensory was conducted; s.e.d. = standard errors of differences between the predicted means; r.d.f. = residual degrees of freedom.

Figure captions

Fig. 1. Graphical illustration of the experimental design.

Fig. 2. Visual illustration of (A) dry aged and (B) wet aged beef loin steaks at 56 days ageing.

Fig. 3. Oxidation measured using (A) TBARS and (B) total carbonyl group content of wet, dry, and wet-then-dry aged beef loins at 35 and 56 days. Values are predicted means \pm SED. Predicted means with different letters (small letters within an ageing time and capital letters within an ageing method) differ significantly ($P < 0.05$).

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Fig. 1

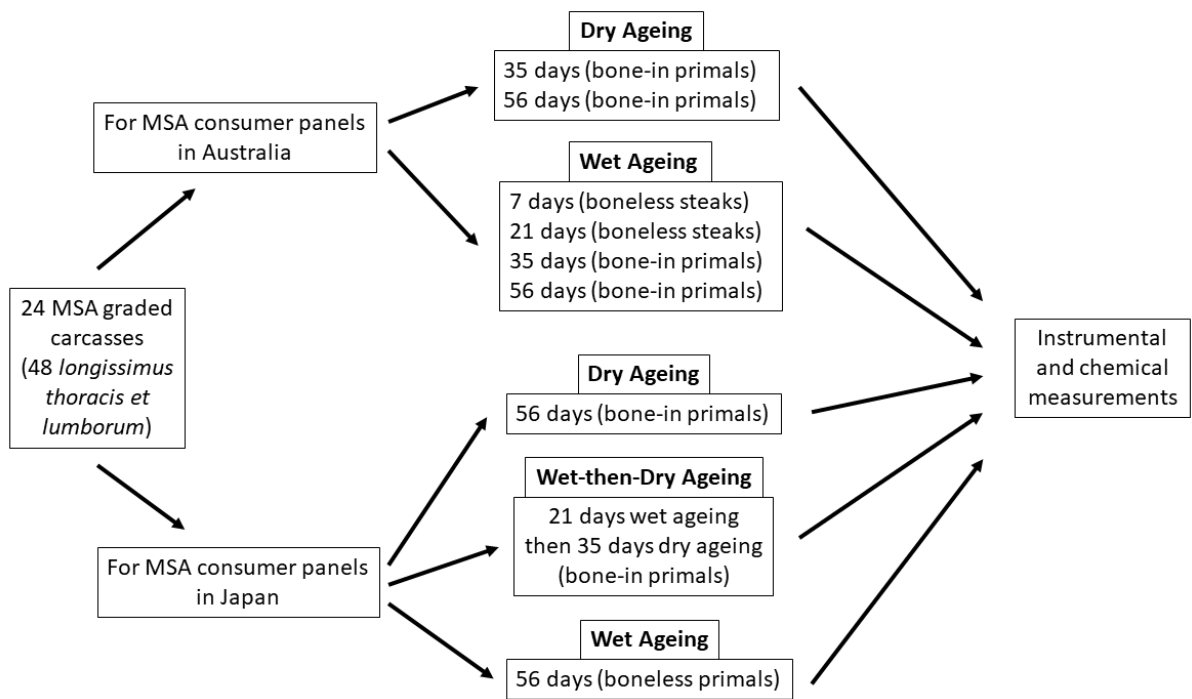
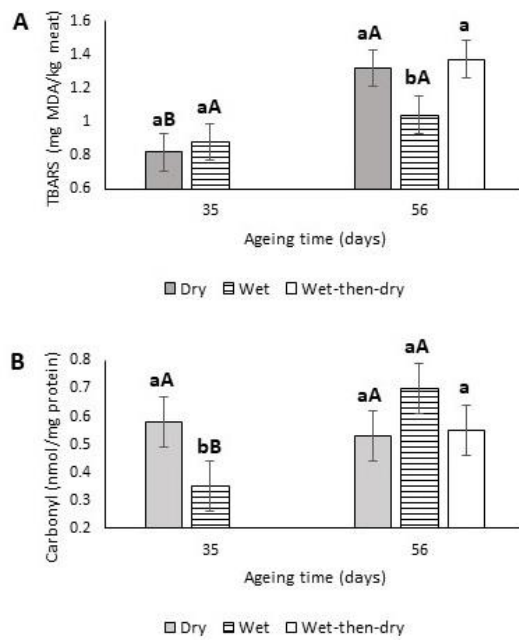


Fig. 2



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Fig. 3



- Dry ageing resulted in beef loins with higher pH and more desirable colour compared to wet ageing
- Dry and stepwise ageing resulted in beef with similar physical and chemical properties
- Australian and Japanese consumers preferred dry aged and stepwise aged beef to wet aged products
- 21 days and 35 days are recommended for wet and dry ageing, respectively, to maximise palatability

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