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Dry Aged Beef – Evaluation of wet age step on quality and yield

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Abstract

Dry aged beef is being marketed as a premium product and is used in a small number of upscale restaurants and retailers in Australia and around the world. Previous studies from the USA using sensory panels failed to show a difference in eating qualities between dry and wet aged beef. Also, although dry ageing of beef is being trialled under different ageing conditions by a few small scale meat processors in Australia, there is a lack of guidelines to ensure food safety and optimal results in terms of eating quality. This project investigates the effect of dry ageing on eating qualities, yield and shelf-life of Australian beef loins in comparison to wet ageing. In addition, recommendations are also made to industry for development of guidelines for beef dry ageing.

Strip loins and cube rolls were either wet aged (bone-out; for 7 days, 21 days, 35 days or 56 days) or dry aged (bone-in; for 35 days or 56 days) or wet aged bone-in, for 21 days then dry aged for 35 days (wet-then-dry).

Australian consumer sensory results, using MSA protocols, showed that dry aged beef loins were scored higher than wet aged products at 35 and 56 days, with 35 day dry aged products receiving optimal scores (MQ4 score of 73.4). The higher scores for dry aged products were consistent in all sensory parameters namely tenderness, juiciness, flavour, overall liking and MQ4 score. Japanese consumer data support the superior eating quality of dry aged beef loins compared to the wet aged counterpart. Sensory results using Japanese consumer panels showed that the wet-then-dry treatment resulted in products with similar sensory qualities compared to the dry aged (only) products and superior compared to the wet aged (only) products.

The higher consumer scores for flavour of dry aged beef correlated with differences in flavour compounds which were analysed by chromatography and mass spectrometry. Dry aged beef had substantially higher concentrations of 3-hydroxy-2-butanone, acetone, pyrazines and hexanal whereas ethanol and acetic acid were much higher in wet aged beef. Ethanol and acetic acid are both key products of anaerobic fermentation while 3-hydroxy-2-butanone, acetone and hexanalare all breakdown products of lipid oxidation. Pyrazines are involved in the formation of Maillard products during cooking. In addition, wet aged beef had a significant reduction in pH after ageing compared to dry aged beef suggesting an acid taste of low pH wet aged beef may have caused the decreased consumer flavour scores. In addition, the dry aged beef had a slight increase in pH, which can contribute to enhanced formation of Maillard reaction products during cooking, which is consistent with higher levels of pyrazines in dry aged products contributing to enhanced flavour. Weight loss due to ageing was generally higher in dry aged products compared to wet aged products. Few differences were seen for colour or total water content between dry and wet aged products. When comparing product at 56 days that has been only dry aged to product undergoing wet-then-dry ageing, there was no difference in point of sale yield (after boning and trimming), colour or water content.

Due to lean tissue shrinkage during dry ageing, changes in fat texture and the colour of lean and fat tissue, sufficient fat cover (at least 20 mm) on beef cuts is needed to maximise yield at point of sale. In addition, dry aged products were more susceptible to spoilage due to bacterial growth, thus ensuring high hygiene standards pre-ageing (during quartering and boning) is particularly important for products destined for dry ageing. Beef primals exported in vacuum bags (wet aged) prior to dry ageing is possible but require more stringent hygiene and process control. A higher airspeed in the chamber at the start of the dry ageing period to accelerate drying and a lower airspeed towards the end to reduce yield loss is recommended. Detailed hazard analysis and cause and control measures were made at each point along the supply and processing chain. It is noted that dry ageing in cheesecloth was excluded in the experimental design as cheesecloth would interfere with airflow and UV exposure.

Executive summary

Dry ageing of Australian beef strip loins and cuberolls resulted in superior eating quality compared to the common wet ageing method in both Australian and Japanese consumers. Flavour chemistry analysis indicated that the higher sensory scores for the dry aged beef products may be explained by an increase in pyrazines ('good' flavour compounds involved in the Maillard reaction) and a lower level of ethanol and acetic acid ('bad' flavour compounds). A two-step ageing method (wet ageing for 21 days followed by dry ageing for 35 days – wet-then-dry method) was also investigated for export for dry ageing purpose. There was little difference in point of sale yield between dry only and wet-then-dry. Sensory results using Japanese consumer panels suggested dry ageing of Australian beef which is wet aged during export to Japan creates products with similar eating quality as that of dry aged (only) and superior to wet aged (only) beef loins. In order to make recommendations for development of industry guidelines for dry ageing, changes in lean tissue shrinkage, fat texture and colour of lean and fat tissue during the two dry ageing methods were closely observed and reported. This project demonstrated the potential to add value to Australian beef both in the domestic and export markets. Close observation of the physical changes of different meat primals during dry ageing only and wet-then-dry ageing processes enabled recommendations to be made to minimise spoilage and weight loss during ageing, thus maximising both point of sale yield and economic returns to processors.

Recommendations have been made for dry ageing of beef along the supply chain; selection of carcasses/primals, hygiene during meat processing, transport and receival of meat, quality of primals needed for dry ageing, and chamber parameters for the two dry ageing processes. It is noted if cheesecloth was to be used for dry ageing, different dry ageing chamber conditions are required as cheesecloth would reduce airflow and UV exposure on the primal surface.

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1 Background

1.1 Ageing of meat

Ageing is a long-established method for improving the tenderness, flavour and overall acceptance of beef. This was traditionally done by 'hanging' the carcase, quarter or primal cut of meat in a cool room or a cool place until it was ready to be sold or consumed. With the advent of vacuum packaging, selected primal cuts could be aged under more controlled conditions, with improvements in yield, ease of processing and transport and the capability of longer storage times. Ageing in a vacuum bag is referred to as 'wet' ageing. A substantial amount of research showed a positive correlation between ageing and tenderness of meat from different species (Huff and Parrish 1993, Dransfield 1994).

1.2 Dry ageing

Dry ageing is the ageing of primal cuts unpackaged, in air under strictly controlled temperature and humidity. There has been an increased interest in dry ageing with research on dry ageing conducted in the United States (Laster *et al.* 2008, Smith *et al.* 2014), CSIRO 2010 Meat Technology Update and more recently MLA funded projects (P.PSH.0679 and P.PSH.0708). However, no research has compared the practice of wet ageing before dry aging, especially on eating quality, secondary shelf life, and also the microbial community.

Some studies have shown no effects of dry ageing on sensory quality (Laster *et al.* 2008, DeGeer *et al.* 2009, Lepper-Blilie *et al.* 2016) while others have shown beneficial effects of dry aging on consumer sensory assessments of quality, mainly on flavour acceptability (Li *et al.* 2014, Stenstrom *et al.* 2014).

Iida *et al.* (2016) showed that in highly marbled beef, the umami (a highly desirable flavour) intensity and glutamic acid and inosine mono phosphate (flavour compounds for umami) compounds were highest after dry ageing for 40 days, when compared to longer or shorter ageing periods. In addition, Kim *et al.* (2016) has shown that seven metabolites, identified as positive flavour precursors, were more prevalent in dry aged beef, relative to wet aged product and that the flavour and overall liking of dry aged meat was preferred by consumers. Furthermore, Kim *et al.* (2016) also identified that wet ageing beef strip loins for 3 weeks at 1°C produced higher shear force (tough meat) than dry ageing for at 1°C for the same period, although the consumer panels did not detect a difference in tenderness between the products.

Australian beef products destined to be dry aged beef in Japan are first vacuum packed, then take 2-3 weeks to reach Japan by boat where they are then subjected to dry ageing. Recently, USA has been competing with Australia in this market and they positioned their dry aged beef as being authentic and premium with no prior wet ageing involved. This concept, driven by the USA, has gained momentum in Japan for steakhouse markets. Kenji Yamamoto ('Yamaken') one of the thought leaders in Japan on Dry Aged beef considers the wet ageing, followed by dry ageing, model as 'only 70% quality', i.e. sub-optimal. He has stated that he is concerned that the vacuum packing retards the meat enzymes and stops the growth of bacteria necessary to improving flavour development (A.Cox 2015; pers comm,). These concerns of inferior quality have not yet been raised or seen with MLA MDC partners and partnership projects (P.PSH.0679 and P.PSH.0708) which both include wet age pre-steps.

2 Project objectives

This project aimed to evaluate the two supply models, dry ageing vs wet then dry ageing, to demonstrate that Australian dry aged beef is neither inferior nor non-authentic. This is an important step to ensure market share for Australian beef is maintained in Japan, and potentially other markets. In addition, development of guidelines based on this project's outcomes can assist suppliers, wholesalers and retailers in developing a consistent approach to dry ageing. Further, creating a data set for MSA will also assist in defining the meat science and commercial factors relating to yield for including or not including a pre-wet ageing step which can assist in terms of technical trade barriers and market access. If consumer assessments of dry aged beef demonstrate that it is significantly higher in acceptability then wet aged beef, it is proposed to include a premium in the MSA model for dry aged product. As well as sensory assessments, this project also aimed to examine molecular differences in the beef meat product resulting from wet and vs ageing at different ageing time points. Biochemical analyses include lipid and protein oxidation, identification and quantification of volatile flavour compounds.

Specific aims of the project are as followed:

- To investigate the effects of dry ageing on eating quality, using the MSA consumer panel methods
- To investigate if a period of wet ageing, followed by dry ageing, influences the quality of the dry aged product (Japanese present system) using consumer panels and biochemical methods
- To compare lipid and protein oxidation in wet and dry aged beef
- To identify and quantify flavour volatile compounds in wet and dry aged beef
- To develop a possible premium for eating quality assured dry aged beef product
- To develop guidelines for industry production of dry aged beef with a wet aged pre-step

3 Methodology

3.1 Animal and carcass collection

Nominated cuts were collected from MSA trial on saleyards/boats and stress. Loins were selected only from non-stressed cattle. Following slaughtering, carcasses (n=24) were selected at 24 hours post mortem to ensure no carcasses fell into dark cutting category (pH > 5.7). Carcasses were graded (Table 1) using MSA grading standards. Strip loins and OP ribs (both bone-in) were removed from both sides and link products outside, fillet, eye round and oyster blade (bone-out) were collected from one side. A total of 48 strip loins and link products were transported under refrigerated conditions to Top Cut Foods (Gold Coast, QLD). The 'link' products are not discussed or presented further as they were collected as a part of the parent project coordinated by Rod Polkinghorne.

3.2 Meat cutting and ageing

Cutting of meat was conducted at Top Cut Foods (Gold Coast, QLD). Bone-out link products (outside, eye round, fillet and oyster blade) allocated for 21 day ageing and boned-out strip loins allocated for 7 and 21 days ageing were cut into steaks (2.5 cm thick), vacuum packed and aged for the designated ageing time. Eight treatments were allocated to the 2 OP ribs and 2 strip loins from each carcass, allowing for randomisation of position within the cuts and sides. The nine treatments within the *longissimus* (OP ribs and striploin) were wet aged (boneless) for 7, 21, 35 and 2 x 56 days, dry aged for 35 and 2 x 56 days and wet aged for 21 days followed by dry aged for 35 days. Boned products (strip loins and cuberolls) were vacuum packed (whole primal) and wet-aged for 7, 21, 35 or 56 days at temperatures ranging between 0-4°C. Primals allocated to dry ageing were kept bone-in and transferred to a dry ageing room and stored for

35 or 56 days. At 21 days post mortem, primals (n=24) were retrieved from wet ageing room, removed from vacuum packaging material, transferred to the dry ageing room and aged for a further 35 days. At the completion of all treatments, the duplicate 56 days wet ageing and 56 days dry ageing samples and the 21 days wet/35 days dry ageing were despatched to Japan by Top Cut Foods Australia. These samples were part of a project coordinated by Peter McGilchrist at Murdoch University and are not referred to further in this report. All steaks and primal were weighed before vacuum packaging for wet ageing or being transferred to the dry ageing room. The experimental design is illustrated in Figure 1.

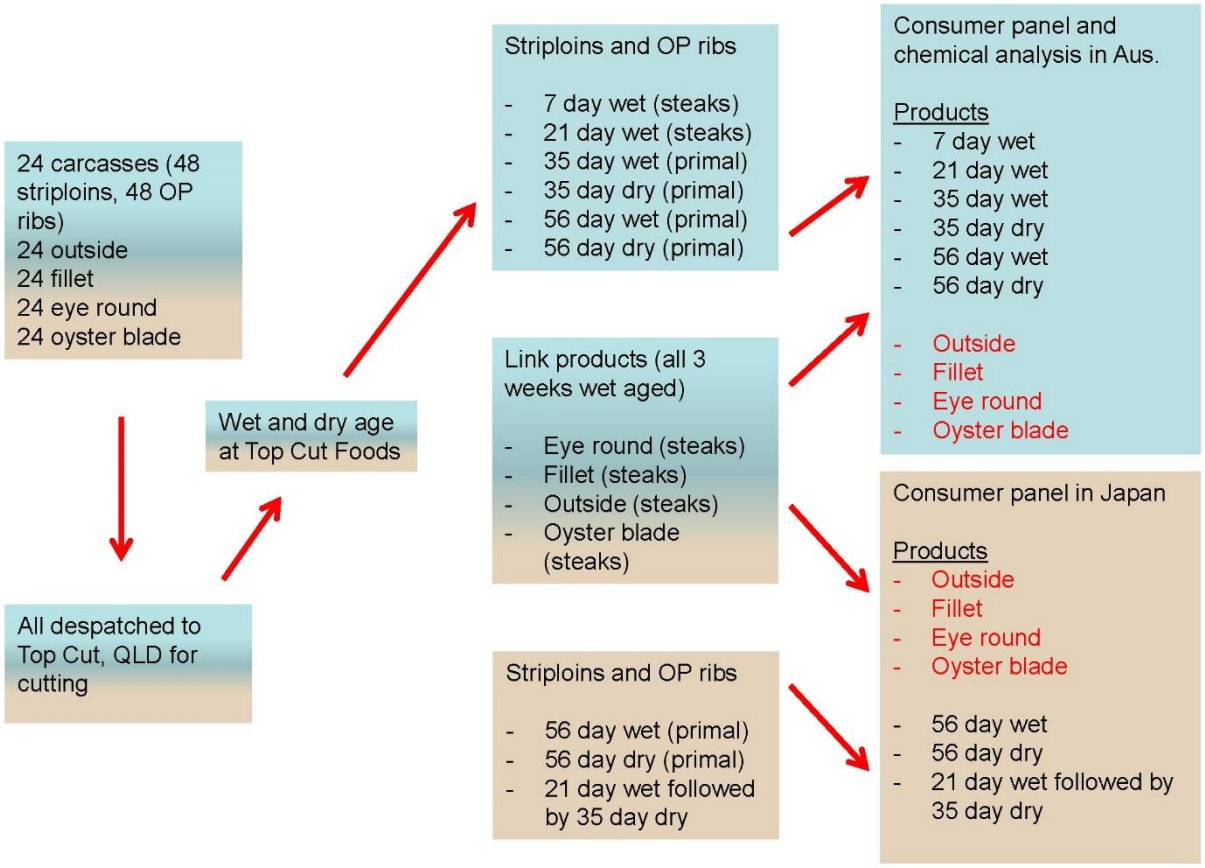

Figure 1. Experimental design.

3.3 Dry ageing room specifications

The chiller used at Top Cut Foods (Gold Coast, QLD) was not a batch chiller where 1 batch enters and remains until the process is complete without stock rotation and the addition of further batches. This type of treatment system is common in industry and requires more monitoring that a single batch chiller. Instead the chiller used for this study was loaded with 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. Due to the rack system, meat products at different heights were exposed to different temperatures, airspeeds and UV at any given time. The initial 7 to 10 days of treatment are the most important as during this period the water migration process begins. If the chamber is opened and additional product loaded into the environment the balance between air and product conditions will be compromised. This will lead to a slowing down of the process and allow more favourable conditions for micro growth which ultimately leads to lower yield.

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 between 53.5% and 100.0% with the average RH was 89.4% over the experimental period. Ideal RH parameters are 70% to 80% at different points during the treatment process in a multi batch chiller. Higher humidity is needed at the beginning and then a reduction to 70% or less towards the end of the process will deliver the best results. The temperature of the chamber varied between 1.3°C and 4.1°C with the average temperature recorded was 2.1°C. Due to the irregular shape of the chiller, air speed measurements varied. In a central position measurements varied between 0.75 meters per second and 1.2 meters per second. The combination of air temperature $\leq$0.5°C, Relative Humidity <80%, an air speed of minimum 0.5 meters per second and up to 2.0 meters per second are ideal. Faster airspeed can also be used but will result in a greater weight loss. For ultimate control, variable fan speed evaporators are used to vary the air speed at distinct stages of treatment.

There were two UV light units fitted to the ceiling of the chiller. These units were activated by a switch on the door which ensures the units are active at all times. One unit is positioned directly in the airflow from the evaporator. This is to treat the air as it leaves the evaporator. The second unit is positioned more towards the rear of the chiller. The use of UV lighting was to discourage microbial growth on meat surface which in turn results in less trimming and a lower yield loss.

3.4 Sampling for sensory and chemical analysis

3.4.1 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.

3.4.2 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.

3.5 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 Ldt., Brisbane, QLD). Temperature compensation was allowed using a TPS temperature probe. After 30 min of blooming, instrumental colour measurement on the surface of 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. The ratio of oxymyoglobin:metmyoglobin (oxy:met) was calculated using reflectance values at wavelength 630nm and 580nm as described by Khliji *et al.* (2010).

3.6 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:

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

3.7 Total yield for dry ageing

Total yield was defined as the total amount of loss from water and trimming after the dry ageing process. Total yield was calculated using the equation below:

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

3.8 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:

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

3.9 Consumer testing

Sensory assessments were conducted according to established MSA protocols (Watson *et al.* 2008). Frozen steaks were thawed at 4°C the day before testing and grilled for 180 seconds 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 familiarizing 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. Each sample was evaluated by ten consumers for odour liking, tenderness, flavour liking, juiciness and overall liking on a 100 mm line scale. A composite overall quality score on a 1 to 100 scale was calculated both as a mean of 10 consumers and as a clipped mean after removing the two highest and two lowest scores for each sample according to validated procedures (Watson et al., 2008). Clipped means were used in subsequent statistical analyses.

3.10 Lipid oxidation assay

Lipid oxidation for all treatment (n = 12, total = 96) was examined using an established thiobarbituric acid reactive substances (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 no. 1 Whatman filter paper. Equal amount of filtrate (1 mL) and 0.02 M TBA solution (1 mL) were mixed in screw cap tube and incubated in water bath at 95°C for 60 min. After incubation, the samples were cooled in ice and 200 µL of each sample was transferred into 96-well plate. The absorbance of samples was measured at 532 nm using Thermo Scientific Multiskan Spectrum spectrophotometer and subtracted with absorbance at 600 nm for correction of nonspecific turbidity.

A standard calibration curve was prepared from 16.7036 µM 1,1,3,3-tetraethoxy-propane (TEP) in Milli-Q water. The standards were mixed with 1 mL of 20 mM TBA and subjected into the same analytical procedures as the beef samples. Results were expressed as mg MDA/ kg of meat.

3.11 Protein oxidation assay

Protein oxidation assay for all treatment (n = 12, total = 96) was conducted according to the method outlined by Lund *et al.* (2007). Beef samples (3.0 g) were homogenized in 15 mL of buffer (pH 7.4) consisting of 2.0 mM $Na_4P_2O_7$, 10 mM Tris, 100 mM KCl, 2.0 mM $MgCl_2$ and 2.0 mM EGTA. The samples were homogenised using a Polytron PT 10-35GT (19000 rpm) for 30 s and washed twice with HCl–acetone (3:100) (v/v) and twice with 10% TCA (w/v). Derivatisation with 10 mM DNPH in 2.0 M HCl and protein blanks were prepared by substituting DNPH with 2.0 M HCl. The tubes were gently agitated for 30 min in the dark. Excess DNPH was removed by washing with 20% TCA and three times with ethanol–ethyl acetate (1:1) (v/v). The pellets were solubilised in 6.0 M guanidine hydrochloride in 20 mM potassium dihydrogen phosphate (pH 2.3) overnight at 4°C. Tubes containing protein blanks were used to determine protein concentration using bovine serum albumin as standard curve. Absorbance at 280 nm and 370 nm of the samples at room temperature was measured and the carbonyl content in nmol/mg protein was calculated. The blank value was subtracted from the corresponding sample value. Duplicate measurements were made for each muscle sample and mean values were used for statistical analysis.

3.12 Volatile analysis

3.12.1 Headspace solid phase microextraction gas chromatography

Samples were selected randomly from 21 days wet aged, 35 day wet and dry aged and 56 day wet and dry aged (n = 12 for each treatment, total = 60) and thawed overnight. Steaks (~ 25 mm thick, 20 g) were grilled using a Silex grill (220°C for 180 sec.s) allowed to rest (3 min). The grilled beef steaks were then roughly cut and weighed and Milli-Q water was added at a ratio of 1:2 (Milli-Q:meat). Samples were macerated using hand-held food processor (X) and 4 g of slurry was transferred into a headspace vial. The internal standard (IS) 4-methylpentanol internal standard was placed into a 200 uL vial insert. Duplicate samples were placed in the auto-injector (AOC-5000, Shimadzu, Rydalmere, Australia). Headspace volatiles were extracted with divinylbenzene/Carboxen/PDMS 23 gauge, 2 cm solid phase microextraction (SPME) fibres (Supelco, Sigma-Aldrich, Castle Hill, Australia) for 40 min at 40°C with gentle agitation. The extracted volatiles were desorbed into a hot injector (250°C) in splitless mode and separated by a gas chromatography–mass spectrometry (QP-2010-Plus GC–MS, Shimadzu). Volatile separation was on a Sol-Gel Wax column (SGE, Ringwood, Australia, 30 m, 0.25 id, 0.25 µm film) with the following temperature programming; initial temperature 35°C was held for 5 min and then heated at 5°C/min to 250°C. The mass spectrometer was programmed to scan the mass range m/z 40–250. Semi-quantitative data were generated using the Shimadzu proprietary software

"LabSolutions" (Version 2.53). Integrated area data were normalised and expressed as a percentage of the IS. Mass spectral matches were conducted with the NIST Mass Spectral Search database.

3.12.2 Gas chromatography olfactometry

The GC-O analyses were conducted using a subset (n = 8, total = 18) of grilled 35 day wet and dry aged beef samples. A panel of trained GC-O assessors or 'sniffers' (n = 8) evaluated the effluent of each of a matching pair (from the same carcass) of wet and dry aged samples. Assessors were trained according to previously described protocols (Frank et al. 2009). Briefly, assessors measured the odour intensity of the GC effluent using a computer mouse and time intensity software (Compusense). The odour intensity throughout the chromatographic run (approximately 20 min) was rated continuously using an unstructured 100-mm line scale on a computer screen, where 0 represented the absence of any perceived odour, 25 was used to indicate a mild intensity odour, 50 moderate intensity, 75 strong and 100 very strong. Odour intensity data were continuously acquired at a rate of 1 Hz. In case of odours persisting for several seconds, assessors were asked to continuously rate the intensity until the odour stimulus disappeared. Simultaneously, assessors were asked to describe 'out loud' the odour quality into a microphone. Assessor descriptions were digitally recorded using the GoldWave audio recording software (GoldWave Inc., St John's, Canada). Time intensity data from each panellist were imported into Excel (Microsoft) and annotated with odour descriptors (when given) and matched to specific volatiles based on compounds identified eluting at the same time; for example, from EI and CI mass spectral data. For each distinct odour event, the integrated area under the time curve was calculated, for example intensity (1–100) × duration (seconds). Thus it was possible for an odour to have a total area under the time curve value greater than 100 units. Replicate area under the time curve data were used for statistical analysis. Peaks detected by less than two assessors were considered noise and deleted from the aromagram. As there was no time delay between the GC-MS and the olfactory port effluent, odours and volatiles could be accurately matched.

3.13 Derivatization of Free Amino Acids and GC–MS (preliminary)

Preliminary semi quantitative free amino acid content was performed using raw samples (n =12, total 96) from the 35 wet and dry aged beef using an established method (Frank *et al.* 2016a). Thawed beef samples (2 g) were immediately suspended in an ice-cold methanol solution (70%), homogenised, and centrifuged. The supernatant was filtered before derivatization. Relative response factors were determined for quantitative ions (m/z) for each analyte, and the concentrations of free fatty acids (mg/100 g) were estimated against the internal standard norvaline (100 µg/mL, m/z 130). Chloroformate derivatives (1 µL) were injected at250 °C (splitless) into the GC−MS system (QP-2010-Plus, Shimadzu) and separated on a SolGel wax column (SGE, Australia, 30 m, 0.25 i.d., 0.25 µm film) using temperature programming: initial temperature 45 °C (held for 2 min) and then heated at 9°C/min to180°C (held for 5 min) and at 40°C/min to 220°C (held for 5 min).

3.14 Proton transfer reaction – mass spectrometry

A high-sensitivity quadrupole model PTR-MS (IONICON Analytik GmbH, Innsbruck, Austria) was used for real-time measurement of the volatile profiles of grilled-beef slurries (see previous sections). Slurry sample (10 g) was transferred into 200 mL Schott bottles and equilibrated to 37°C and gently stirred on a magnetic stirrer. The sample headspace gas was drawn through the inlet tubing at a rate of 100 mL/min, with ~15 mL/min drawn into the reaction chamber of the PTR-MS instrument. The transfer tubing was held at 60°C, the reaction chamber was held at 70°C (2.19 mbar), and the drift tube voltage was set at 600 V. The PTR-MS was used in scan mode (m/z 40-200), and the following additional ions were measured for calibration purposes;

protonated water isotope ($H_3^{18}O^+$; m/z 21), water cluster ($H_3O^+ \cdot H_2O$; m/z 37). Target volatiles were all measured with a dwell time of 50 ms, and the system was programmed to measure the full range of target volatiles every 500 ms, that is, two scans per second Table 1.

4 Results

4.1 Carcass grading

Carcass grading according to the MSA grading standards is present in Table 1. Grading was conducted at 24h post mortem on the longissimus muscle of each carcass. Ultimate pH of all carcasses was below 5.7 to avoid dark cutting meat.

Table 1. Carcass grading data.

Carcass number	Hot carcass weight	Ultimate pH	Meat colour score*	Marbling score*
1	215	5.51	3	220
2	196	5.63	6	340
3	173	5.66	4	420
4	176	5.55	4	200
5	180	5.73	5	400
6	134	5.51	4	290
7	132	5.56	4	210
8	190	5.43	4	400
9	194	5.48	4	350
10	187	5.44	2	460
11	180	5.46	3	290
12	189	5.48	3	270
13	170	5.68	4	230
14	137	5.51	4	260
15	145	5.55	5	250
16	131	5.58	5	280
17	120	5.62	4	330
18	131	5.60	4	370
19	131	5.57	4	Missing
20	126	5.57	3	230
21	127	5.61	2	200
22	118	5.46	3	330
23	156	5.56	4	320
24	131	5.53	4	350

*Data obtained at 24 hours post mortem from the longissimus muscles.

4.2 pH and colour

4.2.1 pH

Table 2. pH of meat after ageing

Ageing type	Days aged					SED	P values		
	1	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control	5.55								
Dry				5.62	5.54	0.01	< 0.001	< 0.001	0.001
Wet		5.43	5.52	5.44	5.28				
Wet-then-dry					5.59				

The pH of wet aged beef was relatively stable up to 21 days of ageing and significantly declined up to 56 days of ageing. In contrast, the pH of dry aged beef at 35 days was higher than any of the wet aged samples and dropped back to approximately 5.5 at 56 days.

4.2.2 Lightness (L*), redness (a*) and yellowness (b*)

Table 3. Instrumental lightness of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.44	0.681	< 0.001	0.983
Dry			34.42	34.88				
Wet	33.46	35.35	35.83	36.33				
Wet-then-dry				35.15				

Table 4. Instrumental redness of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.37	0.026	<0.001	0.198
Dry			24.58	23.38				
Wet	22.93	21.79	25.18	22.89				
Wet-then-dry				22.97				

Table 5. Instrumental yellowness of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.31	0.071	<0.001	0.179
Dry			21.84	20.57				
Wet	21.60	20.08	22.72	22.35				
Wet-then-dry				20.28				

There was no difference in lightness, redness and yellowness of dry and wet aged beef. Within the wet aged samples, L* and b* value increased with ageing time while redness value was highest at day 35. Ageing time had a significant ($P < 0.001$) effect on all three colour parameters of beef whereas ageing method did not.

4.2.3 R630/580, oxy-myoglobin and met-myoglobin

Table 6. Reflectance index ratio (630nm/580nm) of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.24	0.020	<0.001	0.111
Dry			8.57	7.37				
Wet	8.70	7.11	8.56	6.50				
Wet-then-dry				7.05				

Table 7. Oxy-myoglobin (%) of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.68	<0.001	<0.001	<0.001
Dry			93.47	89.59				
Wet	96.70	93.49	93.67	84.79				
Wet-then-dry				88.46				

Table 8. Met-myoglobin (%) of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Control					0.68	<0.001	<0.001	<0.001
Dry			6.53	10.41				
Wet	3.30	6.51	6.33	15.21				
Wet-then-dry				11.54				

Reflectance ratio (630/580), oxy-myoglobin and met-myoglobin were used as indicators of browning effect in meat during ageing. Lower reflectance ratio and oxy-myoglobin and higher met-myoglobin indicate an increase in browning. Significant browning occurred with ageing time in both dry and wet aged samples. No difference in browning was observed between dry and wet aged beef at day 35 or between dry aged only and wet and dry samples at day 56. However, the wet aged only samples at day 56 had the lowest reflectance ratio and oxy-myoglobin and highest met-myoglobin, suggesting the rate of browning after day 35 was lower in dry ageing compared to wet ageing.

4.3 Weight loss, total water content and yield

4.3.1 Weight loss

Table 9. Weight loss (%) of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Dry			11.14	17.41	0.34	<0.001	<0.001	<0.001
Wet	2.86	4.93	6.58	6.04				
Wet-then-dry				16.59				

Weight loss due to ageing was highest in dry aged only samples followed by wet then dry samples and lowest in wet aged only samples. Weight loss also significantly ($P < 0.001$) increased with ageing time in both wet and dry ageing.

4.3.2 Total water content

Table 10. Total water content (%) of meat after ageing

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Dry			73.21	71.74	0.30	0.908	0.004	0.313
Wet	72.83	73.04	72.41	71.65				
Wet-then-dry				72.67				

Total water content was higher in dry aged samples compared to wet aged samples at day 35, however, there was no difference between these sample groups at day 56. The wet and dry aged samples at day 56 had a higher total water content compared to both wet aged only and dry aged only.

4.3.3 Yield (only for dry ageing)

Table 11. Yield (%) at point of sale of meat after ageing

Ageing type	Days aged		SED	P values
	35	56		Ageing type
Dry	43.29	38.68	3.06	0.057
Wet-then-dry		37.52		

The yield at point of sale was calculated for both dry aged only and wet then dry samples after the boning and the dry and darkened outside layer of meat was trimmed. There was no difference in yield at point of sale between 35 and 56 ageing days or between dry ageing only and wet-then-dry ageing.

4.4 Australian consumer data

4.4.1 Tenderness

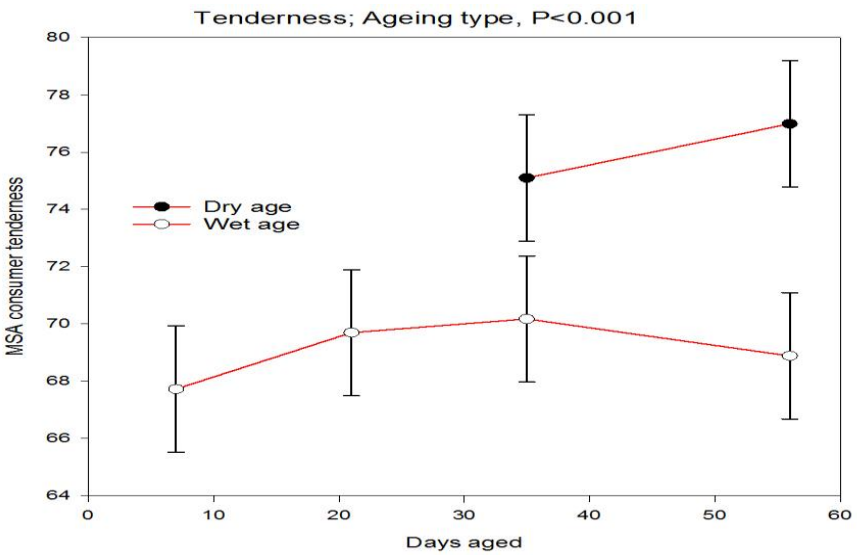

Figure 2. MSA Australian consumer tenderness score for dry or wet aged samples. Dry aged samples were aged for 35 or 56 days. Wet aged samples were aged for 7, 21, 35 or 56 days. Error bars are predicted means ± standard error of difference.

Figure 2 shows a clear difference in consumers' rating for tenderness. Dry aged samples at both days 35 and 56 ageing had a significant (P < 0.001) higher tenderness score compared to wet aged samples stored for the same amount of time. The tenderness score for the dry aged samples appeared to trend upwards, suggesting optimal dry ageing time at 56 days. However, there was little difference in the scores for ageing time in wet aged samples (Table 12).

Table 12. MSA Australian consumer tenderness score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			74.61	76.72	1.814	< 0.001	0.615
Wet	67.38	69.49	69.80	68.42			

SED = Standard error of differences.

4.4.2 Juiciness

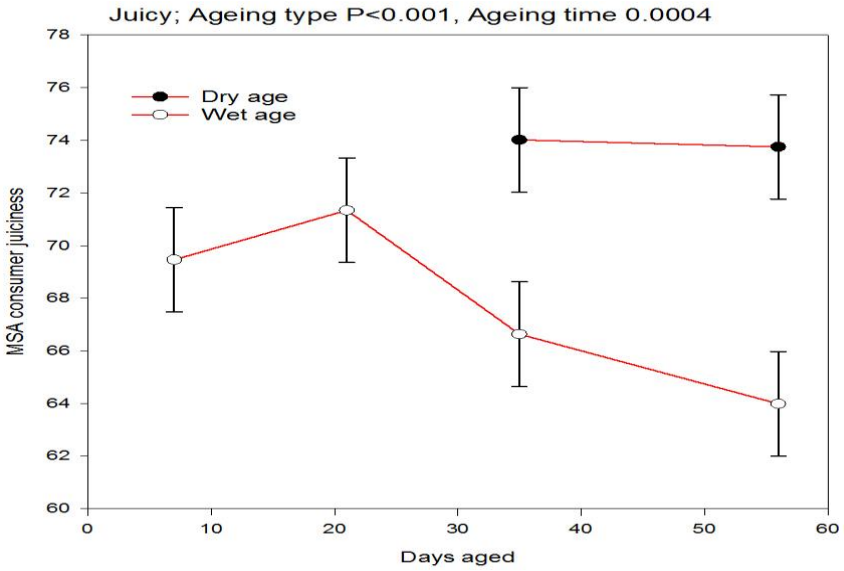

Figure 3. MSA Australian consumer juiciness score for dry or wet aged samples. Dry aged samples were aged for 35 or 56 days. Wet aged samples were aged for 7, 21, 35 or 56 days. Error bars are predicted means ± standard error of difference.

Although having similar total water content (Table 10), juiciness was rated higher than the wet aged samples store for equivalent amount of time (Figure 3). There was no difference in sensory juiciness score between dry aged 35 and 56 days. On the other hand, wet aged samples appeared to be most juicy at 21 days ageing, after which juiciness decreased. There was also a significant (P = 0.006) interaction between ageing type and days aged (Table 13).

Table 13. MSA Australian consumer juiciness score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			73.20	72.97	2.307	< 0.001	0.006
Wet	68.71	70.28	65.88	63.22			

SED = Standard error of differences.

4.4.3 Flavour

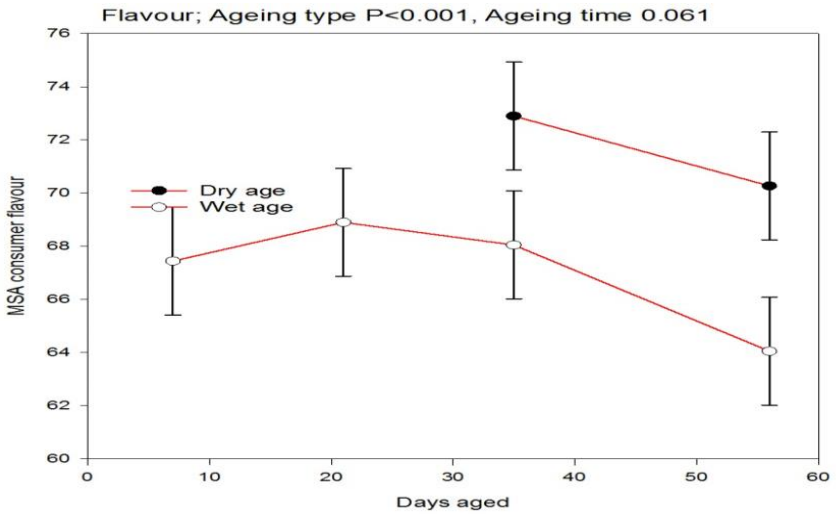

Figure 4. MSA Australian consumer flavour score for dry or wet aged samples. Dry aged samples were aged for 35 or 56 days. Wet aged samples were aged for 7, 21, 35 or 56 days. Error bars are predicted means ± standard error of difference.

Similar to tenderness, dry aged samples received a significantly (P < 0.001) higher score compared to wet aged samples stored for similar amount of time. However, 35 days appeared to be the optimal ageing time for dry ageing as the 56 dry aged samples had a lower sensory score (albeit the difference was not statistically significant). Similarly, 21 days appeared to be the optimal ageing time for wet ageing, with the 56 days wet aged samples received a much lower score.

Table 14. MSA Australian consumer flavour score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			72.14	69.81	1.874	< 0.001	0.06
Wet	66.97	68.69	67.47	63.30			

SED = Standard error of differences.

4.4.4 Overall liking

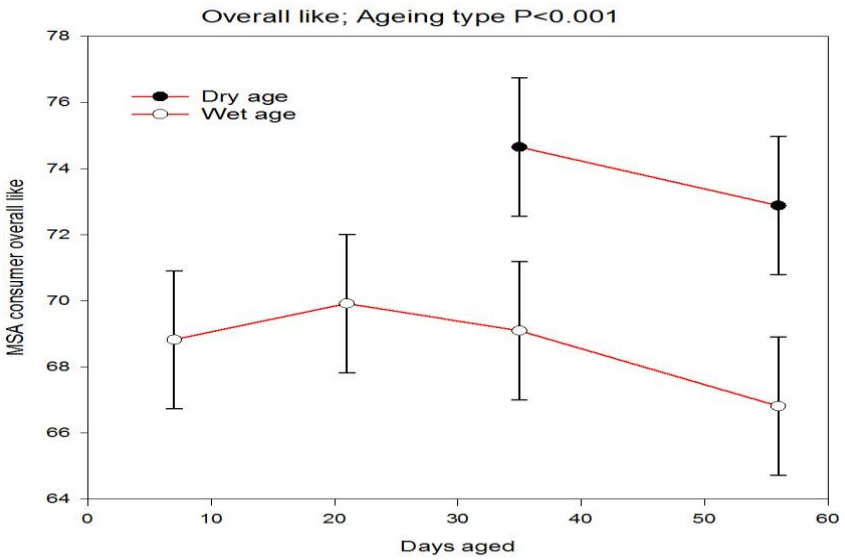

Figure 5. MSA Australian consumer overall liking score for dry or wet aged samples. Dry aged samples were aged for 35 or 56 days. Wet aged samples were aged for 7, 21, 35 or 56 days. Error bars are predicted means ± standard error of difference.

Interestingly, the overall liking scores reflected those of flavour scores. Dry aged samples were rated higher than wet aged regardless of ageing time. There was little difference between the two dry aged periods (35 and 56 days), with the 35 days samples received a slightly higher score. However, a significant difference was observed between the highest (21 days) and lowest (56 days) scores for the wet ageing samples.

Table 15. MSA Australian consumer overall liking score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			73.76	72.34	1.330	< 0.001	0.454
Wet	68.19	69.65	68.40	65.96			

SED = Standard error of differences.

4.4.5 MQ4

Table 16. MSA Australian consumer MQ4 score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			73.39	72.94	1.236	< 0.001	0.396
Wet	67.60	69.34	68.24	65.54			

SED = Standard error of differences.

Meat quality score (MQ4) was calculated as weighted results of the four sensory traits above. A beef description system based on the MQ4 score with some adjustments to the weightings and cut-off values has been shown useful in describing the eating quality of beef for the Japanese consumer (Polkinghorne *et al.* 2014). The MQ4 score of dry aged samples was significantly (P < 0.001) higher than that of wet aged

beef with little difference between the two dry aged periods. However, 21 days ageing appeared to be the optimal ageing time for wet ageing, consistent with the flavor and overall liking results.

4.4.6 Satisfaction

Table 17. MSA Australian consumer satisfaction score for dry or wet aged samples.

Ageing type	Days aged				SED	P values	
	7	21	35	56		Ageing type	Ageing type × days aged
Dry			3.817	3.852	0.065	0.005	0.974
Wet	3.658	3.665	3.645	3.615			

SED = Standard error of differences.

While there was a significant (P = 0.005) difference between dry and wet aged samples, there was no difference between different ageing periods within either dry or wet aged beef.

4.5 Japanese consumer data

The 56 days dry aged, 56 days wet aged and the 21 day wet followed by 35 day dry aged (wet-then-dry) samples were also used for sensory test by Japanese consumers. In collaboration with Prof Takanori Nishimura (Hokkaido University, Japan), untrained sensory panels were recruited from Hokkaido region and tests were conducted in Sapporo, Japan. Data are presented below.

4.5.1 Tenderness

Table 18. MSA Japanese consumer tenderness score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	68.95	51.11	64.32	4.48	<0.001

SED = Standard error of differences.

There was no significant difference in tenderness between dry aged (only) and wet-then-dry samples, indicating that the combined wet-then-dry treatment did not affect sensory tenderness of beef. However, the wet aged samples were scored significantly lower than the other two treatments.

4.5.2 Juiciness

Table 19. MSA Japanese consumer juiciness score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	56.74	48.50	54.12	3.00	0.005

SED = Standard error of differences.

Similar to tenderness results, the juiciness results showed that the wet aged (only) samples received the lowest score out of all three aging types. There was no significant difference between the dry aged (only) samples and the wet-then-dry samples.

4.5.3 Flavour

Table 20. MSA Japanese consumer flavour score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	58.63	40.92	54.25	2.17	<0.001

SED = Standard error of differences.

Flavour score differed significantly amongst the three ageing types with dry aged (only) having the highest score, followed by wet-then-dry samples, followed by the wet aged (only) samples. It is worth noting although statistically significant, the difference in flavour score between these two samples was small when compared with the wet aged (only) samples.

4.5.4 Overall liking

Table 21. MSA Japanese consumer overall liking score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	62.92	43.80	56.92	2.81	<0.001

SED = Standard error of differences.

Overall liking score differed significantly for the three ageing types. The score was highest in the dry aged (only) samples followed by the wet-then-dry samples followed by the wet aged (only) samples.

4.5.5 MQ4

Table 22. MSA Japanese consumer MQ4 score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	62.83	45.60	58.06	2.80	<0.001

SED = Standard error of differences.

MQ4 score was calculated from the four attributes above. The MQ4 for the wet aged (only) samples was significantly lower than that of both the dry aged (only) and the wet-then-dry samples. There was little difference between the dry aged (only) and the wet-then-dry samples.

4.5.6 Satisfaction

Table 23. MSA Japanese consumer satisfaction score.

Ageing type	Dry	Wet	Wet-then-dry	SED	P value
MSA score	2.509	1.954	2.367	0.094	<0.001

SED = Standard error of differences.

There was no significant difference between the dry aged (only) samples and the wet-then-dry samples. However, the satisfaction score for the wet aged (only) samples was significantly lower.

4.6 Comparison of MSA consumer results for Australia and Japan

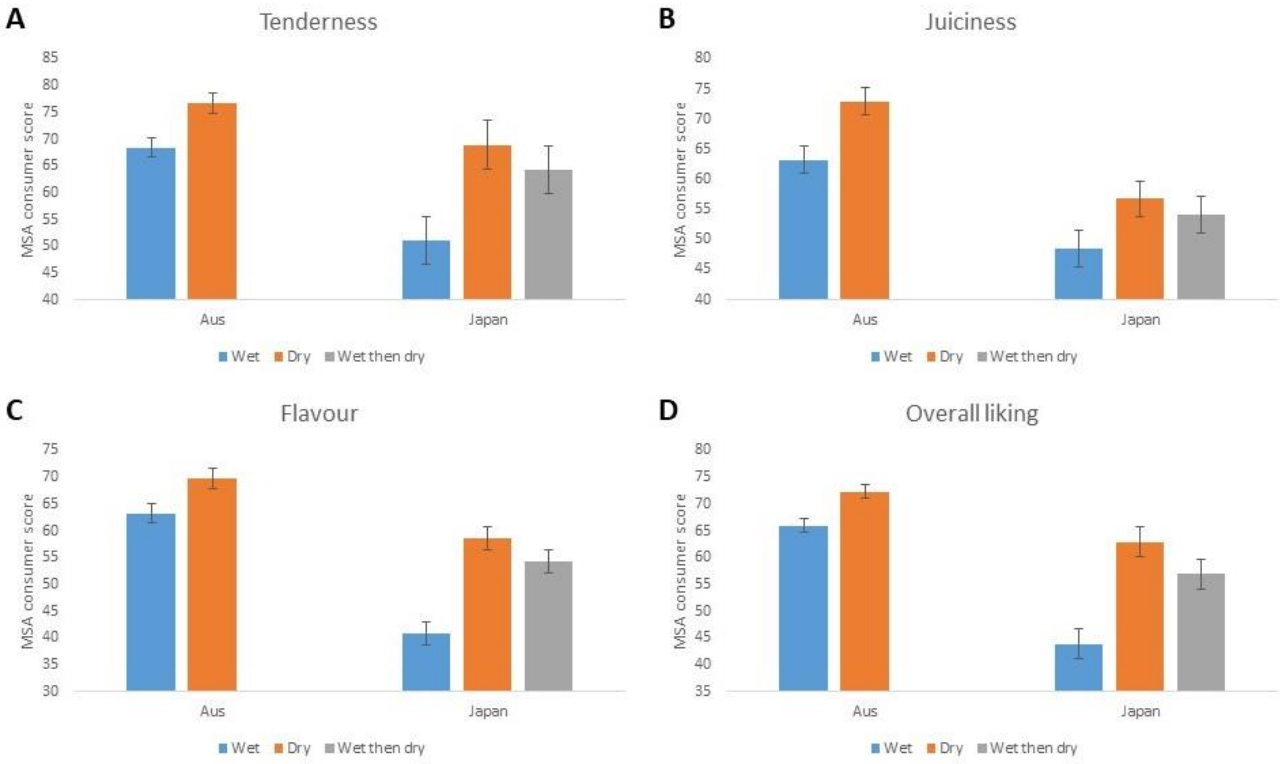

Figure 6. Comparison of MSA consumer scores for four eating quality attributes of wet-, dry- and wet then dry-aged samples between Australian and Japanese consumers. All samples were aged for a total of 56 days. The wet then dry aged samples were only tasted in Japan. Error bars are predicted means ± standard error of difference.

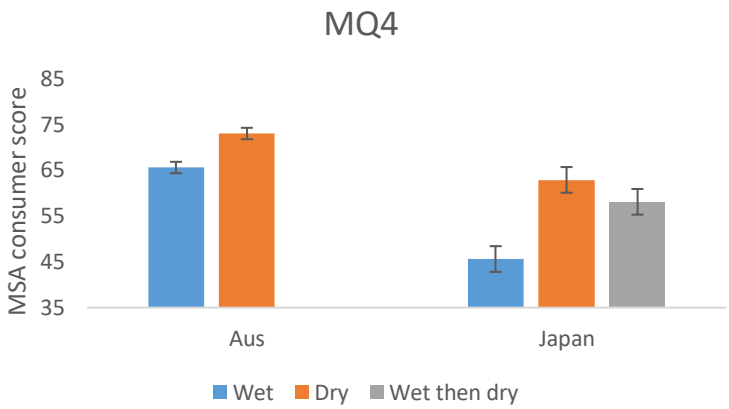

Figure 7. Comparison of MQ4 MSA consumer score for wet-, dry- and wet then dry-aged samples between Australian and Japanese consumers. All samples were aged for a total of 56 days. The wet then dry aged samples were only tasted in Japan. Error bars are predicted means ± standard error of difference.

4.7 Olfactometry differences between grilled wet and dry aged beef

GC-O was performed mainly to check for the presence of distinct taints or off-flavours that may be unique to either the wet or dry aging process and also to elucidate the important odour-impact volatiles present in aged beef flavour extracts. The aromagram profiles of wet and dry aged samples are summarised in Figure 6. While only small differences were measured for most aroma peaks (not labelled), it can be clearly seen that the odour intensity for a number of odour peaks was higher in the dry aged samples, e.g.; 2-methylproponal/acetone, 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, (E)-2-nonenal, 1-octanol. The main grilled beef odour impact volatiles formed corresponded with heat generated pyrazine compounds, well-known components of grilled beef aroma (Cerny and Grosch 1992, Cerny and Grosch 1993). The findings are similar to those reported in (Frank *et al.* 2016b). While it was clear that no off-flavours or unique aroma compounds were formed during dry aging compared to wet aging, a number of odour-active compounds were more intense in the dry-aged samples, supporting the sensory consumer data showing higher flavour scores.

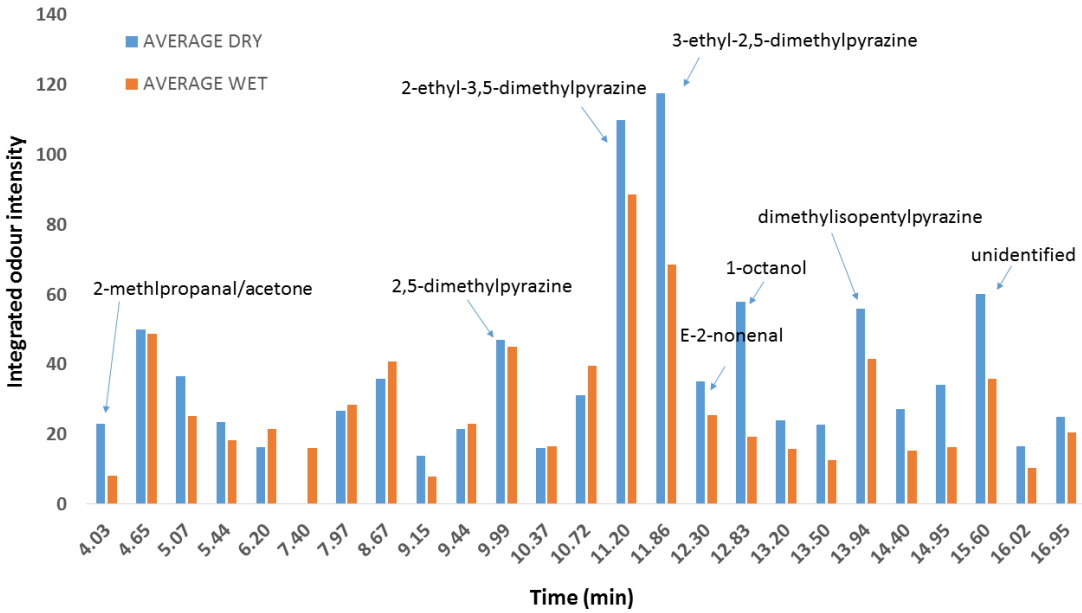

Figure 8. Mean (n=5) gas chromatography olfactory aromagram profiles of freshly grilled dry (blue) and wet (orange) aged beef samples after 35 days.

4.7.1 SPME-GCMS

The SPME method was able to measure more than 80 volatiles compounds, tentatively identified on the basis of electron impact mass spectra (Appendix 9.1). Significant differences were found for nearly every volatile measured for either “aging type” or “days aged” main effects or interactions. Very large differences in particular volatiles were measured according to aging type. Known beef flavour compounds such as 3-hydroxy-2-butanone, acetone and hexanal were all much higher in dry aged beef, whereas ethanol and acetic acid were very high in wet aged beef, indicating that these volatiles are key by-products of different types of bacterial and metabolic activity. Ethanol and acetic acid are both key products of anaerobic fermentation. 3-hydroxy-2-butanone, acetone and hexanal are all breakdown products of lipid oxidation.

4.7.2 PTR-MS

It should be noted that real-time PTR-MS is inherently less sensitive than SPME GC-MS and was only expected to show the main volatile differences between the aging types. For example, only 24 ions (out of 160) differed significantly between dry and wet aged samples, and a similar number of ions changed with time. Of note was the much higher m/z 43 (unknown), m/z 47, 48 (ethanol), m/z 61 (acetic acid), m/z 65 (dimethylsulphide) in wet aged beef, supporting the GC-MS data. In contrast m/z 59 (acetone) and m/z 89 (3-hydroxy-2-butanone) were higher in the dry-aged samples, consistent with the GC-MS findings. It is important to note that most of the ions measured by PTR-MS are likely to be from multiple volatile compounds. A full list of all ions detected by PTR-MS is in Appendix 9.2.

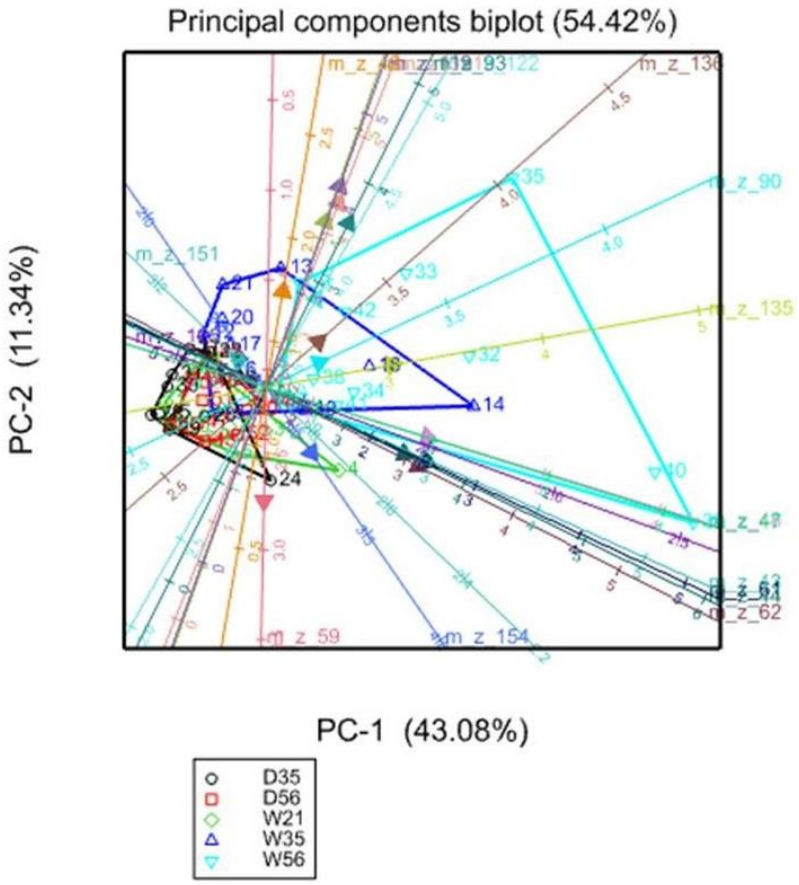

Figure 9. Principle component bilot of volatile ions detected using PTR-MS.

Principle component bilot was used to group volatiles that differed between aging method and time of wet and dry aged samples. Volatile cluster in dry aged samples remained tight regardless of ageing time, whereas clustering variation in wet aged samples increased with time.

4.8 Lipid and protein oxidation of meat

4.8.1 Lipid oxidation

Table 24. TBARS value (mg MDA/kg meat) of wet and dry aged meat after ageing.

Ageing type	Days aged				SED	P values		
	7	21	35	56		Ageing type	Days aged	Ageing type × days aged
Dry			0.82	1.32	0.11	< 0.001	< 0.001	< 0.001
Wet	0.28	0.50	0.88	1.04				
Wet-then-dry				1.37				

Lipid oxidation of meat affects both shelf-life and flavour. Lipid oxidation increased with time in both wet and dry aged samples. Dry ageing induced more lipid oxidation than wet ageing at day 56 but no significant difference was observed for samples at day 35. Lipid oxidation in the combined wet and dry aged samples at day 56 was higher than those in wet ageing only and similar to those in dry ageing only. It is noted that the level of lipid oxidation in all samples was significantly lower than the cut-off limit for acceptability (2 mg MDA/kg meat) (Campo *et al.* 2006).

4.8.2 Protein oxidation

Table 25. Total carbonyl content (nM DNPH/mg protein) of wet and dry aged meat after ageing.

Ageing type	Days aged		SED	P values		
	35	56		Ageing type	Days aged	Ageing type × days aged
Dry	0.58	0.53	0.09	< 0.001	< 0.001	0.191
Wet	0.35	0.70				
Wet-then-dry		0.55				

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). Increase in carbonyl content indicates increased oxidation.

Protein oxidation in dry aged sample peaked at 35 days with no further increase up to 56 days. Similar level of protein oxidation was observed for the wet then dry aged samples at 56 days. In contrast, protein oxidation in wet aged samples increased continuously over the ageing period. Carbonyl content of wet aged samples at day 56 was twice as much as day 35 and higher than both dry aged only and wet then dry aged samples.

4.9 Guideline development for industry production of beef dry ageing

4.9.1 Process map of dry ageing 'only'

STEP	CRUCIAL POINTS
1. Receive (Carcase or Side)	• Temp Control • Handing Hygiene • Transport Hygiene • Time Post Slaughter
2. Portion/Quarter	• Equipment Hygiene • Handling Hygiene • Personal Hygiene • Cutting Lines
3. Load Dry Ageing Chamber-	• Equipment Hygiene • Personal Hygiene • Temperature Control
4. Commence Dry Ageing	• Product Spacing • Relative Humidity • Air Speed • Temperature
5. Trim/Package	• Temperature Control • Cross Contamination • Personal Hygiene • Equipment Hygiene
6. Dispatch	• Temperature Control

4.9.2 Process map of wet ageing prior to dry ageing (wet-then-dry)

STEP	CRUCIAL POINTS
1. Receive (Vacuum Packed Primals)	• Temp Control • Loose Packaging • Time Post Slaughter/packing
2. Unpack product Load into chamber	• Equipment Hygiene • Personal Hygiene • Temperature Control • Loose Packaging
3. Commence Dry Age	• Product Spacing • Relative Humidity • Air Speed • Temperature
4. Trim/Package	• Temperature Control • Cross Contamination • Personal Hygiene • Equipment Hygiene
5. Dispatch	• Temperature Control

4.9.3 Dry ageing only

4.9.3.1 The importance of sufficient fat cover prior to the dry ageing process

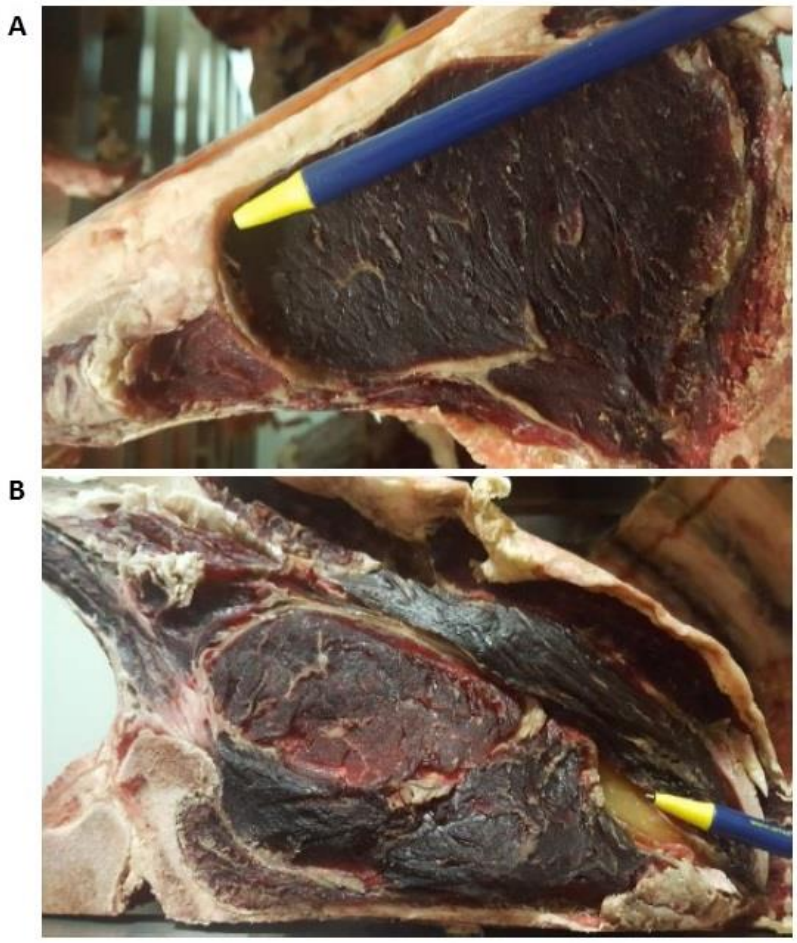

Figure 10. Representative photos (side view) of (**A**) a strip loin and (**B**) a cuberoll with sufficient fat cover at day 21 during dry ageing.

Figure 8A illustrates that the lean tissue had started to shrink longitudinally and pulled away from under the fat cover by day 21. The colour of lean tissue also significantly darkened and the surface rapidly dried out and hardened. The colour and dryness of the longissimus were different to those of the surrounding muscles. Similar shrinkage and colour change was also observed for cuberolls (Figure 8B). In addition to lean tissue retraction, the nuchal ligament (paddywhack) also shrunk.

It is noted that at this point in time the surface of lean tissue had not completely sealed to form a ‘cap’ and shrinkage was still continuing.

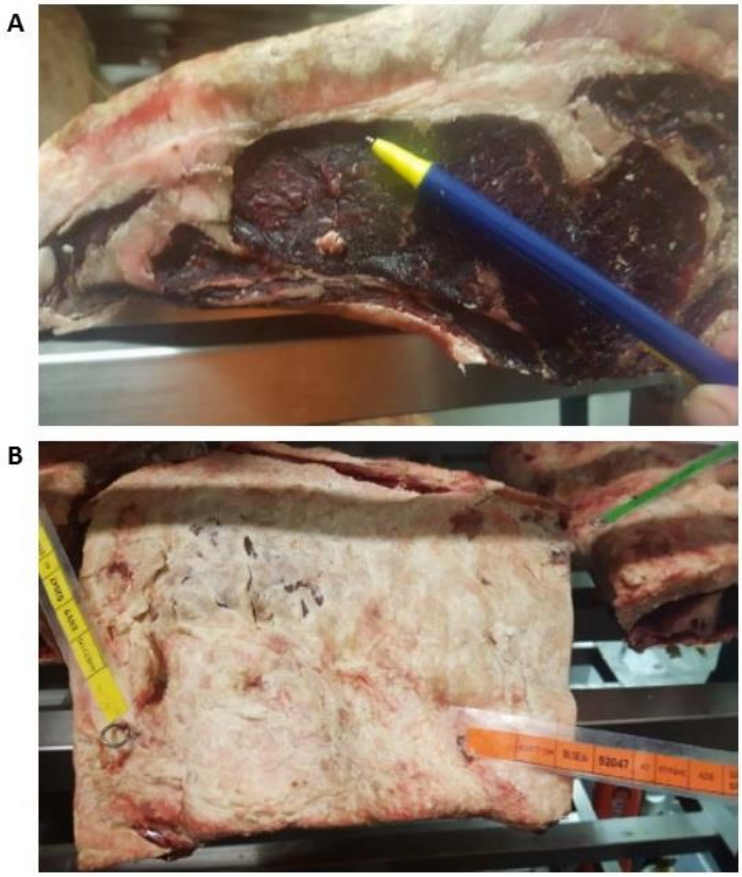

Figure 11. Representative photos of (A) side view and (B) top view of a strip loin with sufficient fat cover at day 21 during dry ageing.

At day 21, beef quarters with sufficient fat cover had visible lean tissue shrinkage (Figure 9A) and the fat cover had started to lose its flexibility and oiliness to become more chalky and crumbly (Figure 9B). The colour changed to be whiter with patches of redness. Figure 9A also shows two distinct layers on the fat cover with the outer layer having more texture and colour changes than the inner layer which remained more oily and flexible. The 'normal' texture and colour of the inner layer is important for trimming for retail purpose after ageing.

These changes in lean (Figure 8) and fat (Figure 9) tissues seen by day 21 of dry ageing indicated good airflow, temperature and UV exposure. It is noted that dry ageing in cheesecloth was not included in the experimental design. However, it is expected that cheesecloth would interfere with airflow and UV exposure on the primal surface, thus meat dry aged in cheesecloth would require different dry ageing chamber conditions.

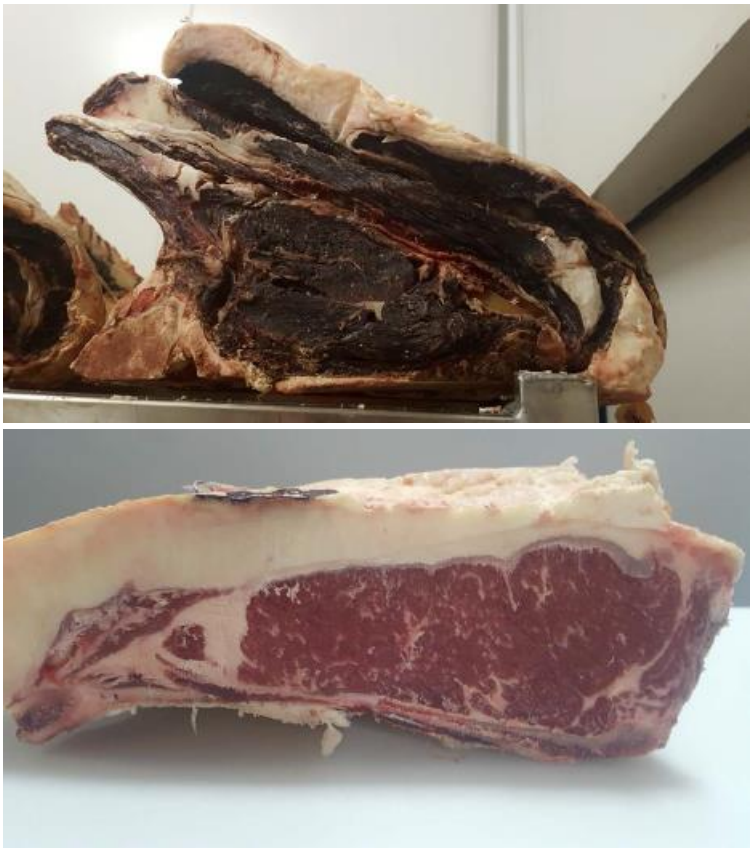

Figure 12. Representative photos of (A) a cuberoll and (B) a strip loin ready for retail trimming at day 35 of dry ageing.

At day 35, a sealed cap had formed on lean tissues of both strip loins and cuberoll (Figure 10A), indicating a good dry ageing process. It is noted that lean tissue shrinkage and texture and colour changes of the fat cover had substantially slowed down by this point. During trimming and boning for retail ready meat, the crumbly and inflexible outer layer of the fat cover as seen in Figure 10B was trimmed while the oily and white inner layer was left on the loins to improve yield.

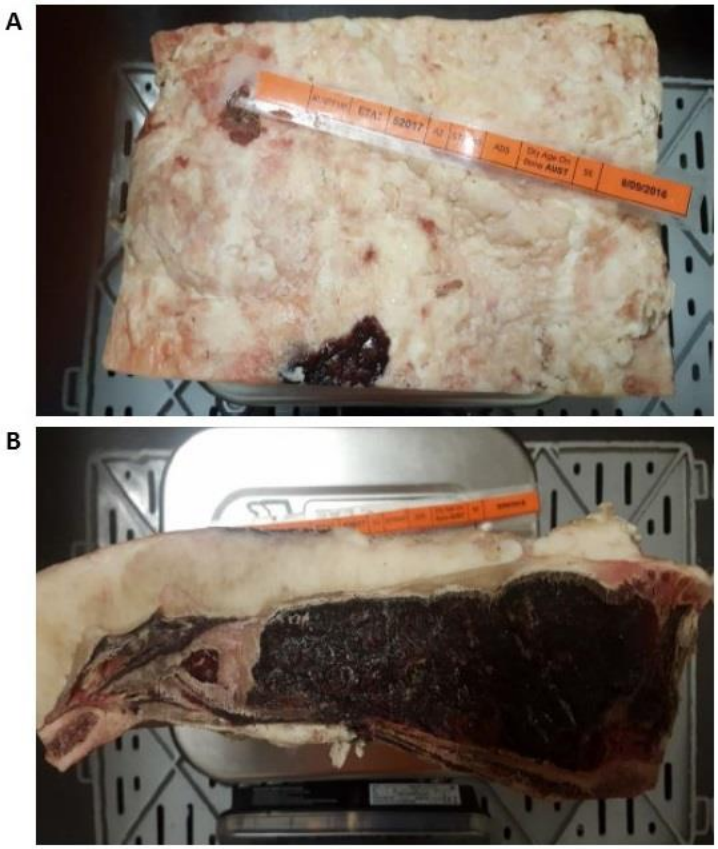

Figure 13. Representative photos of (A) top view and (B) side view of a strip loin with sufficient fat cover at day 56 during dry ageing.

Compared to day 21, the quarters at day 56 had a completely sealed cap on the lean tissue and mostly white fat cover (Figure 11).

Figure 14. A representative photo (side view) of a strip loin with insufficient fat cover at day 21 during dry ageing.

Compared to the quarters with a thick fat cover, insufficient fat cover resulted in more shrinkage of lean tissue (Figure 12) by day 21 which would likely lead to lower yield of the finished product. Also separation between the lean and fat tissues was greater in quarters with a thin fat cover. Due to the change in texture and colour of fat during dry ageing, sufficient trimming to the 'normal' inside layer of fat for retail purpose

would be required. A thin layer of fat cover may result in loss of all fat and even lean tissue at point of sale, thus significantly affecting yield.

4.9.3.2 The importance of hygiene during quartering prior to dry ageing

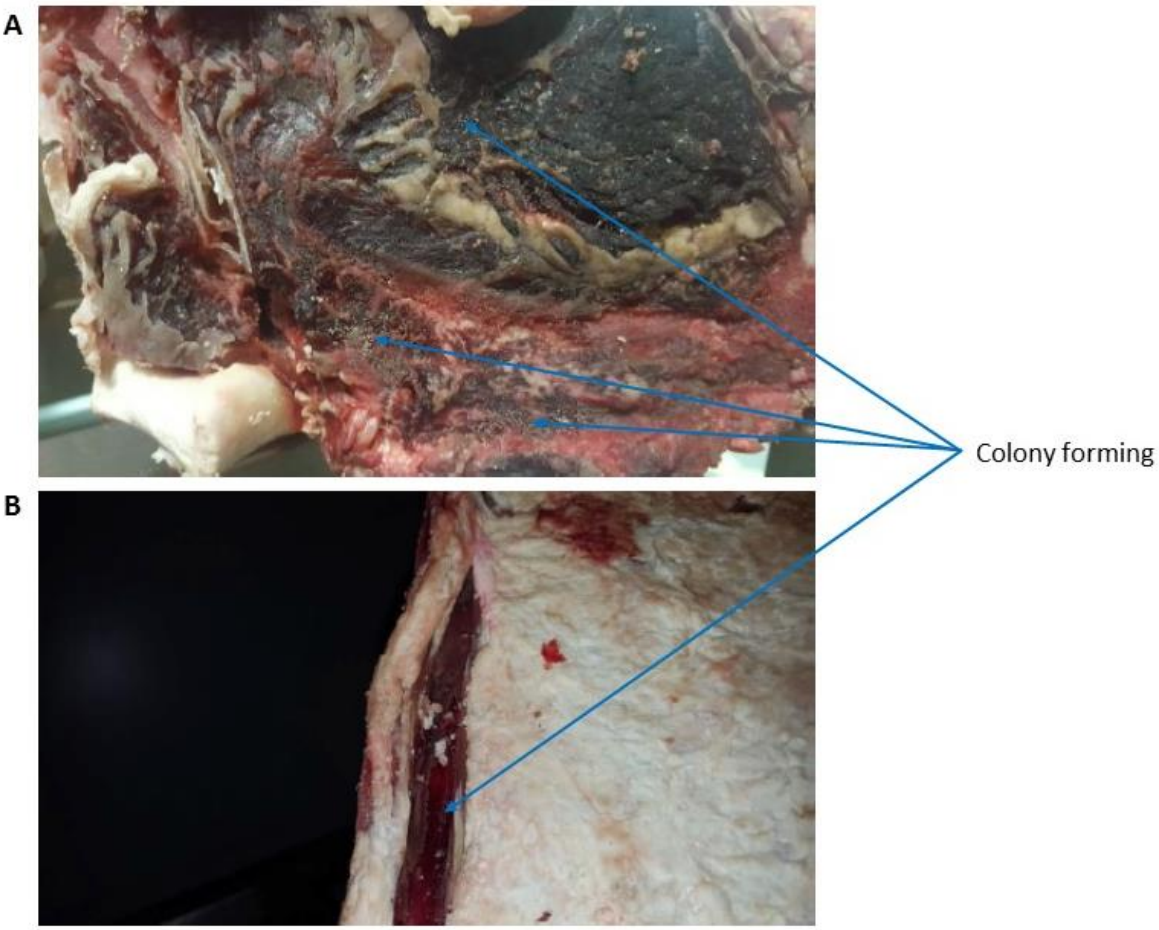

Figure 15. Representative photos of (A) colonies forming on a cuberoll and (B) knife mark and colonies on a strip loin at day 21 during dry ageing.

Hygiene is an important aspect of the meat industry. This is especially true for dry ageing in which, unlike wet ageing, oxygen level is high, thus suppressing aerobic bacterial growth with correct temperature, airflow and UV is essential. Figure 13 shows early signs of spoilage, most likely the result of dirty knife during quartering. Monitoring of spoilage is essential in the first three weeks of dry ageing. Quarters with bacterial colonies need to be removed at this point and airspeed increased to increase drying rate and discourage bacterial growth. It is noted that the colonies shown in Figure 13 was suppressed later in the dry ageing process due to good airflow and rapid drying of lean, emphasising the importance of airflow in minimising effect of poor hygiene.

Important indications during the first 3 weeks of dry ageing for monitoring purpose. This timeframe is critical in making sure good dry ageing process.

1. Signs of leaning pulling away and surface drying rapidly
2. Fat became whiter and chalky and less flexible
3. Nuchal ligament shrinkage.

4.9.4 Wet aged for 21 days prior to dry aging for 35 days (wet-then-dry)

4.9.4.1 The importance of sufficient fat cover due to fat staining

In relation to the differences in the process map for the production of dry aged beef which has been wet aged prior the following points need consideration.

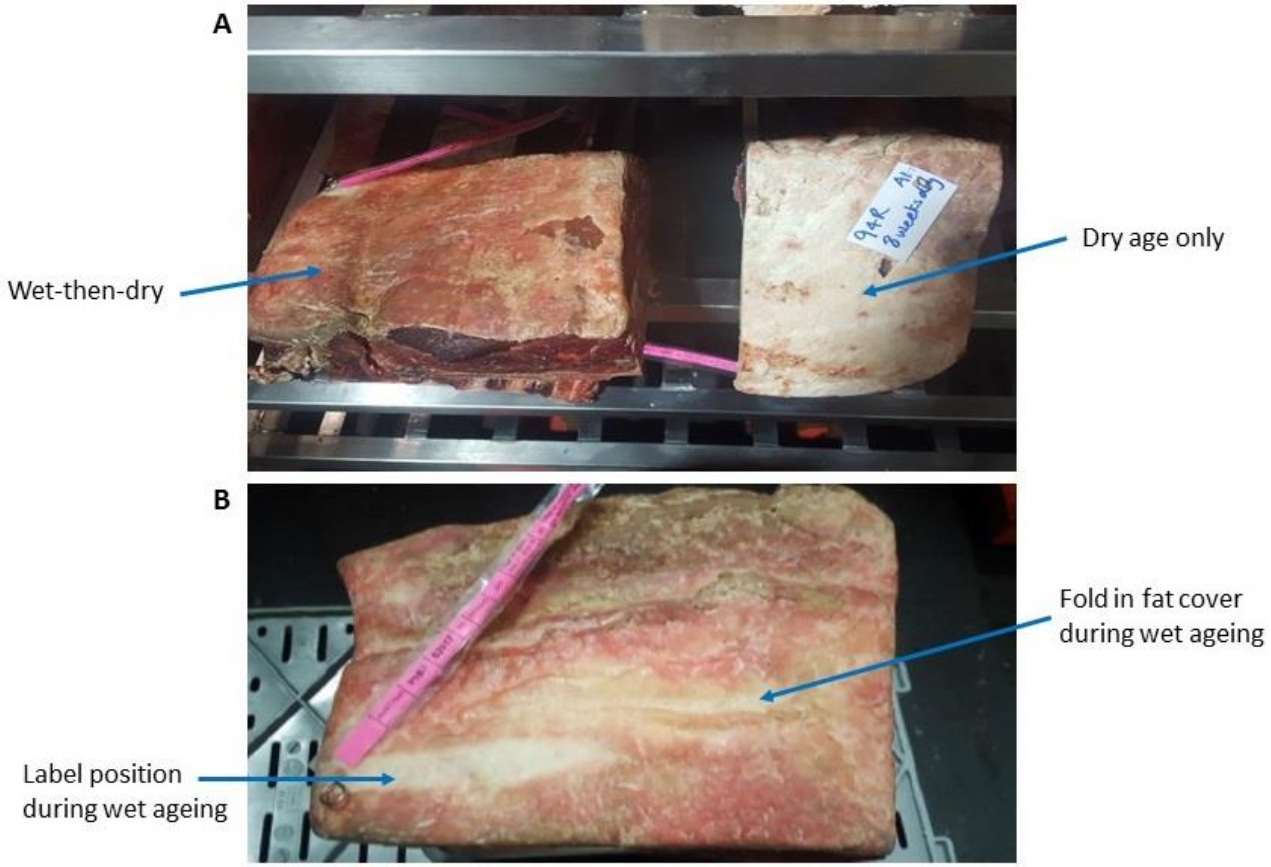

Figure 16. Representative photos of (**A**) wet then dry quarters (left) and dry age only quarter (right) at day 24 and (**B**) wet then dry quarters with label on top of or folds in the fat cover during wet ageing.

In addition to shrinkage of lean tissue and texture change of the fat cover as seen with the dry ageing only process, the fat cover on quarters that were wet-the-dry aged were also stained. Figure 14A shows staining of the fat cover on a wet-then-dry aged quarter (left) compared to the white fat cover of a dry aged only quarter at day 24. Wet aged product had a 'head start' on certain cold tolerant (psychrotrophic) microbes which are anaerobic (prefer a lack of oxygen) such as *Lactococcus* and *Lactobacillus.* These microbes are classified as lactic acid producing microbes and are commonly found on vacuum packaged meat but not so commonly found on chilled meat in aerobic conditions. Under some circumstances the wet ageing period can allow the fat surface to absorb the blood which appears to result in staining of the fat. However, the colour observed on the fat cover of the wet-then-dry quarters was not consistent with colour of blood-to-

fat stain. In addition, where there was a label on the fat cover during the wet ageing period, the fat stain did not appear during the dry ageing process (Figure 14B). It is currently not understood the cause of the fat stain on the wet-then-dry products or the reason for lack of stain where the surface was not exposed during wet ageing.

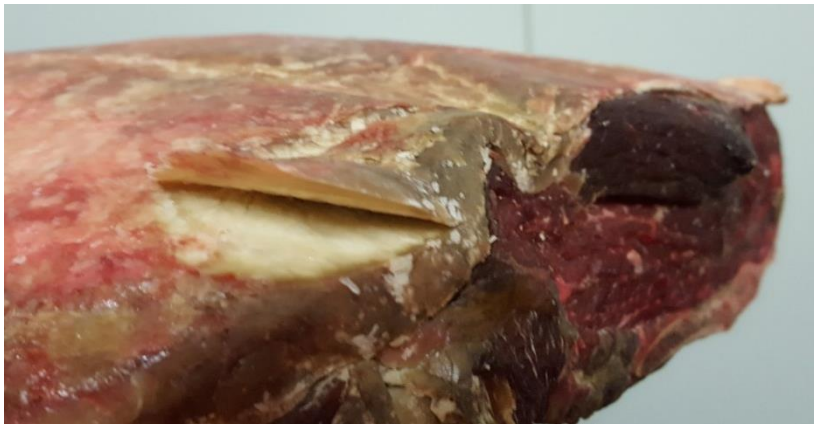

Figure 17. A representative photo of wet-then-dry quarter with sufficient fat cover at day 24.

Figure 15 demonstrates the thickness of the fat stain was approximately 1.5-2mm at day 24 (3 days in to the dry ageing process) on a wet-then-dry aged quarter. Due to this fat stain, more trimming for retail purpose was required for the finished product, emphasising the need for a thicker layer of fat cover on quarters destined for wet-then-dry ageing process compared to dry ageing only process. The increase in trim loss resulted in a lower yield for wet-then-dry products.

Figure 18. A representative photo of wet-then-dry quarter with insufficient fat cover at day 56.

In the case of quarters with insufficient fat cover (Figure 16), the stain was seen to penetrate the lean tissue which resulted in a substantial decrease in yield at point of sale due to heavy trimming.

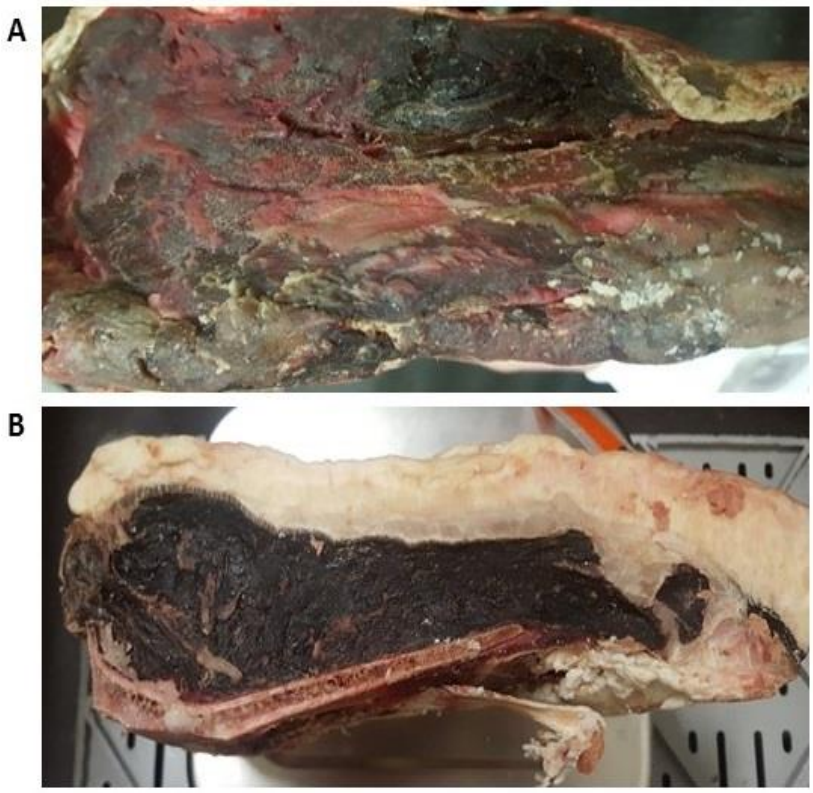

Figure 19. Representative photos of (**A**) wet-then-dry and (**B**) dry aged only strip loins at day 35.

Compared to a dry aged only quarter (Figure 17B), those that went through a wet-then-dry ageing process (Figure 17A) were more susceptible to excess spoilage due to microbial growth at day 35, demonstrating the importance of hygiene during quartering.

4.9.5 Chamber parameters

A dry ageing chamber (Figure 18) used for dry ageing of beef which has been wet aged prior has differing ideal parameters than that of product to be dry aged alone. An increase in air speed will assist in the initial surface drying phase of treatment. This is important due to the fact that no natural migration of moisture had been allowed in the lead up to the drying process in non-vacuum product.

Figure 20. Chamber evaporator & operating UV unit

The combination of air temperature ≤0.5°C, Relative Humidity <80%, an air speed of minimum 0.2 m/s and the use of UV lighting encourages drying of the surfaces and results in substantial reduction of microbial surface growth which in turn results in less trimming required and a higher final yield.

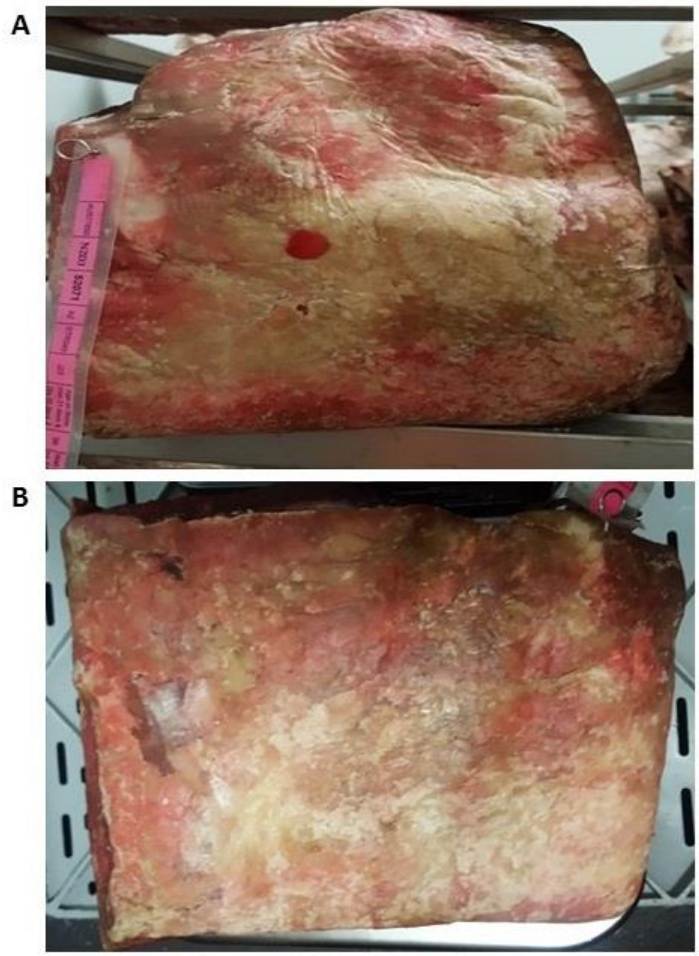

Figure 21. Representative photos of wet-then-dry quarter in (A) with insufficient fat cover at day 56.

Wet-then-dry product placed on rack in a high airflow position and exposed directly to UV light treatment (Figure 19A) had a drier surface compared to those in more sheltered positions (Figure 19B). The level of relative humidity in the chamber is also more critical to encourage this drying. The initial 7 to 10 days of treatment are the most important as during this period the water migration process begins. If the chamber is opened and additional product loaded into the environment the balance between air and product conditions will be compromised. This will lead to a slowing down of the process and allow more favourable conditions for micro growth which ultimately leads to lower yield.

Chamber conditions for product that is dry aged alone are more forgiving as the product has an established weight loss from the time of slaughter without the additional disrupting step of vacuum packaging.

The use of cheese cloth or other packaging is not recommended as this impedes airflow to the surface of the product as well as providing a more favourable environment for the growth of microbes due to it holding moisture close to or in contact with the surface.

4.9.6 Pre Dry Age Hygiene

The emphasis on strict hygiene procedures cannot be over stated. In the Standard process map for vacuum packed products steps are as follows;

- Transport
- Trimming/slicing
- Cooking

However when wet aged product is destined for a further dry aging process there are additional processes such as unpacking and dry ageing prior to the product being trimmed and sold to the consumer for cooking. Processors producing products intended for further dry ageing need to consider these additional steps in their hazard analysis.

Figure 17 shows visible differences in contamination levels between two cuts made during the same quartering process on the corresponding day of dry age treatment. Vacuum packaging encourages the growth of anaerobic microbes. Tighter controls over hygiene practices are needed for the processing of products that are to be dry aged post wet ageing.

4.9.7 Summary of differences between the two ageing processes

Below are the summary of the differences between dry ageing only and wet-then-dry ageing products

Table 26. Differences between dry ageing only and wet-then-dry ageing products

	Dry ageing only	**Wet-then-dry**
Stock type	Vacuumed product	Vacuumed product
Post slaughter time limits	5 days post slaughter	Vacuum pack asap after slaughter 21 days max wet age
Advantages	More forgiving to changes to conditions Less Lactic Acid bacteria at start of treatment	Allows more flexibility on stock levels
Disadvantage	Short lead time to dry age	Requires more process control More critical with fat layer on the outside Stricter pre-ageing hygiene control to reduce spoilage risks More trimming at the end Staining of fat
Critical process control or recommendations	Careful observation in the first 3 weeks	Higher air speed especially at the start of dry ageing. Lower air speed

	Stricter hygiene during quartering	later in the process to lower yield loss. Careful observation in the first 3 weeks Only high Fat (min 20 mm) coverage primal are recommend

The wet-then-dry ageing method is possible but requires special attention:

- More stringent hygiene during quartering
- Fat cover min 20 mm to allow for stain removal
- Higher air velocity during first 10 days of treatment

4.9.8 Generic WRAC table and hazard analysis tables

The use of a critical control point (CCP) decision tree in conjunction with the weighted risk assessment criteria (WRAC) analysis process (Table 27) would indicate the need for tighter than standard hygiene specs to be developed for vacuum packed product which is to be further dry aged. Individuals will need to apply their own specifics to generate the correct information.

The assessment of the significance of Food Safety hazards uses the following matrix of the severity (consequences) and the likelihood (frequency). A value of 1-10 indicates a significant Food Safety or Quality Issue (i.e. above the line), which signifies that control measure(s) must be put in place – CCP status control measures & that monitoring & records must be kept. Food Safety issues that are not significant will have values of 11 – 25. It is up to the HACCP team members to determine whether it makes good sense to have any control measures in place (i.e. CCP status control measures). ***A CCP is a step in a process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.***

HAZARD SEVERITY

1. Fatality occurrence
2. Serious Illness
3. Product Recall
4. Customer Complaint
5. Not significant

HAZARD LIKELIHOOD

A. Common repeating
B. Known to occur or 'it has happened'
C. Could occur or 'I've heard of it happening'
D. Not expected to occur
E. Practically impossible

Table 27. Weighted risk assessment criteria (WRAC) analysis.

	LIKELIHOOD				
SEVERITY	A	B	C	D	E
1	1	2	4	7	11
2	3	5	8	12	16
3	6	9	13	17	20
4	10	14	18	21	23
5	15	19	22	24	25

4.9.9 Hazard analysis

Table 28. Hazard, cause and control measures of both dry ageing only and wet-then-dry ageing methods.

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
1 Receival	Micro: Excessive microbiological load/growth on incoming meat.	Poor slaughter practices	Supplier Approval Program	SP 2+D=**12**
		Temperature > 5 degrees	Temperature control. Maintain meat temperature below 5 degrees	SP 2+D=**12**
		Time post slaughter	Reject product which out of date	SP 2+D=**12**
	Physical or microbial contamination	Packaging damaged or meat contacted unclean surface	Inspection of delivered meat by trained personnel	SP 4+D=**21**

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
2 Storage	Micro: Growth of micro-organisms	poor temperature control	Temperature control. Maintain meat temperature below ≤ 2.0 degrees	SP 2+D=**12**
		product past shelf life	Stock control – product traceability	SP 2+D=**12**
	Micro: Cross Contamination	Incorrect storage (under hanging meat)	Stock rotation	SP 5+D=**24**

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
3 Unpack / load racks	Micro: Cross Contamination	Contamination from food handlers	GMP Personal Hygiene	SP 2+D=**12**
		Contamination from premises or equipment	GMP hygiene & sanitation.	SP 2+D=**12**
	Micro: Growth of micro-organisms	Product temperature rises during processing	Product to be processed in a temperature controlled environment without delay	SP 2+D=**12**
		Insufficient space between cuts restricting airflow	Trained staff performing temp checks and rack spacing	SP 2+D=**12**
	Chemical contamination	Contamination from premises or equipment	GMP hygiene & sanitation.	SP 5+D=**24**

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
4 Dry ageing	Micro: (pathogen) growth	Incorrect temperature control	Chiller temperature to remain between -0.5 – 1.0°C	SP 2+D=**12**
		Incorrect relative humidity control	Relative humidity to be 85% - 75%	SP 4+D=**21**

		Incorrect airflow control	Airflow to be 0.2- 0.5 mtrs/second	SP 4+D=**21**
		No use of UV lights	UV lights in use	SP 4+D=**21**
		Contamination from premises or equipment	GMP hygiene & sanitation.	SP 2+D=**12**
	Microbiological growth	Product temperature rises during processing	Product to be processed in a temperature controlled environment without delay	SP 2+D=**12**
	Contamination from cleaning chemical	Inadequate sanitation	GMP hygiene & sanitation	SP 4+D=**21**

PROCESS STEP	**HAZARD**	**CAUSE**	**CONTROL MEASURES**	**CCP / SP WRAC NO.**
5 Trim	Microbiological Cross Contamination	Trimmed off cuts come into contact with finished product	GMP trimming	CCP 2+C=**8**
		Contamination from premises or equipment	GMP hygiene & sanitation.	SP 2+D=**12**
	Microbiological growth	Product temperature rises during processing	Product to be processed in a temperature controlled environment without delay	SP 2+D=**12**
	Contamination from cleaning chemical	Inadequate sanitation	GMP hygiene & sanitation	SP 4+D=**21**

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
6 Packaging	Microbiological Cross Contamination	Contaminated packaging or equipment	GMP Packaging	SP 2+D=**12**
	Microbiological growth	Product temperature rises during processing	Product to be processed in a temperature controlled environment without delay	SP 2+D=**12**
	Contamination from cleaning chemical	Inadequate sanitation	GMP hygiene & sanitation	SP 4+D=**21**

PROCESS STEP	HAZARD	CAUSE	CONTROL MEASURES	CCP / SP WRAC NO.
7 Storage	Microbiological growth	Poor temperature control	Temperature control. Maintain meat temperature $\leq$ 2.0 degrees	CCP 2+B=**5**
		Product past shelf life	Stock control – product traceability	SP 2+D=**12**

CCP= Critical Control Point, SP= Supporting Program

5 Discussion

5.1 Physical measurements

Apart from ultimate pH which is well known to play an essential role in various eating qualities of meat, pH of meat during ageing is important for activity of endogenous proteases such as calpains which are involved in the ageing process of meat. In addition, meat pH affects Maillard reaction during cooking which is responsible for flavour of cooked meat (Madruga and Mottram 1995). The pH of dry aged beef was significantly higher than that of wet aged samples at both days 35 and 56. Further investigation is required to understand if the difference in pH of wet and dry aged beef contributed to difference in flavour observed.

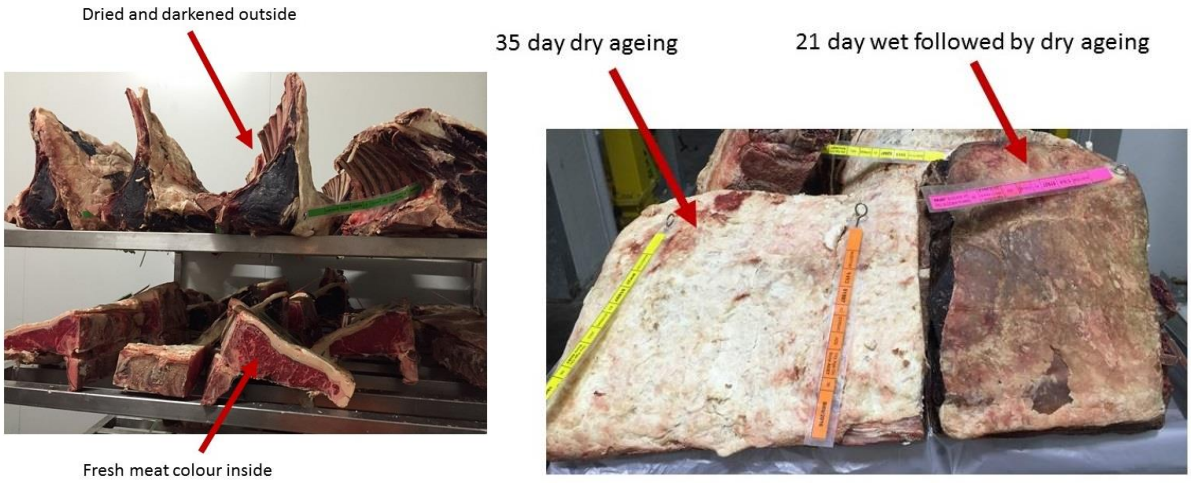

Figure 22. Representative photos of meat during the dry ageing process.

Although there was no difference in total water content of wet aged and dry aged (at point of sale), dry ageing lead to a significant loss in final yield (approximately 43%) due to surface drying and the need to trim off darkened meat. It should be noted that in this project wet ageing was conducted with boneless meat whereas dry ageing was performed with bone-in meat. A previous study (Kim *et al.* 2016) comparing wet and dry aged beef found that wet and dry aged beef had final yields of 55% and 46%, suggesting that most of the loss at point of sale was in bone weight. The difference in yield between the two ageing process is important for industry in terms of cost recovery and profitability.

5.2 Sensory results

Dry aged beef in this project received higher scores amongst Australian consumers for all eating attributes namely tenderness, juiciness, flavour, overall liking which were reflected in higher MQ4 scores regardless of ageing time. This in in contrast to results from previous studies from the USA and New Zealand. US studies (Laster *et al.* 2008, Smith *et al.* 2008, Smith *et al.* 2014) comparing wet and dry aged beef from US Choice and US Select grades showed no difference in sensory evaluation.

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.* 2008).

Consumer sensory conducted in Japan suggested that dry ageing of beef for 56 days significantly enhanced all eating qualities of beef amongst the Japanese consumers, in agreement with the Australian consumer data. It is noted that all eating attributes of dry and wet aged beef was consistently lower in Japanese consumers compared with Australian consumers. In order to investigate opportunities for dry ageing of Australian beef in Japan, the 21 days wet aged followed by 35 days dry aged (wet-then-dry) treatment was included for the Japanese consumers. The wet-then-dry samples received similar sensory scores for tenderness and juiciness and slightly lower (statically different) scores for flavour, overall liking and MQ4. The study of Polkinghorne *et al.* (2014) comparing sensory evaluation of beef between Australian and Japanese consumers found that grilled steaks were significantly downgraded by Japanese consumers regardless of muscle type, consistent with results found in this project. In addition, Polkinghorne *et al.* (2014) also found that juiciness as a sensory trait was more important for Japanese consumers than Australian consumers. In this project, tenderness, juiciness, MQ4 and satisfaction scores did not differ significantly between the dry aged (only) and the wet-then-dry samples for the Japanese consumers. In addition, the average difference in MSA score for all sensory attributes and MQ4 between wet-then-dry and wet aged (only) samples for the Japanese consumers was 11.32 and 12.46 respectively (Tables 18-22). These results together indicate that the wet-then-dry ageing treatment provides an opportunity to create premium products with Australian beef in Japan without significantly affecting eating quality.

The effect of ageing time on eating qualities was also investigated for both wet and dry aged samples. For dry aged samples, there was no significant difference between 35 and 56 days aged meat regardless of eating attribute. The study of Campbell *et al.* (2001). Data for wet ageing in this project, however, suggest that ageing beyond 21 days provided no additional benefit to tenderness and was detrimental to juiciness, flavour and overall liking.

5.3 Flavour chemistry results

The significant difference in flavour between wet and dry 35 days aged samples were investigated using SPME and PTR-MS. Difference in concentration in many volatiles were identified between wet and dry aged samples.

The volatile data from SPME showed very clearly higher amounts of pyrazine compounds in the dry aged beef samples compared to the wet aged. Pyrazines are important heat induced Maillard reaction products with strong grilled and roasted odours. Their formation is dependent on the amount of substrate present (mainly free amino acids and carbonyl groups from lipids) as well as the moisture content of the meat. The naturally lower moisture at the surface of dry aged beef probably facilitated the formation of pyrazines. From the volatile and GC-O data, it appears that the higher concentration of key Maillard reaction products such as the pyrazines are mainly responsible for the more intense flavour in dry aged beef. Of interest, the pyrazines were generally much higher at day 35 for both dry and wet aged samples, in agreement with the sensory consumer data, where a clear maximum flavour score was measured at 35 days, which decreased at day 56.

5.4 Oxidation results

Correlation between lipid oxidation and production of free radicals to colour and shelf life of meat is well established (Faustman *et al.* 2010). An increase in lipid oxidation also leads to more rancidity, thus reducing shelf life. 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. It is noted that MDA is only a part of the total odour complex (Tarladgis *et al.* 1960). Also volatile odour compounds produced by mechanisms other than lipid oxidation also contribute to the rancid flavour of meat (Casaburi *et al.* 2015). It is noted that the level of MDA of all samples in this study was well below the 2 mg/kg meat recommended by Campo *et al.* (2006), suggesting an acceptable lipid oxidation level for shelf life.

6 Conclusions/recommendations

6.1 Conclusions

- Dry ageing of Australian beef loins produced superior eating products compared to wet ageing when tested with both Australian and Japanese consumers.
- Optimal eating qualities were observed for 35 days dry aged beef for Australian consumers.
- The combined treatment of 21 days wet ageing followed by 35 days dry ageing did not significantly affect eating quality compared to dry ageing (only) treatment.
- The combined treatment of 21 days wet ageing followed by 35 days dry ageing significantly improved eating quality of Australian beef loins compared to the wet ageing (only) treatment.
- The difference in sensory flavour profiles between dry ageing (only) and wet ageing (only) methods may be explained by difference in the concentration of a number of volatiles associated with flavours of grilled beef.
- Lipid oxidation and protein oxidation was at acceptable level in beef aged up to 56 days regardless of ageing methods.
- Wet-then-dry aged products were more susceptible to bacterial growth.
- Quarters exposed to good airflow and UV were drier and less likely to have colonies on the surface compared to those in more sheltered positions in the dry ageing chamber.

6.2 Recommendations

- The significant difference in sensory eating qualities between wet and dry aged Australian beef demonstrates the potential of dry ageing to value-add to the Australian red meat industry.
- Limiting dry ageing time to 35 days may reduce production costs and at the same time producing optimal eating qualities.
- Fat cover of at least 20mm is recommended for primals destined for dry ageing.
- Hygiene is essential during quartering prior to dry ageing due to susceptibility to microbial growth. This is especially important for the wet-then-dry ageing process.

- A higher airspeed during the first ten day of dry ageing is needed especially for the wet-then-dry treatment is critical.
- A lower airspeed towards the end of dry ageing is recommended to reduce yield loss, especially for the wet-then-dry treatment.

7 Key messages

- Dry ageing of Australian beef loins produced products with superior eating qualities without affecting shelf-life of meat.
- Australian beef which is wet aged before dry aging produces products with similar eating quality as that of dry aged.
- Total yield at point of sale in dry aged meat is lower than wet aged meat, thus increasing the cost for processors. However, the higher cost at retail may be justified by a superior product.
- Monitoring the dry ageing process in the first two weeks is important to minimise wastage due to bacterial spoilage.
- Sufficient fat layer is needed to avoid trimming of lean tissue and thus increasing point of sale yield.

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9 Appendix

9.1 SPME-GCMS

Table 9.1. Effect of ageing method (dry or wet) and time (35 or 56 days) on volatile compounds identified in SPME of beef samples. Values are means after adjustment for intramuscular fat (covariate), n=12 for each ageing method and time treatment. All IDs are based on electron impact mass spectral database matches and have not yet been confirmed with standard compounds.

Volatile	m/z	Ageing type	Days aged		P value			SED		
			35	56	Ageing type	Days aged	Ageing type × days aged	Ageing type	Days aged	Ageing type × days aged
ALCOHOLS/DIOLS										
Ethanol	45	Dry	80	195	**<0.001**	0.088	0.158	239.7	239.1	339.7
		Wet	2329	3174						
1-pentanol	55	Dry	292	572	0.628	**<0.001**	0.125	24.7	24.6	35
		Wet	321	517						
1-penten-3-ol	57	Dry	105	6	**0.023**	**0.003**	**<0.001**	12	12	17.1
		Wet	19	31						
1-hexanol	56	Dry	236	206	**0.018**	**0.036**	0.236	24.2	24.1	34.3
		Wet	198	106						
2-ethyl hexanol	57	Dry	170	179	**0.001**	**0.006**	**0.002**	18.9	18.8	26.7
		Wet	335	181						
4-butoxybutanol	57	Dry	54	19	**0.006**	**<0.001**	**0.008**	4.5	4.5	6.4
		Wet	24	19						
1-heptanol	70	Dry	**121**	**123**	**<0.001**	0.33	0.205	9.6	9.6	13.6
		Wet	87	63						

1-octanol	56	Dry	106	97	0.245	**0.003**	**0.024**	6.8	6.7	9.6
		Wet	115	70						
2-phenylethanol	91	Dry	25	11	**0.002**	0.101	0.577	11.3	11.3	16
		Wet	79	52						
Benzylalcohol	108	Dry	364	184	**<0.001**	**0.029**	0.118	43.5	43.4	61.6
		Wet	51	20						
4-methylphenol	107	Dry	13	2	0.078	**0.005**	0.065	6.5	6.5	9.3
		Wet	39	1						
3-methylphenol	107	Dry	23	8	0.934	**<0.001**	0.824	2.2	2.1	3.1
		Wet	22	9						
Ethylphenol-1	107	Dry	10	1	0.101	**0.039**	**0.004**	1.6	1.6	2.3
		Wet	1	4						
Ethylphenol-2	107	Dry	6	0	**0.017**	**0.021**	**0.008**	1	1	1.4
		Wet	0	1						
KETONES/DIONES										
Acetone	43	Dry	**383**	**176**	**<0.001**	**<0.001**	**0.014**	17.4	17.4	24.7
		Wet	205	101						
2,3-butanedione	86	Dry	1067	1018	0.898	**0.004**	**0.018**	46.6	46.5	66.1
		Wet	1201	889						
2-butanone	72	Dry	963	440	0.343	**<0.001**	0.447	58.9	58.8	83.5
		Wet	976	546						
2-pentanone	86	Dry	1060	992	0.925	**0.005**	**0.044**	46.7	46.6	66.2
		Wet	1169	886						
2-heptanone	58	Dry	40	4	0.154	**0.02**	**0.002**	5.4	5.4	7.6
		Wet	26	36						

2-octanone	58	Dry	61	33	**0.003**	**<0.001**	0.128	3.8	3.8	5.4
		Wet	39	24						
2-nonanone	58	Dry	47	32	0.199	0.218	0.296	6.6	6.6	9.4
		Wet	49	49						
1,4-butanediol	42	Dry	69	21	0.695	**<0.001**	**0.007**	6.1	6.1	8.6
		Wet	47	40						
1,3-butanediol	45	Dry	54	54	**<0.001**	0.628	0.582	15.4	15.4	21.8
		Wet	237	254						
3-hydroxy-2-butanone	45	Dry	2183	1915	**<0.001**	0.08	0.832	153.4	153	217.4
		Wet	473	138						
2-methyl-3-octanone	99	Dry	134	378	**<0.001**	**<0.001**	**0.002**	20.9	20.8	29.6
		Wet	104	177						
butyrolactone	86	Dry	700	256	0.651	0.07	**0.015**	93.3	93.1	132.3
		Wet	388	495						
PYRAZINES/ MAILLARD										
2-methylpyrazine	94	Dry	391	157	**<0.001**	**<0.001**	0.513	25.6	25.5	36.2
		Wet	245	46						
2,5-dimethylpyrazine	108	Dry	292	107	**0.005**	**<0.001**	0.546	26.1	26.1	37.1
		Wet	181	29						
2,6-dimethylpyrazine	108	Dry	1345	587	**<0.001**	**<0.001**	0.782	89.6	89.4	127
		Wet	882	73						
2,3-dimethylpyrazine	108	Dry	142	63	**<0.001**	**<0.001**	0.513	8.3	8.2	11.7
		Wet	80	12						
2-ethyl-5-methylpyrazine	121	Dry	248	97	**<0.001**	**<0.001**	0.151	13.3	13.2	18.8
		Wet	143	33						

2-ethyl-6-methylpyrazine	121	Dry	510	190	**<0.001**	**<0.001**	**0.075**	27.7	27.6	39.2
		Wet	275	64						
trimethyl pyrazine	122	Dry	1019	456	**<0.001**	**<0.001**	0.251	48.2	48.1	68.4
		Wet	570	125						
3-ethyl-2,5-dimethylpyrazine	135	Dry	654	289	**0.002**	**<0.001**	0.45	44.4	44.3	63
		Wet	503	68						
2-ethyl-3,5-dimethylpyrazine	135	Dry	221	86	**<0.001**	**<0.001**	0.095	11.4	11.4	16.1
		Wet	116	22						
1-diethyl methylpyrazine	149	Dry	49	20	**<0.001**	**<0.001**	0.68	3.5	3.5	5
		Wet	29	3						
2-diethyl methylpyrazine	149	Dry	22	8	**<0.001**	**<0.001**	**0.014**	1.3	1.3	1.8
		Wet	6	0						
3,5-dimethyl-2-isobutylpyrazine	122	Dry	34	10	**0.001**	**<0.001**	0.244	3.2	3.2	4.6
		Wet	15	0						
dimethyl isopentylpyrazine	122	Dry	**96**	**40**	**<0.001**	**<0.001**	0.675	8	7.9	11.3
		Wet	53	4						
ALDEHYDES										
2-methylpropanal	72	Dry	496	215	0.46	**<0.001**	0.487	37.7	37.6	53.4
		Wet	440	213						
2-methylbutanal	57	Dry	3869	1574	0.83	**<0.001**	0.788	420.9	419.8	596.6
		Wet	3659	1596						
3-methylbutanal	58	Dry	1230	607	0.073	**<0.001**	0.342	138.6	138.2	196.4
		Wet	1641	741						

hexanal	56	Dry	1366	3974	**<0.001**	**0.001**	**<0.001**	262.5	261.8	372
		Wet	1293	847						
heptanal	70	Dry	345	24	0.383	**0.005**	**0.047**	56.8	56.6	80.4
		Wet	167	104						
decanal	57	Dry	70	52	**0.01**	**0.009**	0.491	6.9	6.9	9.8
		Wet	53	25						
nonanal	57	Dry	797	758	0.914	**0.028**	**0.057**	68.9	68.8	97.7
		Wet	934	598						
octanal	84	Dry	183	237	0.131	0.549	**0.002**	16.1	16.1	22.9
		Wet	224	140						
(E,E)-2,4-hexadienal	81	Dry	78	104	0.133	0.065	0.736	10.9	10.9	15.4
		Wet	64	82						
2,5-dimethylbenzaldehyde	134	Dry	1007	1099	**0.004**	0.866	0.462	171.1	170.6	242.4
		Wet	1758	1588						
long chain aldehyde	57	Dry	38	21	**0.001**	0.924	0.052	7.6	7.6	10.8
		Wet	56	73						
benzaldehyde	105	Dry	703	368	0.102	**<0.001**	0.143	46	45.9	65.2
		Wet	714	526						
4-ethylbenzaldehyde	134	Dry	34	12	**<0.001**	**0.002**	**0.002**	2.8	2.7	3.9
		Wet	10	11						
5-methylfurancarboxaldehyde	110	Dry	1	0	0.124	0.135	0.163	0.2	0.2	0.3
		Wet	0	0						
furfural	96	Dry	19	6	0.28	0.512	**0.045**	4.6	4.6	6.6
		Wet	14	23						

SULPHUR COMPOUNDS										
methanethiol	47	Dry	65	15	**0.024**	**<0.001**	0.251	10.7	10.7	15.2
		Wet	106	30						
carbon disulphide	76	Dry	163	53	0.247	**0.001**	**0.038**	16.7	16.7	23.7
		Wet	145	115						
dimethyl sulphide	62	Dry	156	93	0.064	0.48	**0.001**	18.9	18.8	26.7
		Wet	115	217						
dimethyl disulphide	94	Dry	62	20	0.126	0.08	0.103	11.5	11.4	16.2
		Wet	61	61						
dimethyl trisulphide	79	Dry	18	21	0.833	0.323	0.715	2.3	2.3	3.3
		Wet	18	20						
methional	76	Dry	19	24	**0.034**	**0.007**	**<0.001**	3.3	3.3	4.7
		Wet	44	15						
methionol	106	Dry	8	1	0.408	**0.008**	0.642	2.4	2.4	3.4
		Wet	11	2						
2-acetyl-2-thiazoline	129	Dry	16	4	**0.027**	**<0.001**	0.806	1.8	1.8	2.6
		Wet	11	0						
benzothiazole	135	Dry	450	113	0.213	**0.001**	**0.038**	51.2	51.1	72.6
		Wet	262	170						
ALKANES										
octane (?)	85	Dry	11	23	**<0.001**	0.596	0.266	22.6	22.5	32
		Wet	264	223						
long chain alkane-2	57	Dry	1400	1192	**0.028**	0.262	0.703	253.1	252.5	358.7
		Wet	2147	1740						
trimethy loctane	57	Dry	980	811	**0.004**	0.123	0.521	158.8	158.4	225.1

		Wet	1666	1286						
MISCELANEOUS		Wet	592	315						
trimethyl amine	58	Dry	276	89	**<0.001**	**<0.001**	**0.003**	21.5	21.4	30.5
		Wet	52	31						
2-acetylpyrrole	94	Dry	91	35	0.117	**<0.001**	0.766	8.5	8.5	12.1
		Wet	79	18						
2-pentylfuran	81	Dry	55	44	0.091	0.223	0.754	10.7	10.7	15.2
		Wet	38	21						
Pyridine	79	Dry	397	295	0.198	**<0.001**	0.182	28.6	28.5	40.5
		Wet	397	214						
Pyrrole	67	Dry	54	25	0.174	**0.005**	**0.003**	4.1	4.1	5.8
		Wet	33	35						
ACIDS										
acetic acid	60	Dry	293	316	**<0.001**	**<0.001**	**<0.001**	70.9	70.7	100.4
		Wet	**1150**	**1903**						
hexanoic	60	Dry	213	170	**0.004**	0.359	0.656	31.3	31.2	44.4
		Wet	314	299						
octanoic acid	60	Dry	78	19	0.055	**0.037**	**0.038**	12.8	12.8	18.2
		Wet	76	78						
2-ethylbutanoic acid	88	Dry	22	46	**0.049**	**<0.001**	0.121	7.4	7.4	10.5
		Wet	27	75						
ESTERS										
butyl formate	56	Dry	1527	936	**0.048**	**<0.001**	0.556	97.3	97.1	138
		Wet	1688	1216						
methyl acetate	74	Dry	145	73	0.418	**<0.001**	0.436	8.9	8.9	12.6

		Wet	130	72						
methyl butanoate	74	Dry	882	547	**0.036**	**0.002**	0.123	57.4	57.2	81.3
		Wet	926	785						
methyl-2-methylbutanoate	88	Dry	211	128	0.061	**0.002**	0.158	14.8	14.7	20.9
		Wet	220	182						
methyl heptanoate	74	Dry	843	962	0.648	0.431	0.707	101.6	101.4	144.1
		Wet	929	970						
methyl octanoate	74	Dry	13	2	**0.007**	0.067	0.695	6	6	8.6
		Wet	35	20						
methyl propanoate	88	Dry	109	61	**0.018**	**<0.001**	0.55	6.9	6.9	9.8
		Wet	133	76						
ethyl nonanoate	74	Dry	62	83	0.231	0.788	**0.012**	6.7	6.7	9.6
		Wet	91	71						
methylsalicylate	120	Dry	78	11	**0.038**	**0.002**	**0.012**	9.7	9.6	13.7
		Wet	26	18						

Data are semi-quantitative (i.e. relative arbitrary concentration unit). SED = standard error of differences. Significant differences ($P < 0.05$) are indicated in bold.

9.2 PTR-MS

Table 9.2. Effect of ageing method (dry or wet) and time (35 or 56 days) on volatile compounds identified in PTR-MS of beef samples. Values are means after adjustment for intramuscular fat (covariate), n=12 for each ageing method and time treatment.

m/z	Ageing method	Ageing time (days)			P values			SED
		21	35	56	Ageing type	Days aged	Ageing type × days aged	
40	Dry		89	99	0.747	0.092	0.844	11.5
	Wet	85	84	101				
41	Dry		6518	7541	0.477	**0.005**	0.234	1088.4
	Wet	6299	6174	8969				
42	Dry		15428	9737	0.186	**<0.001**	0.171	3181.9
	Wet	4282	21508	10339				
43	Dry		6520	6960	**<0.001**	**0.012**	0.052	2212.1
	Wet	7307	9094	16245				
44	Dry		400	444	**0.003**	**0.011**	0.118	67.3
	Wet	454	472	680				
45	Dry		116120	82584	0.232	0.051	0.702	19453.7
	Wet	97502	136049	99312				
46	Dry		3884	3111	0.188	0.056	0.753	455.7
	Wet	3416	4367	3558				
47	Dry		1751	3738	**<0.001**	**0.025**	0.180	22851.9
	Wet	7994	53349	104871				
48	Dry		143	166	**<0.001**	**0.035**	0.170	565.8
	Wet	322	1374	2648				
49	Dry		952	315	0.496	0.098	0.077	271
	Wet	359	740	828				
50	Dry		229	227	0.188	0.834	0.830	13
	Wet	216	218	214				
51	Dry		362	282	0.265	0.752	0.849	214.1
	Wet	484	510	486				
52	Dry		42	34	0.413	0.579	0.653	9.2
	Wet	45	45	43				
53	Dry		142	175	0.181	0.418	0.745	36
	Wet	152	118	139				
54	Dry		65	61	0.255	0.550	0.622	45.6
	Wet	104	120	84				
55	Dry		88389	54987	0.157	0.748	0.987	98709.4
	Wet	110794	197359	162200				
56	Dry		509	548	0.251	0.859	0.644	349
	Wet	688	927	744				
57	Dry		1439	1703	0.086	0.840	0.752	995.4
	Wet	1935	2955	2793				
58	Dry		333	374	0.068	0.125	0.667	45.3
	Wet	290	260	331				
59	Dry		27409	37723	**<0.001**	0.241	0.156	4443.2
	Wet	26506	20709	22341				

60	Dry		1168	1522	0.351	0.488	0.075	238.3
	Wet	1051	1327	1071				
61	Dry		1861	2403	**<0.001**	**0.023**	0.058	1435.2
	Wet	3492	3588	8419				
62	Dry		135	145	**0.003**	**0.019**	0.057	36.6
	Wet	158	167	289				
63	Dry		2944	2612	**0.042**	0.614	0.809	627.2
	Wet	2901	3824	3752				
64	Dry		115	83	**0.033**	0.317	0.226	24.8
	Wet	95	132	149				
65	Dry		-379	1258	**0.016**	0.287	0.314	25075
	Wet	1073	26423	64689				
66	Dry		29	73	**0.025**	0.286	0.272	730.1
	Wet	53	664	1866				
67	Dry		222	261	0.054	0.164	0.241	129.6
	Wet	235	293	554				
68	Dry		71	64	0.927	0.969	0.361	8
	Wet	65	64	69				
69	Dry		7631	7635	0.973	0.146	0.194	1366.4
	Wet	6659	6457	8968				
70	Dry		478	498	0.915	0.129	0.269	78
	Wet	432	414	557				
71	Dry		905	890	0.418	**0.033**	0.170	162.3
	Wet	643	832	1159				
72	Dry		125	126	0.717	0.149	0.218	13.3
	Wet	113	116	142				
73	Dry		19584	21606	0.366	0.891	1.000	7924.1
	Wet	22235	24287	26199				
74	Dry		963	881	0.169	0.682	0.580	141.4
	Wet	758	762	798				
75	Dry		476	574	0.106	0.525	0.654	146.5
	Wet	578	598	793				
76	Dry		68	65	0.951	0.945	0.226	6.1
	Wet	65	62	70				
77	Dry		187	338	0.523	0.903	0.702	186.6
	Wet	361	327	372				
78	Dry		40	53	0.275	0.911	0.248	17
	Wet	50	68	52				
79	Dry		169	244	0.127	0.351	0.922	185.7
	Wet	560	380	450				
80	Dry		52	50	0.548	0.775	0.934	9.9
	Wet	60	57	56				
81	Dry		297	290	0.796	0.303	0.601	47
	Wet	360	288	317				
82	Dry		49	50	0.624	0.333	0.475	6
	Wet	53	44	53				
83	Dry		1905	3307	0.523	0.180	0.515	1659.5
	Wet	2386	1878	4746				
84	Dry		178	266	0.743	0.250	0.930	85
	Wet	194	154	257				

85	Dry		351	380	0.958	0.300	0.638	43.2
	Wet	342	333	398				
86	Dry		69	78	0.262	0.058	0.190	6.3
	Wet	59	70	66				
87	Dry		8199	7286	0.123	0.690	0.240	1372.5
	Wet	6111	5441	6937				
88	Dry		485	470	0.229	0.594	0.581	79.1
	Wet	385	385	435				
89	Dry		856	1168	**0.013**	**0.030**	0.054	512
	Wet	995	1064	2786				
90	Dry		186	207	**0.047**	**0.048**	0.133	21.4
	Wet	208	193	263				
91	Dry		849	878	**0.003**	0.059	0.293	70.8
	Wet	843	946	1075				
92	Dry		163	168	0.065	0.381	0.631	9.5
	Wet	167	173	185				
93	Dry		388	505	0.073	**<0.001**	0.086	79.5
	Wet	358	391	716				
94	Dry		163	169	0.105	**0.028**	0.128	13
	Wet	159	164	201				
95	Dry		349	326	0.095	0.995	0.472	34.7
	Wet	315	283	299				
96	Dry		166	118	0.514	0.131	0.063	15.5
	Wet	132	138	134				
97	Dry		249	328	0.878	0.296	0.885	85.9
	Wet	279	249	351				
98	Dry		73	79	0.476	0.369	0.690	8.9
	Wet	74	65	78				
99	Dry		231	276	0.745	0.192	0.704	25.9
	Wet	249	232	263				
100	Dry		56	57	0.639	0.184	0.173	4.5
	Wet	52	50	59				
101	Dry		709	908	0.459	0.595	0.716	193.3
	Wet	805	657	753				
102	Dry		113	121	0.651	0.868	0.642	13.6
	Wet	113	113	114				
103	Dry		921	910	0.114	0.353	0.222	102.4
	Wet	785	708	882				
104	Dry		126	134	0.519	**0.046**	0.276	7.9
	Wet	122	115	136				
105	Dry		519	368	0.311	0.077	0.359	128.4
	Wet	275	527	532				
106	Dry		551	567	0.127	0.637	0.927	28.9
	Wet	525	520	531				
107	Dry		1571	1702	0.791	**<0.001**	0.752	88.1
	Wet	1395	1535	1711				
108	Dry		1627	1661	0.084	0.954	0.525	71.7
	Wet	1592	1569	1544				
109	Dry		555	511	0.683	0.289	0.659	26.1
	Wet	524	538	512				

110	Dry		145	139	0.749	0.081	0.905	6.9
	Wet	129	144	137				
111	Dry		135	149	0.776	0.800	0.665	17.7
	Wet	142	145	148				
112	Dry		64	67	0.674	0.909	0.596	5.5
	Wet	67	68	67				
113	Dry		125	128	0.673	0.531	0.436	11.9
	Wet	131	121	139				
114	Dry		68	60	0.510	0.672	0.170	5.3
	Wet	59	59	63				
115	Dry		229	286	0.288	0.158	0.444	25.3
	Wet	273	266	291				
116	Dry		43	46	0.060	0.338	0.520	5
	Wet	43	52	51				
117	Dry		176	280	0.449	0.177	0.547	57.6
	Wet	216	169	226				
118	Dry		76	78	0.735	**0.019**	0.217	5.7
	Wet	86	69	82				
119	Dry		97	108	**0.002**	0.246	0.827	22.3
	Wet	104	148	163				
120	Dry		690	710	**0.021**	0.694	**0.038**	20.3
	Wet	693	687	650				
121	Dry		291	313	**<0.001**	**0.001**	0.440	40.7
	Wet	263	425	494				
122	Dry		90	102	**0.002**	**0.003**	0.402	7
	Wet	87	112	116				
123	Dry		211	189	0.225	0.426	0.860	19.5
	Wet	186	190	173				
124	Dry		537	541	0.058	0.703	0.676	21.9
	Wet	514	501	516				
125	Dry		982	954	0.249	0.716	0.625	37.9
	Wet	935	935	935				
126	Dry		1251	1208	0.347	0.929	0.289	53.1
	Wet	1239	1245	1286				
127	Dry		106	113	**0.010**	0.613	0.893	10.2
	Wet	115	127	132				
128	Dry		51	53	0.513	0.636	0.557	4.5
	Wet	53	51	56				
129	Dry		78	91	0.937	0.199	0.870	11.7
	Wet	72	79	90				
130	Dry		37	32	0.234	0.950	0.352	4.4
	Wet	36	38	40				
131	Dry		43	47	0.080	0.099	0.545	6.3
	Wet	43	49	58				
132	Dry		28	24	0.174	0.926	0.306	3.6
	Wet	27	29	31				
133	Dry		31	29	**<0.001**	0.255	**0.015**	4
	Wet	38	37	48				
134	Dry		28	28	0.778	0.269	0.128	3.3
	Wet	26	32	25				

135	Dry		44	43	**<0.001**	**<0.001**	**0.008**	6.6
	Wet	44	55	82				
136	Dry		27	31	**0.012**	0.405	0.514	3.4
	Wet	30	35	36				
137	Dry		92	72	0.415	0.061	0.455	10.4
	Wet	93	80	72				
138	Dry		49	53	0.290	0.869	0.751	5.3
	Wet	49	46	47				
139	Dry		41	45	0.096	0.067	0.790	4.9
	Wet	37	49	50				
140	Dry		26	28	0.876	0.901	0.150	3.2
	Wet	26	30	25				
141	Dry		44	40	0.759	0.854	0.241	4.7
	Wet	41	41	46				
142	Dry		36	36	0.790	0.870	0.774	3.7
	Wet	37	38	36				
143	Dry		118	130	0.859	0.908	0.790	25.8
	Wet	134	126	128				
144	Dry		37	39	0.353	0.713	0.496	4.4
	Wet	33	36	33				
145	Dry		32	38	0.227	0.259	0.944	4.7
	Wet	35	37	42				
146	Dry		35	36	0.231	0.661	0.847	3.6
	Wet	36	32	32				
147	Dry		29	29	0.119	0.221	**0.033**	2.9
	Wet	27	20	30				
148	Dry		27	31	0.646	0.262	0.702	3.6
	Wet	25	27	29				
149	Dry		32	34	0.449	**0.039**	0.324	3.2
	Wet	29	31	38				
150	Dry		24	27	0.082	0.113	0.543	3
	Wet	28	26	31				
151	Dry		38	33	0.935	**0.002**	0.077	3.5
	Wet	36	43	28				
152	Dry		28	25	0.463	0.917	0.558	3.9
	Wet	27	29	29				
153	Dry		29	29	0.074	0.224	0.148	3.4
	Wet	32	30	37				
154	Dry		23	27	0.063	0.013	0.181	3
	Wet	30	24	34				
155	Dry		30	30	0.796	0.606	0.354	3.1
	Wet	29	33	28				
156	Dry		26	23	0.361	0.607	0.180	2.4
	Wet	27	26	27				
157	Dry		29	28	0.454	0.449	0.540	3.4
	Wet	28	33	29				
158	Dry		28	23	0.969	0.156	0.680	3.4
	Wet	26	29	22				
159	Dry		26	30	0.661	0.197	0.767	3.8
	Wet	31	26	31				

160	Dry		26	25	0.837	0.472	0.974	2.9
	Wet	29	26	25				
161	Dry		27	24	0.230	0.360	0.712	2.7
	Wet	25	29	27				
162	Dry		25	23	0.624	0.370	0.171	3
	Wet	27	23	27				
163	Dry		24	27	0.909	0.468	0.772	3.2
	Wet	23	25	26				
164	Dry		23	26	0.200	0.141	0.589	2.8
	Wet	27	25	30				
165	Dry		32	25	0.826	**0.014**	0.899	3.2
	Wet	30	31	25				
166	Dry		24	25	0.817	0.852	0.507	3
	Wet	26	26	24				
167	Dry		20	28	0.898	0.665	**0.010**	2.9
	Wet	25	26	22				
168	Dry		24	25	0.344	0.536	0.557	2.7
	Wet	24	27	27				
169	Dry		30	26	0.061	0.536	0.603	3.3
	Wet	24	24	22				
170	Dry		28	25	0.274	0.567	0.375	3
	Wet	24	24	24				
171	Dry		24	31	0.116	0.634	**0.031**	2.5
	Wet	23	27	24				
172	Dry		20	22	0.061	0.912	0.338	3.1
	Wet	25	26	23				
173	Dry		22	28	0.939	**<0.001**	0.300	3
	Wet	21	21	32				
174	Dry		22	24	0.663	0.584	0.289	2.8
	Wet	23	22	20				
175	Dry		24	27	0.653	0.579	0.996	3.7
	Wet	25	22	24				
176	Dry		23	22	0.530	0.363	0.226	3
	Wet	23	27	22				
177	Dry		26	25	0.189	0.481	0.635	3.1
	Wet	31	29	26				
178	Dry		23	27	0.201	0.469	0.962	3
	Wet	23	20	23				
179	Dry		25	26	0.721	0.954	0.795	2.9
	Wet	26	25	25				
180	Dry		22	25	0.726	0.773	0.092	2.5
	Wet	21	25	21				
181	Dry		21	22	**0.006**	0.793	0.692	3.1
	Wet	25	26	29				
182	Dry		25	29	0.067	0.316	**0.011**	2.6
	Wet	22	27	20				
183	Dry		26	27	0.062	0.737	0.857	3.1
	Wet	22	22	22				
184	Dry		25	23	0.585	0.825	0.427	3
	Wet	25	24	25				

185	Dry		26	25	0.649	0.419	0.454	3.2
	Wet	29	23	26				
186	Dry		25	23	0.394	0.667	**0.048**	2.8
	Wet	22	19	25				
187	Dry		26	26	0.235	0.245	0.640	3.2
	Wet	29	25	23				
188	Dry		20	24	0.382	0.127	0.793	2.9
	Wet	26	22	27				
189	Dry		23	22	0.896	0.558	0.653	3.1
	Wet	25	22	24				
190	Dry		25	26	0.120	0.174	0.269	3.2
	Wet	26	20	25				
191	Dry		28	22	**0.017**	0.642	0.111	3.2
	Wet	23	18	20				
192	Dry		20	27	0.721	0.365	0.163	3.7
	Wet	23	23	23				
193	Dry		23	21	0.260	0.715	0.053	2.9
	Wet	24	21	27				
194	Dry		24	24	0.073	0.204	0.867	3.2
	Wet	26	20	19				
195	Dry		24	24	0.915	0.842	0.996	2.6
	Wet	22	24	23				
196	Dry		25	28	0.278	0.356	0.665	3.2
	Wet	27	24	26				
197	Dry		24	22	0.088	0.901	0.836	2.7
	Wet	24	27	26				
198	Dry		24	25	0.887	0.963	0.443	2.9
	Wet	25	26	24				
199	Dry		23	24	0.320	0.122	0.846	3
	Wet	26	21	22				
200	Dry		25	30	0.360	0.106	0.633	3.5
	Wet	21	24	26				

Data are semi-quantitative (i.e. relative arbitrary concentration unit). SED = standard error of differences. Significant differences ($P < 0.05$) are indicated in bold.